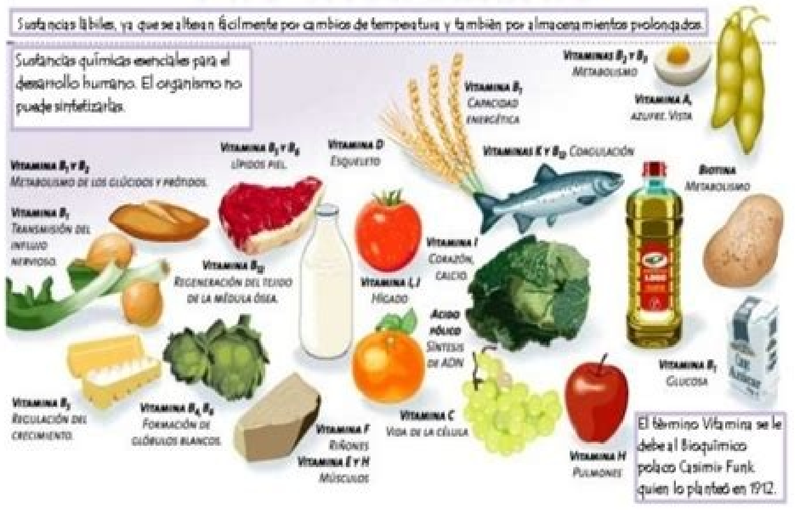
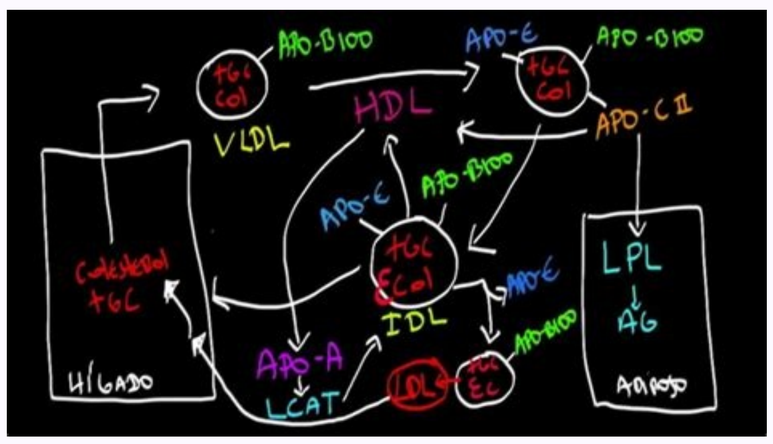
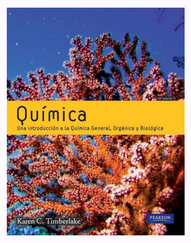
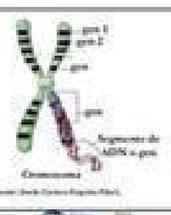
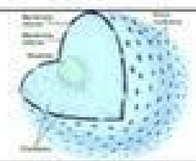
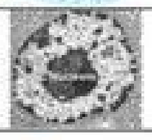
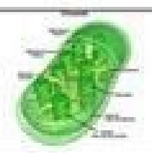
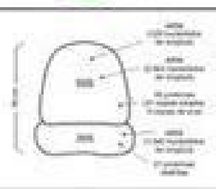
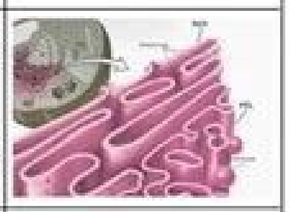
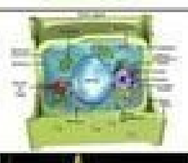
Sintesis de los lipidos pdf

LAS VITAMINAS







COMPONENTES CELULARES				
COMPONENTE CELULAR	ESTRUCTURA	FUNCIÓN	RELACIÓN	IMAGEN
Pared celular	Red de mocrofibrillas de celulosa, polisacáridos y glipoproteínas.	Protege y da soporte a la célula.	Se encarga de proteger a los otros organelos dentro de la célula.	
Flagelos	Un microtúbulo rodeado por microtúbulos en pares fusionados que interactúan por medio de brazos proteicos.	Mueven la célula mediante fluidos.	Gracias al movimiento que proporciona la membrana introduce alimentos a la célula, de ahí otros componentes se benefician.	
Membrana plasmática	Bicapa fosfolipídica, proteínas integrales y globulares.	Aísla el contenido de la célula del ambiente, regula entrada y salida de materiales, comunica con otras células.	Permite el paso que sustancias que proveen de "alimento" a los diferentes organelos.	
Material genético	Formado por A, C, G, T, un grupo fosfato unido a una azúcar (ribosa o desoxirribosa).	Contiene información necesaria para construir la célula y controlar la actividad celular.	En el núcleo se duplica, posteriormente se traduce y gracias a esto hay transcripción que permite la formación de proteínas para la supervivencia de la célula en su conjunto.	
Cromosomas	Formado por constricción primaria (centrómero), constricción secundaria y telómero).	Contiene y controla todo el uso del DNA.	Se encargan de dirigir como se expresa el DNA. Sólo se observan durante la división celular. Prácticamente no interactúan directamente con otros organelos.	
Núcleo	Formado por la envuelta nuclear, poros nucleares.	Contiene al nucléolo y cromatina (eucromatina, heterocromatina)	se enlaza con el RE, permite la salida de ribosomas y ARNm que posteriormente llegará a los ribosomas.	
Nucléolo	Esta dentro del núcleo.	Sintetiza ribosomas.	Exporta los ribosomas al citoplasma. Dentro del ribosoma, se llevará a cabo la síntesis de proteínas.	
Mitocondrias	Membrana interna, membrana externa, matriz y crestas	Produce energía por metabolismo aeróbico	Recibe la glucosa proveniente de los cloroplastos. Provee de energía a la célula en general, ya que aquí se sintetizan los componentes energéticos más importantes.	
Cloroplasto	Membrana interna, membrana externa, tilacoide, conector de tilacoide, estroma	Realizan fotosíntesis.	Exporta la glucosa sintetizada a la mitocondria.	
Ribosoma	Subunidad menor, subunidad mayor	Síntesis de proteínas	Traduce la información proveniente de los distintos RNA. Posteriormente, las proteínas formadas, son excretadas de la célula hacia la matriz extracelular.	
Retículo endoplásmico	Sacos aplanados, cisternas, en el caso de RE contiene ribosomas	Sintetiza componentes de la membrana celular (proteínas y lípidos)	Aprovecha los materiales presentes en el citoplasma para formar lípidos y proteínas, posteriormente exporta al aparato de golgi, lo sintetizado a través de vesículas de transmisión.	
Aparato de Golgi	Cisternas intermedias, vesículas de transporte, vesículas de transición	Modifica, y empaca proteínas y lípidos, sintetiza algunos carbohidratos	Recolecta las vesículas provenientes del RE, y madura lípidos y proteínas. Estos lípidos y proteínas formaran parte de la membrana celular.	
Lisosomas	Enzimas digestivas intracelulares	Digestión de componentes inservibles de la célula.	Se forma a partir de segmentos del aparato de golgi. Posteriormente degradara componentes provenientes de la membrana.	
Vacuola central	Agua y pared de la vacuola	Contiene agua y desechos; brinda presión de turgencia como soporte a la célula	Guarda materiales de desecho provenientes de muchos otros organelos dentro de la célula.	
Citoesqueleto	Microtúbulos, microfilamentos, filamentos intermedios	Da forma y soporte a la célula; coloca y mueve partes de la célula	Se relaciona con otros organelos, ya que los mueve a los lugares apropiados.	
Centriolos	Pequeños microtúbulos	Producen los microtúbulos de cilios y flagelos.	Forman las fibras de uso durante la división celular. Sin dichas fibras, no sería posible la división.	

INTEGRANTES:

- Alcocer Ramírez Andrea.
- Barranco García Luis Enrique.
- Cruz Reyes Miguel Ángel.
- Prieto Herrera Antonio.
- Ramírez Reyes Daniel de Jesús
- Reyes Velázquez Bruno David

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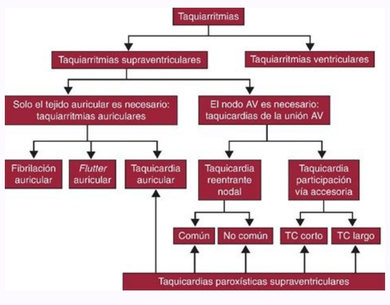

Sintesis de los lipidos en el organismo. Sintesis de metabolismo de los lipidos. Resumen de la sintesis de los lipidos. Que es perfil de lipidos. Cuáles son los síntomas de un golpe de calor. La sintesis de los lipidos se lleva a cabo en.

Según la definición de "LIPID MAPS" (LIPID Metabolites And Pathways Strategy), un lípido es cualquier molécula insoluble en agua y soluble en solventes orgánicos. En las células hay moléculas que cumplen estos requisitos y que se denominan lípidos biológicos. Incluyen a una gran variedad de lípidos tales como ácidos grasos, ceras, monoglicéridos, diglicéridos, triglicéridos, fosfolípidos, esfingolípidos, esteroles, terpenos, prenoles, eicosanoides, vitaminas solubles en grasas, entre otros. En las células los lípidos tienen tres funciones básicas: ser componentes estructurales básicos de las membranas biológicas, almacén de energía y actuar como moléculas señalizadoras, es decir, transportadoras de información. En esta página nos vamos a centrar sobre todo en los lípidos que están relacionados con las membranas biológicas. Modelos de membrana La organización de las membranas celulares está determinada por las características de sus componentes, fundamentalmente por los lípidos. Los otros componentes importantes de las membranas celulares son las proteínas, principales actores en las funciones celulares asociadas a las membranas, y los glúcidos. Sin embargo, la diversidad de los lípidos de membrana (hay más de mil tipos diferentes) y su organización espacial (formando un bicapa) hacen a estas moléculas esenciales. Los lípidos constituyen aproximadamente el 50 % del peso de las membranas, con unos 5 millones de moléculas por µm2. Se estima que aproximadamente el 5 % de los genes de una célula están dedicados a producir sus lípidos. Los lípidos definen las propiedades físicas de las membranas. La longitud y el grado de saturación de sus ácidos grasos regulan la fluidez y el grosor de la membrana. Hay una distribución desigual de tipos de lípidos entre las dos monocapas creando lo que se denomina asimetría de membrana. En la membrana plasmática las cargas asociadas a las partes hidrofílicas de los lípidos de la monocapa interna contribuyen a crear un gradiente eléctrico entre la cara externa y la interna, y por tanto a modular el potencial

eléctrico. Mediante interacciones electroquímicas , los lípidos son capaces de modular la actividad de las proteínas de membrana y de segregarse lateralmente, en el plano de la membrana, creando dominios funcionales (como las balsas de lípidos) que hacen a una misma membrana heterogénea y multifuncional. Las diferentes membranas de la células tienen diferente composición de lípidos, lo que es importante para definir las propiedades de tales compartimentos y para establecer la identidad de estos. 1. Tipos Figura 1. Principales lípidos presentes en las membranas celulares. En las células eucariotas se ha encontrado una diversidad enorme en los lípidos de membrana. Sin embargo, atendiendo a su estructura molecular, se pueden clasificar en tres grandes familias: los glicerolípidos, los esfingolípidos y los esteroles (Figura 1) Glicerolípidos Son los lípidos más abundantes (del 70 %) de las membranas celulares. Se caracterizan por poseer cadenas de ácidos grasos unidos a una molécula de glicerol (Figura 2). En la mayoría de los casos hay dos cadenas de ácidos grasos, de 13 a 19 átomos de carbono de longitud, una saturada unida al carbono 1 del glicerol y la otra insaturada unida al carbono 2, mientras que al carbono libre del glicerol se le une un grupo fosfato, y al grupo fosfato se le pueden unir una variedad de grupos polares, tales como la colina, etanolamina, serina, inositol, etcétera. A estos lípidos se les llama glicerofosfolípidos, constituyen la mayoría de los fosfolípidos de las membranas celulares y son sus propiedades físicas. Los dobles enlaces (insaturado) hacen que la cadena de ácido graso se doble y, aunque restrinja las posibilidades de movimiento de la cadena, un aumento de la proporción de estos dobles enlaces aumenta la fluidez de la membrana puesto que provoca más separación entre moléculas. Los ácidos grasos constituyen la parte hidrofóbica (fobia por el agua) de los glicerofosfolípidos y son los que constituyen la parte interna de las membranas. Figura 2. Estructura y tipos de glicerofosfolípidos más abundantes de las membranas eucariotas. Los glicerofosfolípidos son el tipo de lípido más abundante de las membranas celulares, pero también forman parte de las lipoproteínas del suero y actúan como surfactante en los pulmones. Por otra parte, las células pueden almacenar grandes cantidades de lípidos en su interior en modo de gotas de lípidos. Estos glicerolípidos (sin fosfato), denominados neutros, son principalmente triacilglicéridos, donde los tres carbonos del glicerol están unidos a cadenas de ácidos grasos. Por último, hay algunos glicerolípidos sin grupo fosfato que tienen monosacáridos unidos al glicerol, son los glicoglicerolípidos, los cuales son muy escasos en los animales pero muy frecuentes en las plantas y las bacterias. Los principales tipos de glicerofosfolípidos son : ácido fosfatídico (PA), fosfatidil colina (PC), fosfatidil etanolamina (PE), fosfatidil serina (PS), fosfatidil glicerol (PG), fosfatidil inositol (PI), cardiolipina (CL) presente en la mitocondria, ácido lisofosfatídico (BMP/LBA) abundante en lisosomas, endosomas tardíos y multivesiculares. Los glicosilfosfatidilinositoles (GPI) son glicolípidos que se unen covalentemente a proteínas por su extremo carboxilo. Los GPI son ubicuos en las células eucariotas. Sirven de puntos de anclaje para muchas proteínas de membrana. GPI también pueden encontrarse como lípidos libres. Constan de fosfatidil inositol, glucosamina, tres manosas, y etanolamina fosfato. La proteína se une a la etanolamina fosfato por un enlace amida. Esfingolípidos Los esfingolípidos deben su nombre a que poseen una molécula de esfingosina, un alcohol nitrogenado con una cadena carbonada larga, a la cual se le une una cadena de ácido graso, formando la estructura básica denominada ceramida (Figura 3). La ceramida es biológicamente activa en las células y se encuentra como tal en las membranas celulares, pero en pequeña concentración. Aunque se habla de ceramida como una sola molécula, en realidad es un grupo de moléculas que se diferencian en la longitud de sus cadenas de ácidos grasos. A esta estructura de ceramida se añaden otras moléculas como azúcares o grupos fosfato que dan lugar a los diferentes esfingolípidos. Si se une a la ceramida un grupo fosfato más una colina o etanolamina tenemos a la esfingomielina (serían fosfolípidos). Si se unen sacáridos tenemos a los glicoesfingolípidos. Los glicoesfingolípidos se pueden dividir en dos grandes grupos: glucoesfingolípidos y galactoesfingolípidos. Los primeros tienen una glucosa como primer glúcido, mientras que los segundos tienen una galactosa. Los galactoesfingolípidos abundan en la mielina del tejido nervioso y también se expresan en intestino, testículos y riñón. La configuración con un solo monosacárido da lugar a los glicoesfingolípidos denominados cerebrósidos. Figura 3. Estructura y algunos esfingolípidos abundantes de las membranas eucariotas. Si se añade a la glucosa una cadena de azúcares de al menos tres monosacáridos, siendo uno de ellos el ácido siálico, tenemos a los esfingolípidos denominados gangliósidos. La diversidad de glicoesfingolípidos es enorme y diferente tanto en la composición de glúcidos como en su orden, pero también en la longitud de los ácidos grasos que heredaron de la ceramida. En las células animales la gran mayoría de los glucolípidos son glicoesfingolípidos. Esteroles Figura 4. Colesterol Los esteroles son el tercer grupo de lípidos más importante en las membranas biológicas. Su estructura química posee tres regiones: un grupo hidroxilo o alcohol en el carbono 3, cuatro anillos carbonados y una cadena alifática (Figura 4). El colesterol es el esterol más importante de las células animales y el tercer tipo de lípido más abundante en la membrana plasmática (desde el 10 al 40 % del total de lípidos). Aparece en pequeñas proporciones en las membranas de los orgánulos celulares como en las del retículo endoplasmático (1-5 %), en las membranas del aparato de Golgi (del 10 %). Sin embargo, hay algunas membranas con alta concentración de colesterol, que puede llegar al 50 %. Incluso pueden llegar hasta más del 50 % en las membranas de las vesículas fibrosas de las lentes del ojo. El colesterol se integra bien entre los fosfolípidos con su cabeza polar hacia las cabezas polares de los fosfolípidos y el resto de la molécula entre los ácidos grasos. No aparece en las membranas de las plantas, en algunas células eucariotas, ni en las bacterias, pero estas células tienen otro tipo de esteroles. En plantas es el fitosterol el esterol más abundante y en los hongos se encuentra el ergosterol. En las gotas de lípidos de las células animales se suelen acumular una gran cantidad de ésteres de colesterol como sustancias de reserva. El colesterol, y los esteroles en general, son esenciales para la integridad y funcionamiento de las membranas eucariotas puesto que contribuyen a la modulación de la rigidez, la fluidez y la permeabilidad de las membranas, sobre todo la membrana plasmática. También participan en ciertos procesos metabólicos vitales como la síntesis de hormonas esteroideas o de sales biliares, entre otras. El colesterol contribuye también a modular la actividad de los receptores acoplados a proteínas G y facilita la transducción de señales y el tráfico vesicular. Junto con los esfingolípidos parecen contribuir a formar heterogeneidades laterales (balsas de lípidos) en la distribución molecular, sobre todo en las membranas plasmáticas. 2. Distribución en membranas Figura 5. Proporción aproximada de los lípidos totales de un célula típica de mamífero (tomado de Vance 2015) Las diferentes membranas de las células eucariotas tienen una composición lipídica característica y en ocasiones muy diferente entre sí, incluso entre partes diferentes de la misma membrana, es decir, heterogénea por dominios. Se ha propuesto que la propia identidad de un orgánulo viene determinada no sólo por las proteínas que posee sino por el juego de lípidos que compone sus membranas. Por ejemplo, la membrana plasmática tiene una composición lipídica diferente a la membrana del retículo endoplasmático o a la del aparato de Golgi. Mantener estas identidades no es fácil puesto que tienen que contrarrestar el continuo intercambio de membranas mediado por vesículas y transportadores (Figura 5). Aunque haya lípidos similares en las diferentes membranas, hay diferencias importantes entre ellas (Figura 6). Por ejemplo, todas la membranas tienen una gran proporción de fosfatidil colina, pero este glicerolípido es más abundante en las membranas del retículo endoplasmático y en la membrana interna de la mitocondria. Las cadenas de ácidos grasos de los lípidos son en general más cortas y con más insaturaciones en el retículo, lo que le confiere un carácter fluido y hace a su membrana más delgada. En las membranas post-Golgi, es decir, membrana plasmática y endosomas, la concentración de esfingolípidos y colesterol es mayor que en el retículo y en las membranas del aparato de Golgi. El fosfoinosítido PI(4)P abunda en el aparato de Golgi y se ha usado tradicionalmente para identificar este orgánulo. Las mitocondrias tienen, aparte de otros más extendidos lípidos de membrana, cardiolipina, que sintetizan ellas mismas. Es interesante que en los procesos de maduración de algunos compartimentos, como es el caso de los endosomas, se cambia progresivamente la composición de alguno de sus lípidos. El ácido lisofosfatídico es abundante en las vesículas internas de los cuerpos multivesiculares y endosomas tardíos, representando hasta el 15 % del total de lípidos. Figura 6. Distribución de los lípidos más abundantes en diferentes compartimentos membranosos celulares. Las flechas azules delgadas indican trasiego de lípidos transportados por las vesículas, las rojas gruesas la transferencia de lípidos entre membranas muy próximas, las flechas rojas delgadas indican la transferencia de lípidos entre membranas mediante transportadores proteicos, los cuales actúan entre varios compartimentos (no indicado). En la imagen inferior se representa el incremento de la concentración de colesterol desde el retículo endoplasmático hasta la membrana plasmática. PC: fostatidil colina, PE: fosfatidil etanolamina, PI: fosfatidil inositol, PS: fosfatidil serina, SM: esfingomielina, ISL: esfingolípido inositol, CL: cardiolipina, MBP: bis monoacilglicerol fosfato (modificado de van Meer et al., 2008). También hay variaciones laterales de lípidos que hacen que una misma membrana tenga dominios funcionales. Otros fosfolípidos como PI(3,4,5)P3 se acumulan en la zona de fagocitosis y en el frente de avance de las células que se mueven, y están ausentes en la parte apical de las células epiteliales. Estas heterogeneidades pueden depender también de procesos de síntesis y degradación locales. La segregación de lípidos diferencial entre distintas membranas puede deberse a diferentes mecanismos: síntesis, degradación y modificaciones químicas locales, o a transporte diferencial entre membranas. Síntesis diferencial La concentración de determinadas especies de lípidos viene condicionada por su lugar de síntesis. Por ejemplo, los esfingolípidos se terminan de ensamblar en el aparato de Golgi y son los compartimentos post-Golgi donde más abundan, pero están ausentes en el retículo endoplasmático. La fosfatidilcolina se sintetiza en el retículo endoplasmático y es ahí donde más abunda. Sin embargo, no siempre es cierto puesto que, por ejemplo, el colesterol se sintetiza en el retículo endoplasmático, pero es más abundante en membranas post-Golgi. Esto es debido a que es transportado rápidamente a otras membranas. Selección y transporte. Los lípidos deben ser transportados entre membranas puesto que no difunden libremente por el citosol. Ello implica que el transportador puede seleccionar qué lípidos acarrea de un lado a otro cambiando así las proporciones de determinadas especies de lípidos. Estos transportadores son fundamentalmente las vesículas, las cuales forman sus membranas con lípidos de las propias membranas del compartimento de partida. Por ejemplo, las vesículas que van desde el Golgi a la membrana y a los endosomas están enriquecidas en esfingolípidos y colesterol, respecto la concentración de estas moléculas en las propias membranas del Golgi. También hay transportadores de lípidos individuales que son proteínas que son capaces de extraer un lípido de una membrana, transportarlo por el citosol y colocarlo en otra membrana. Contactos entre membranas. Las membranas de determinados orgánulos pueden observarse extremadamente próximas. Hay proteínas que se colocan entre las membranas de dos orgánulos cuando están muy próximas espacialmente, haciendo de puente para el trasiego de lípidos entre las membranas de ambos orgánulos. Degradación y reciclado diferencial. Todas las membranas sufren un proceso de reciclado de lípidos, bien por degradación in situ de los lípidos o por su salida en vesículas de reciclado. 3. Asimetría La asimetría es la distribución diferencial de lípidos entre las dos monocapas de lípidos que componen las membranas. Mientras en el retículo endoplasmático ambas monocapas son muy similares, en las membranas del lado trans del aparato de Golgi y los compartimentos post-Golgi suelen tener una clara diferencia de composición entre monocapas. Figura 7. Esquema de las flipasas, flopasas y escramblasas. Los lípidos pueden pasar de una monocapa a otra mediante proteínas transportadoras: las flipasas llevan lípidos hacia la monocapa citosólica, las flopasas hacia la monocapa externa y las escramblasas en ambos sentidos. Figura 8. Distribución asimétrica de lípidos en la membrana plasmática. La fosfatidil colina y la esfingomielina se localizan principalmente en la monocapa externa, mientras que la fosfatidil serina, la fosfatidil etanolamina y el fosfatidil inositol se encuentran en la monocapa citosólica. 4. Dominios laterales Figura 9. Dominios de membrana. Figura 10. Balsas de lípidos. Las denominadas balsas de lípidos se propusieron en 1997. Son microdominios del orden de nanómetros, muy dinámicos, ricos en esfingolípidos y colesterol, y su visualización con microscopios de fuerza atómica parece demostrar su existencia. Las caveolas son otro tipo de dominio lateral. Son porciones de la membranam, fundamentalmente la membrana plasmática, en las cuales abundan el colesterol, esfingolípidos y unas proteínas denominadas caveolinas. Estas regiones se invaginan para fomar, en muchos casos, vesículas de endocitosis, llevando consigo receptores y proteínas transmembrana, los cuales son atrapados en estas regiones gracias a la composición diferente de lípidos. Sin embargo, las caveolas parecen tener también otras funciones como controlar la composición molecular de la membrana o contrarrestar tensiones mecánicas. Otro mecanismo para generar heterogeneidades laterales es la interacción de los lípidos con las proteínas trasnsmebrana. Ciertas proteínas transmembrana se desplazarían por la membrana asociadas a un conjunto de lípidos, a los cuales atraerían por interacciones electroquímicas, de manera que estos lípidos formarían una periferia molecularmente diferente al resto de la membrana. Aunque tradicionalmene se ha pensado que la monocapa externa y la interna de la membrana son independientes a la hora de distribuir sus heterogeneidades lipídicas respectivas, hay evidencias que pueden influirse entre sí. Una manera es por la presencia de proteínas transmembrana que afectan simultáneamente a ambas monocapas, pero también puede ocurrir sin la intervención de proteínas. Una posibilidad de sincronización de heterogeneidades entre las dos monocapas puede ser debida a la longitud de las cadenas de ácidos grasos como las de algunos esfingolípidos, las cuales puede ser de hast 24 átomos de carbono (normalmente son 18) con lo que se insertarían entre los ácidos grasos de los lípidos de la otra hemicapa afectando a la distribución lipídica. Otra manera de sincronizar monocapas es mediante agrupaciones de lípidos de cadena larga en una capa, en la otra suele haber otros de cadena corta para homogeneizar el espesor de la membrana. 5. Síntesis Existen multitud de enzimas para la síntesis de los diferentes tipos de lípidos localizadas principalmente en el citosol, en las membranas del retículo endoplasmático, del aparato de Golgi, y, en el caso del colesterol, en otros orgánulos. Hay que destacar que los lípidos son difícilmente excretables por la célula, al contrario que otras moléculas hidrosolubles. Por tanto, la célula debe disponer también de un arsenal enzimático para catabolizar los lípidos que va sintetizando continuamente. La acumulación de cualquier especie de lípido de manera incontrolada implica toxicidad para la célula. Los tres tipos de lípidos más abundantes de las membranas de las células, glicerolípidos, esfingolípidos y esteroles, empiezan su proceso de síntesis en el retículo endoplasmático. Glicerofosfolípidos Los glicerofosfolípidos se sintetizan en el retículo endoplasmático gracias a unas enzimas localizadas en sus membranas que tienen el centro activo hacia la el citosol (Figura 11). Sólo una parte de algunos glicerofosfolípidos se sintetiza en el aparto Golgi y la fosfatidil etanolamina se puede también sintetizar en las mitocondrias. El proceso comienza con la llegada de una cadena de ácido graso, transportada por el citosol, que se inserta en la monocapa externa de la membrana del retículo por una proteína denominada proteína de unión a ácidos grasos. Estos ácidos grasos se activan mediante la adición de un coenzima-A. La unión de dos ácidos grasos activados a una molécula de glicerol 3 fosfato por una acil transferasa produce una molécula denominada ácido fosfatídico. El ácido fosfatídico pierde el grupo fosfato y se convierte en diacil glicerol. La fosfatidil colina, fosfatidil serina, y el fosfatidil inositol se sintetizan mediante la adición de CDP-colina, CDP-serina o CDP-inositol al diacil glicerol, respectivamente. La fosfatidil serina, en mamíferos, se sintetiza a partir de la CDP-colina o de la fosfatidil colina o fosfatidil etanolamina. Los enzimas responsables de este proceso están concentradas en las membranas del retículo endoplasmático, en las zonas de contacto entre el retículo y las mitocondrias. Figura 11. Esquema de la síntesis de la fosfatidil colina en las membranas del retículo endoplasmático. El ácido fosfatídico, que es la base para todos los fosfolípidos al dar diacilglicerol, se sintetiza mayoritariamente en el retículo endoplasmático, y una pequeña proporción en la membrana externa de la mitocondria. También se puede formar en otros compartimentos por catabolismo de glicerofosfolípidos preexistentes o por fosforilación del diacilglicerol. Los glicerofosfolípidos se pueden sintetizar en otros componentes por modificación química de lípidos preexistentes, y esto ocurre en diferentes compartimentos. Hay reacciones muy variadas y en compartimentos diferentes. La fosfatidil etanolamina sirve para producir la fosfatidil serina en el retículo endoplasmático. La fosfatidil serina, la fosfatidil colina, y fosfatidil etanolomina, pueden cambiar sus cabezas hidrofílicas por otras para convertirse en otro lípido. Por ejemplo, una fosfatidil colina puede cambiar la colina por una serina y se convierte en una fosfatidil serina. Además, otros cambios pueden ser modificaciones químicas de la cabeza hidrofílica. Así, la fosfatidil etanolamina también se puede formar desde la fosfatidil serina, y la fosfatidil etanolamina desde la fosfatidil serina. De la misma manera se pueden perder las cabezas hidrofílicas y tener de nuevo diacil gliceroles. El ácido lisofosfatídico se sintetiza mayoritariamente en los cuerpos multivesiculares por modificaciones de otros lípidos. En algunas ocasiones esta transferencia se produce entre lípidos de diferente tipo. Así, la fosfatidil colina es donante de la fosfocolina para producir esfingomielinas en el aparato de Golgi. El fosfatidil inositol puede ser fosforilado en tres sitios de su cabeza para dar diferentes fosfolípidos con lo que se consigue una familia de fosfoinosítidos. No se sintetiza a partir del diacilglicerol sino de CDP-diacilglicerol. El fosfatidil inositol también puede glicosidarse en el lado citosólico de la membrana plasmática, recibir una fosfoetanolamina de una fosfatidil etanolamina, cambiar hacia el interior y unirse covalentemente a una proteína por su COOH terminal. Por ello estos glicerofosfolípidos siempre aparecen en la hemicapa citosólica. Esfingolípidos La base molecular sobre la que se construyen los esfingolípidos es la ceramida. Ésta se sintetiza en las membranas del retículo endoplasmático por una serie de pasos a partir un aminoácido, principalmente la serina y acil-coenzima A, que da di-hidro-esfingosina, y a la que se une un ácido graso para dar ceramida (Figura 12). Después de su formación, de la ceramida es trasportada desde el retículo endoplasmático al aparato de Golgi para formar esfingolípidos más complejos. Este trasvase se realiza por dos mecanismos: en vesículas o mediante transportadores de ceramida (CERT). Sin embargo, en el caso de los galactoesfingolípidos, la enzima responsable de añadir la galactosa se encuentra en las membranas del retículo endoplasmático. Los galactoesfingolípidos suelen sulfatarse. Figura 12. Esquema de la síntesis de la síntesis de esfingolípidos. No se indica la síntesis de galactosil esfingolípidos en el retículo endoplasmático (modificado de Thidar et al., 2013). Una vez en el lado cis del aparato de Golgi la ceramida puede seguir dos rutas: síntesis de esfingomielina mediante la adición de un grupo fosfato más una colina, o la adición de una glucosa. Las esfingomielinas son las más abundantes de los esfingolípidos de membrana y son absolutamente imprescindibles para la viabilidad de los organismos y de sus células. La esfingomielina se genera por la trasferencia de un grupo fosfo colina desde un lípido fosfatidil colina a una ceramida, generándose un diacilglicerol y una esfingomielina. Los glucoesfingolípidos son trasladados al dominio trans del aparato de Golgi para convertirse en glicolípidos mediante la adición de más monosacáridos por enzimas que los transfieren (glicosil trasnferasas). Es destacable que los animales sin la capacidad de producir glucoesfingolípidos no son viables, pero sí los cultivos in vitro de sus células. El fosfatidil inositol PI(4)P es fundamental para la síntesis de esfingolípidos en el aparato de Golgi por interaccionar con proteínas que participan en su síntesis. Colesterol El colesterol que hay en las células puede tener dos orígenes: sintetizado por la propia célula o venir desde el exterior celular como parte de las partículas de lipoproteínas que contienen colesterol esterificado (Figura 13). En este segundo caso, la fuente principal de estas partículas es el hígado, en las cuales se empaqueta el colesterol de la dieta en forma de lipoproteínas. Se estima que el 80 % del colesterol de una célula animal se incorpora por endocitosis mediada por receptor. Todos los tejidos animales pueden conseguir colesterol de estas dos fuentes, propio o externo, excepto el tejido nervioso que sólo tiene colesterol propio porque las lipoproteínas no pueden cruzar la barrera hematoencefálica. La síntesis de colesterol por la célula es una larga ruta metabólica en la que participan numerosas enzimas citosólicas, localizadas en otros orgánulos, y otras asociadas a las membranas del retículo endoplasmático. En el retículo se encuentran la hidroximetilglutaril reductasa y las implicadas en los últimos pasos de la ruta metabólica antes de obtener el colesterol. El colesterol es rápidamente transportado a otras membranas de la célula tras su síntesis en las membranas del retículo. La síntesis de colesterol está regulada por su concentración, de modo que cuando es excesiva se degradan las enzimas responsables de su síntesis. La principal enzima responsable de este mecanismo de detección es la hidroximetilglutaril reductasa. El exceso de colesterol en el retículo produce su esterificación y se almacena en las gotas de lípidos. Figura 13. Esquema de la síntesis de la síntesis de colesterol. El proceso tiene numerosas reacciones químicas. Se destacan las enzimas localizadas en la membrana del retículo endoplasmático. 5.1. Mitocondrias En las mitocondrias se encuentran lípidos típicos de otras membranas de la célula. La composición de lípidos en las mitocondrias es importante sobre todo en la membrana interna para las proteínas que participan en la fosforilación oxidativa o respiración, principal centro de producción de ATP de la célula. En las membranas de las mitocondrias abundan la fosfatidil colina, la fosfatidil etanolamina, el fosfatidil inositol y la fosfatidil serina, que se encuentran también en otras membranas. Pero poseen lípidos exclusivos como el fosfatidil glicerol y la cardiolipina. Las mitocondrias poseen concentraciones muy bajas de esfingolípidos y colesterol. La función que tiene el colesterol en la membrana plasmática, donde mantiene un equilibrio entre fluidez e hidrofobicidad (impermeabilidad), lo hace la cardiolipina en a membrana interna de la mitocondria. Aquí la hidrofobicidad es importante puesto que el gradiente de protones creado durante la fosforilación oxidativa ha de mantenerse haciendo a la membrana relativamente impermeable. La cardiolipina es esencial para mantener la estructura y el potencial de la membrana mitocondrial interna, además de para crear un ambiente adecuado para las proteínas de la cadena respiratoria. Esto es importante puesto que en esta membrana la proporción de proteínas es muy alto respecto a la de lípidos. Por ejemplo, la ausencia de cardiolipina produce malformaciones o desestabiliza algunos complejos proteicos de la cadena respiratoria. La cardiolipina es además es necesaria para importar esas proteínas a la membrana (téngase en cuenta que algunas se sintetizan en el citosol). Las mitocondrias se fusionan entre sí y se dividen. Esto requiere unir y separar membranas constantemente. La cardiolipina, el ácido fosfatídico y el fosfatidil etanolamina son necesarios para estos procesos. La fosfatidil colina y la fosfatidil serina se sintetizan exclusivamente en el retículo endoplamático, y deben importarse a las mitocondrias. La fosfatidil etanolamina se puede obtener en la mitocondria a partir de la fosfatidil serina o importarse desde el retículo. La cardiolipina se sintetiza en la mitocondria. Independientemente del lugar de síntesis, los lípidos tienen que pasar de una membrana de la mitocondria a la otra, y esto parece favorecerse por los contactos directos entre ambas membranas. Los contactos entre membranas funcionan también para el trasiego de lípidos entre el retículo endoplasmático y las mitocondrias. Curiosamente, no se han encontrado todavía trasnportadores de lípidos que aporten o saquen lípidos de las membranas de las mitocondrias. 5.2. Cloroplatos Este orgánulo es el principal centro de producción de cadenas de ácidos grasos de las células vegetales. Estos ácidos grasos se exportan al retículo endoplasmático, donde se utilizan para la síntesis de otros lípidos. Entre ellos está el diacil glicerol, que debe volver al cloroplasto donde se sintetizarán los galactosil glicerolípidos. Al igual que ocurre con las mitocondrias, la comunicación entre el retículo y el cloroplasto es esencial para mantener la composición lipídica típica. Los cloroplastos tienen una composición particular de lípidos, entre los que se encuentran los galacto glicerolípidos, los cuales sólo se sintetizan y se encuentran en los cloroplastos. Los galactolípidos son esenciales para los cloroplastos, sobre todo para la membrana de los tilacoides donde mantienen las condiciones para el funcionamiento de la cadena fotosintética. Pueden representar entre el 25 y el 50 % de todos los lípidos de la membrana tilacoidal, y hasta el 60 % de los lípidos de las membranas interna y externa del cloroplasto. El único fosfolípido sintetizado en el cloroplasto, el fosfatidil glicerol, y tiene una función crítica en el centro del fotosistema II. La composición lipídica de las cianobacterias, que comparten ancestro con los cloroplastos, tiene una composición similar. 6. Funciones Estructura de la membrana La naturaleza química de los glicerofosfolípidos hace que formen espontáneamente bicapas lipídicas, como las de las células. La fosfatidil colina es la principal responsable de esta propiedad, siendo además el lípido más abundante de los animales (¡excepto en Drosophila!), pero también las otras especies de lípidos tienen la capacidad de formar bicapas. La composición de glicerofosfolípidos, esfingolípidos y colesterol de una membrana, tanto en la diversidad de sus cabezas como en la longitud de sus ácidos grasos, afecta a la fluidez, grosor, curvatura y carga electrostática de dicha membrana. Pero también sirven para anclar y asociar otras moléculas como proteínas. La fosfatidil colina forma en torno al 50 % de los lípidos de las membranas eucariotas y suelen poseer una cadena saturada y otra con un enlace insaturado. Su forma cilíndrica les hace ideales como principales componentes estructurales de las membranas. Pero los lípidos de membrana, además de su función estructural, afectan a otros aspectos de la membrana. Así, los esfingolípidos, al contrario que las fosfatidil colina, suelen tener sus cadenas saturadas y son más largas por lo que se agrupan formando áreas más compactas y la membrana es más gruesa. El colesterol tiene dos efectos, impide que las cadenas de ácidos grasos se empaqueten demasiado, se impide el estado de gel sólido, pero también en las membranas más fluidas aporta más rigidez por reducir al flexibilidad de las cadenas insaturadas, lo que aumenta la anchura de la membrana y su impermeabilidad. La mayor concentración de colesterol, esfingolípidos y una mayor saturación de las cadenas de ácidos grados hace a la membrana plasmática, más rígida, más ancha, y por tanto más impermeable, que otras membranas. Esto podría ser un mecanismo para segregar proteínas con dominios transmembrana de diferente longitud entre diferentes compartimentos celulares. Los que se encuentran en el retículo son más cortos que los que hay en al membrana plasmática. Las membranas del retículo endoplasmática y del lado cis aparato de Golgi son más estrechas y más fluidas. Hay otros glicerlípidos cuya estructura molecular adopta un volumen de cono. El ácido fosfatídico es poco abundante en las membranas (1 a 2 %), pero su forma molecular es en forma de cono. La fosfatidil etanolamina es un componente básicamente estructural, al igual que fosfatidil colina. También tiene una forma cónica. Esta forma molecular podría facilitar procesos de curvatura y fusión de membranas. Cualquier curvatura de la membrana crea una hemicapa cóncava y otra convexa. Aquellos lípidos cónicos, es decir, aquellos que tienen una cabeza pequeña comparada con el volumen que ocupan sus ácidos grasos, inducen o favorecen curvaturas convexas, mientras que aquellos con cabezas grandes favorecen curvaturas cóncavas. Esto que se cumple en membranas artificiales no parece que sea la principal fuerza en las membranas celulares, puesto que aquí las proteínas tienen una gran importancia, pero podrían colaborar. La proporción entre lípidos cilíndricos y cónicos está bien regulada en la célula. Por ejemplo, si se inhibe la síntesis de fosfatidil serina, la célula incrementa la producción de fosfatidil inositol. La presencia de determinados lípidos en una membrana y la localización en una de sus hemicapas o su concentración en una heterogeneidad lateral condiciona la asociación de ciertas proteínas, lo que determina la funcionalidad de la propia membrana. Así, el glicosil-fosfoinositol, derivado del fosfoinositol. sirve para anclar proteínas de forma permanente a la membrana plasmática, normalmente a la hemicapa extracelular. La distribución de Los glicerofosfolípidos se con carga negativa (lípidos aniónicos) se concentra en la cara citosólica de la membrana plasmática por lo que tiene una carga neta, la cual tienen dos funciones, contribuir al potencial de membrana y crear un ambiente iónico adecuado para la asociación de ciertas proteínas a la membrana, y para la actividad de las propias proteínas transmembrana. Algunos lípidos como el fosfatidil inositol y la fosfatidil serina son aniónicos, es decir, tienen carga neta negativa, y algunos esfingolípidos son catiónicos, aunque la mayoría de los lípidos no tienen carga neta. Sin embargo, en la membrana plasmática los lípidos aniónicos se localizan en la hemicapa citosólica. Esto es interesante porque la asimetría de membrana crea una distribución diferente de cargas entre ambas superficies de la membrana, que contribuye al potencial de membrana. Se ha visto que en ausencia de iones la membrana plasmática es capaz de producir un potencial de membrana por sí misma debido a la mayor concentración de cargas negativas en la monocapa interna. Esta carga aniónica local también afecta a proteínas transmembrana puesto que sus dominios intracelulares (en el caso de la membrana plasmática) son sensibles a los ambientes eléctricos y de esta manera se modula su actividad. Por ejemplo, en el caso de los canales aumentando o disminuyendo la probabilidad de cierre o apertura. Además, facilita la asociación específica de proteínas que necesitan un ambiente eléctrico determinado y que es aportado por la naturaleza química de las cabezas de los lípidos. Hay proteínas como K-Ras, Src, o Rac1, que son policatiónicas y por tanto serán atraídas por lugares de la membrana donde abunden estos lípidos. Los fosfo inosítidos bi a tri fosforilados contribuyen de manera importante a la carga aniónica de la membrana donde se encuentran. Por el contrario, la cara citosólica de el retículo endoplasmático presenta una carga neta neutra ideal para reacciones bioquímicas. Para ver el efecto de los lípidos en la modificación de la permeabilidad y fluidez de la membrana ver esta página Señalización Los lípidos afectan a la comunicación celular y participan en ella. Esto puede ser bien porque creen ambientes o plataformas donde las cascadas de señalización se vean favorecidas o siendo ellos mismos las propias señales. En el primer caso tenemos como ejemplo a la balsas de lípidos, asociaciones principalmente de esfingolípidos y colesterol, que crean ambientes diferentes en la membrana en los que algunos receptores son capaces de realizar mejor sus mecanismos de transducción tras unirse al ligando. O se favorece la interacción de proteínas implicadas en una cascada de señalización por favorecer su proximidad física. Estos dominios de membrana son muy plásticos, es decir, se crean, se destruyen y cambian de tamaño con mucha facilidad. Hay otros tipo de señalización en los que intervienen directamente los lípidos. Por ejemplo, la rotura de la asimetría lipídica en la membrana plasmática funciona como una señal de que hay alteraciones en la célula. Por ejemplo, las células que sufren apoptosis, muerte celular programada, exponen rápidamente la fosfatidil serina en la monocapa externa y esto es una señal de "cómeme" para que los macrófagos eliminen esa célula. También es importante para el inicio de la coagulación sanguínea. Incluso algunos virus con membrana exponen en su monocapa externa fosfatidil serina y fostatidiletanolamina para ser incorporados a las células con más facilidad por macropinocitosis o fagocitosis. Hay más ejemplos. El lípido fosfatidil inositol PI(4,5)P2, localizado en la hemicapa interna, al ser degradado por ciertas fosfolipasas se divide en dos moléculas, una de las cuales, IP3, viaja por el citosol y actúa como segundo mensajero, afectando a la liberación de calcio desde el retículo endoplasmático. La fosfatidil colina es una fuente de ácido araquidónico, el cual se transforma en prostaglandinas. Formación de vesículas / tráfico vesicular Los lípidos son relevantes en la ruta vesicular. En los procesos de endocitosis mejor estudiados, endocitosis dependiente de clatrina, fagocitosis y macropinocitosis, los fosfolípidos participan como elementos activos. La endocitosis mediada por clatrina necesita PI(4,5)P2 durante las fases de nucleación, selección de cargas y ensamblaje de la cubierta, pero debe desaparecer de la zona durante la escisión y eliminación posterior de la cubierta. La endocitosis mediada por clatrina se inicia en zonas ricas en PI(4,5)P2, los cuales reclutan las moléculas necesarias para iniciar la nucleación, entre las que se encuentran GPTasas y moléculas tipo BAR que deforman la membrana, tales como al anfifisina y endofilina. Pero también otras proteína relacionadas con la formación de la vesícula son capaces de unirse a este fosfolípido, tales como la dinamina y las propias proteínas adaptadoras AP-2, las cuales se unen a la vez a la carga y a la cubierta de clatrina. Sin embargo, los procesos de escisión y eliminación de la cubierta de la vesícula una vez liberada la vesícula requieren una disminución drástica de la concentración de PI(4,5)P2 en las membranas. Esto se lleva a cabo por fosfatasas. Estas fosfatasas se reclutan durante la formación de la vesícula. Es obvio por qué esto es necesario puesto que si esta molécula produce una atracción de moléculas para formar la vesícula, es necesaria su eliminación para que se pierda la afinidad de las moléculas de la cubierta por la membranas. Es interesante que la eliminación de el PI(4,5)P2 de las membranas ya curvadas ayuda en el proceso de escisión. La fosfatidil etanolamina es importante durante la formación del autofagosoma puesto que permite la unión moléculas adaptadoras solubles. También interactúa con proteínas de la membrana produciendo modificación de su actividad. PI(4,5)P2 también participa en la fagocitosis mediante su asociación con la actina y probablemente favorezca su polimerización, pero tiene que ser eliminado en la última fase la formación del fagosoma. PI(4,5)P2 es convertido o degradado en varias moléculas, entre ellas PI(3.4.5)P3, que se acumula en el fagosoma naciente donde prodía reclutar la proteína motora miosina. La maduración del fagosoma es similar a la de los endosomas, pero en los fagosomas también se tienen que reclutar proteínas que acaben con los patógenos ingeridos, como la generación de ROS. PI(3)P es importante para el reclutamiento de estas enzimas. PI(4)P es muy abundante en el aparato de Golgi, y además de colaborar en la síntesis de esfingolípidos, es capaz de captar la proteínas adaptadoras AP-1 al lado trans. Esto regula el tráfico vesicular anterógrado. PI(3)P está está asociado con la maduración de los endosomas. Se puede formar a partir de PI(3,4,5)P3, PI(3,4)P2, o por quinasas. Entre las quinasas está la Vps34, la cual se asocia con los endosomas tempranos. Las proteínas que participan en la maduración de los endosomas tienen lugares de unión para el PI(3)P. PI(3)P va siendo degradado a medida que maduran los endosomas y una vez que éstos han reclutado una importante maquinaria proteica. La degradación ocurre por varios mecanismos, siendo uno de ellos la fosforilación, convirtiéndose en PIP(3.5)P2, que es un marcador de endosomas tardíos. Por último, en los cuerpos multivesiculares PI(3)P es forzado a formar parte de las vesículas intraluminares. El ácido lisofosfatídico se produce también en los endosomas en maduración. Este lípido favorece la formación de las vesículas intraluminares, incluso en ausencia de ESCRT. Bibliografía ↷ Bibliografía Alberts A, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2007. Molecular Biology of the Cell. 5th editon. Garlan Science. ISBN: 9780815341055. Becker WM, Kleinsmith LJ, Hardin J, Raasch J. 2003. The world of the cell. 6th. San Francisco: Benjamin Cummings. ISBN-10: 0321716027 ISBN-13: 9780321716026. Bohdanowicz M, Grinstein S. 2013. Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis 6th. Physiological reviews. 93: 69-106. Carquin M, D'Auria L, Pollet H, Bongarzone ER, X Tyteca D. 2016. 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Page 2 La organización y propiedades de las membranas celulares está determinada por las características de sus componentes, lípidos, proteínas y carbohidratos. Sin embargo, la diversidad (hay más de mil tipos de lípidos diferentes) y su organización espacial (formando un bicapa) hacen a los lípidos esenciales. Así, ellos definen las propiedades físicas de las membranas. La longitud y el grado de saturación de sus ácidos grasos regulan la fluidez y el grosor de la membrana, y su distribución desigual crea asimetría en las membranas. En la membrana plasmática las cargas asociadas a sus partes hidrófilicas contribuyen a crear un gradiente eléctrico entre la cara externa y la interna, y por tanto a modular el potencial eléctrico. Mediante interacciones electroquímicas son capaces de modular la actividad de las proteínas de membrana. Se ha postulado que las interacciones moleculares entre ciertos lípidos producen la segregación de dominios espaciales y funcionales en áreas restringidas de la membrana que afectan también a la localización de las proteínas y a sus funciones. Son las denominadas balsas de lípidos o "lipid rafts". Pero además pueden actuar como segundos mensajeros que abandonan la membrana, viajan a compartimentos intracelulares y desencadenan respuestas celulares. Modelos de membrana Los lípidos constituyen aproximadamente el 50 % del peso de las membranas, con unos 5 millones de moléculas por μm2. Las membranas celulares de una célula eucariota contienen más de 1000 tipos de lípidos que aparecen en distinta proporción según el tipo de membrana que estemos considerando. Se estima que aproximadamente el 5 % de los genes de una célula están dedicados a producir sus lípidos. Figura 1. Principales lípidos presentes en las membranas celulares. Los lípidos de membrana se caracterizan por poseer una parte apolar o hidrófoba que constituye la parte interna de la membrana y por una parte hidrofílica que está en contacto con el medio acuoso. Por ello se dice que son moléculas anfipáticas. Se clasifican en tres grupos según su estructura y composición molecular: glicerofosfolípidos (también denominados glicerolípidos, fosfoglicéridos o simplemente fosfolípidos), los esfingolípidos y los esteróles (Figura 1). 1. Fosfoglicéridos o glicerofosfolípidos Son los lípidos más abundantes (representan más del 70 %) de las membranas celulares y estructuralmente constan de tres partes: dos cadenas de ácidos grasos, glicerol, un grupo fosfato al que se unen moléculas de diversa naturaleza y que aportan gran parte de la variabilidad de estos lípidos (Figura 2). Las cadenas de ácidos grasos contienen de 13 a 19 átomos de carbono de longitud. La mayoría de los enlaces entre estos carbonos son simples y por tanto se dice que son enlaces saturados. Sin embargo, más de la mitad de estos ácidos grasos tienen al menos un doble enlace entre dos átomos de carbono, hablamos entonces de ácidos grasos insaturados. Los dobles enlaces hacen que la cadena de ácido graso se doble y, aunque restrinja las posibilidades de movimiento de la cadena, un aumento de la proporción de estos dobles enlaces aumenta la fluidez de la membrana puesto que provoca más separación entre moléculas. Los ácidos grasos constituyen la parte hidrofóbica (fobia por el agua) de los glicerofosfolípidos y son los que constituyen la parte interna de las membranas. Figura 2. Estructura y tipos de glicerofosfolípidos más abundantes de las membranas eucariotas. El glicerol hace de puente entre los ácidos grasos y la parte hidrofílica (apetencia por el agua). Este componente hidrofílico está formado por un grupo fosfato al que se pueden unir una variedad de moléculas, tales como la etonalamina, colina, serina, glicerol, inositol, el inositol 4,5-bifosfato, etcétera. Estos componentes son los que dan nombre a los distintos tipos de glicerofosfolípidos. El tipo fosfatidilcolina representa más del 50 % de los fosfoglicéridos en las membranas eucariotas. 2. Esfingolípidos Deben su nombre a que poseen una molécula de esfingosina, un alcohol nitrogenado con una cadena carbonada larga, a la cual se le une una cadena de ácido graso, formando la estructura básica denominada ceramida (Figura 3, ver figura). A la ceramida se le une una parte hidrofílica que puede de ser de diversa naturaleza. Por tanto queda una estructura similar a la de los glicerofosfolípidos, dos cadenas hidrofóbicas unidas a una estructura hidrofílica. Los esfingolípidos constituyen la mayoría de los denominados glicolípidos de las membranas, presentes mayoritariamente en las células animales, es decir, lípidos que poseen uno o más azúcares unidos formando parte de su zona hidrofílica. Otro tipo de esfingolípidos son las esfingomielinas que poseen una etanolamina o una colina fosforiladas en sus zonas hidrofílicas. Los esfingolípidos son más abundantes en las membranas plasmáticas que en las de los orgánulos, y se les propone como lo principales responsables, junto con el colesterol, de la segregación lateral de la membrana en dominios moleculares (balsas de lípidos). Figura 3. Estructura y algunos esfingolípidos abundantes de las membranas eucariotas. 3. Esteroles Figura 4. Colesterol El colesterol es el esterol (Figura 4) más importante de las células animales y el tercer tipo de lípido más abundante en la membrana plasmática (hasta el 25 % del total de lípidos), mientras que aparece en pequeñas proporciones en las membranas de los orgánulos celulares como las del retículo endoplasmático (1 %), mitocondrias y lisosomas. El colesterol no aparece en las membranas de las plantas, en algunas células eucariotas, ni en las bacterias, pero estas células tienen otro tipo de esteroles. Los estéroles son esenciales para la integridad y funcionamiento de las membranas eucariotas. Sirven para modular la rigidez, la fluidez y la permeabilidad. Además, contribuyen también a modular la actividad de los receptores acoplados a proteínas G y facilitan la transducción de señales y el tráfico vesicular. El colesterol se localiza entre las cadenas de ácidos grasos de los otros lípidos. Es importante para la organización de la membrana, sobre todo la plasmática, puesto que junto con los esfingolípidos parece contribuir a formar heterogeneidades laterales y también participa en ciertos procesos metabólicos vitales como la síntesis de hormonas esteroideas o de sales biliares, entre otras. 4. La distribución de lípidos depende de la membrana Figura 5. Proporción aproximada de los lípidos totales de un célula típica de mamífero (tomado de Vance 2015) Aunque hay tres grandes grupos de lípidos de membrana (glicerolípidos, esfingolípidos y esteroles), hay miles de tipos moleculares diferentes de lípidos distribuidos por las membranas celulares (Figura 5). La composición de lípidos varía entre las membranas de los diferentes compartimentos membranosos de la célula. Se propone que la propia identidad de los orgánulos viene determinada por la composición de sus membranas, tanto proteínas como lípidos. Por ejemplo, la membrana plasmática tiene una composición lipídica diferente a la membrana del retículo endoplasmático o a la del aparato de Golgi. Estas diferencias se mantienen a pesar del flujo constante de lípidos desde sus compartimentos de síntesis, principalmente el retículo endoplasmático, hasta otras membranas como la plasmática o los endosomas, estando este transporte mediado por vesículas, transportadores y contactos directos entre membranas. La cantidad y tipo de lípidos varía entre membranas (Figura 6). Por ejemplo, aunque todas la membranas tienen fosfatidil colina, este glicerolípido es más abundante en las membranas del retículo endoplasmático. En la membranas post-Golgi, es decir, membrana plasmática y endosomas, la concentración de esfingolípidos y colesterol es mayor que en el retículo y en las membranas del aparato de Golgi. Las mitocondrias tienen, aparte de otros más extendidos, lípidos de membrana propios como el fosfatidilglicerol y la cardiolipina, que sintetizan ellas mismas. Figura 6. Distribución de los lípidos más abundantes en diferentes compartimentos membranosos celulares. Las flechas azules delgadas indican trasiego de lípidos transportados por las vesículas, las rojas gruesas la transferencia de lípidos entre membranas muy próximas, las flechas rojas delgadas indican la transferencia de lípidos entre membranas mediante transportadores proteicos, los cuales actúan entre varios compartimentos (no indicado). En la imagen inferor se representa el incremento de la concentración de colesterol desde el retículo endoplasmático hasta la membrana plasmática. PC: fostatidil colina, PE: fosfatidil etanolamina, PI: fosfatidil inositol, PS: fosfatidil serina, SM: esfingomielina, ISL: esfingolípido inositol, CL: cardiolipina, MBP: bis monoacilglicerol fosfato (modificado de van Meer et al., 2008). Esto significa que deben existir mecanismos de segregación por compartimentos membranosos de las diferentes especies de lípidos de membrana. Se han propuesto diversos mecanismos que contribuyen a esta distribución desigual de lípidos: Síntesis diferencial. La concentración de determinadas especies de lípidos viene condicionada por su lugar de síntesis. Por ejemplo, los esfingolípidos se terminan de ensamblar en el aparato de Golgi y son los compartimentos post-Golgi donde más abunda, pero están ausentes en el retículo endoplasmático. La fosfatidilcolina se sintetiza en el retículo endoplasmático y es ahí donde más abunda. Sin embargo, no siempre es cierto puesto que, por ejemplo, el colesterol se sintetiza en el retículo endoplasmático, pero es más abundante en membranas post-Golgi. Esto es debido a que es transportado rápidamente a otras membranas. Además, durante los procesos de síntesis de unos lípidos se emplean a otros como donantes de partes moleculares por lo que a la vez que se sintetiza una nueva especie lipídica desaparece otra, y todo contribuye a cambiar la composición lipídica de la membrana de ese compartimento. Selección y transporte. Los lípidos deben ser transportados entre membranas puesto que no difunden libremente por el citosol. Ello implica que el transportador puede seleccionar qué lípidos acarrea de un lado a otro cambiando así las proporciones de determinadas especies de lípidos. Estos transportadores son fundamentalmente las vesículas, las cuales forman sus membranas con lípidos de las propias membranas del compartimento de partida. Por ejemplo, las vesículas que van desde el Golgi a la membrana y a los endosomas están enriquecidas en esfingolípidos y colesterol, respecto la concentración de estas moléculas en las propias membranas del Golgi. También hay transportadores de lípidos individuales que son proteínas que son capaces de extraer un lípido de una membrana, transportarlo por el citosol y colocarlo en otra membrana. Contactos entre membranas. Las membranas de determinados orgánulos pueden observarse extremadamente próximas. Hay proteínas que se colocan entre las membranas de dos orgánulos cuando están muy próximas espacialmente, haciendo de puente para el trasiego de lípidos entre las membranas de ambos orgánulos. Esto ocurre entre membranas de compartimentos que no están comunicados mediante vesículas, por ejemplo, entre el retículo endoplasmático y las mitocondrias. Pero también entre compartimentos comunicados por vesículas como el retículo endoplasmático y el lado trans del aparato de Golgi, entre el retículo endoplasmático y los endosomas, o entre el retículo endoplasmático y la membrana plasmática. Por ejemplo, el complejo proteico CERT (ceramide transfer protein) transfiere ceramida desde las membranas del retículo endoplasmático al lado trans del aparato de Golgi. Degradación y reciclado diferencial. Todas las membranas sufren un proceso de reciclado de lípidos, bien por degradación in situ de los lípidos o por su salida en vesículas de reciclado. En ambos procesos es posible un mecanismo de selección de unos tipos de lípidos respecto a otros que condicionará la población de lípidos de la membrana. 5. Asimetría Da asimetría es la distribución diferencial de lípidos entre las dos monocapas de lípidos que componen las membranas. Mientras en el reticulo endoplasmático ambas monocapas son muy similares,pero no idénticas, en cuanto a la composición lipídica, las membranas del lado trans del aparato de Golgi y los compartimentos post-Golgi suelen tener una clara diferencia de composición entre monocapas. Para saber más ver asimetría de membrana. Bibliografía ↷ Bibliografía Bissig C, Gruenberg J. 2013. Lipid sorting and multivesicular endosome biogenesis. Cold Spring Harbour perspectives in biology. 5:a016816. Janmey PA, Kinnunen PKJ. 2006. Byophisical properties of lipids and dynamic membranes. Trends in cell biology. 16:538-546. Vance JE. 2015. Phospholipid synthesis and transport in mammalian cells. Traffic. 16:1. van Meer G, Voelker DR, Feigenson GW. 2008. Membrane lipids: where are they a how they behave. Nature reviews in molecular cell biology. 9:112-124. Page 3 Los glúcidos presentes en las membranas están unidos covalentemente a los lípidos formando los glicolípidos y a las proteínas formando las glicoproteínas de membrana. Algunos proteoglicanos insertan sus cadenas de aminoácidos con radicales hidrófobos en la membrana, quedando los glicosaminoglicanos hacia el exterior (Figura 1). Aunque existen glúcidos en las membranas intracelulares, tanto glicolípidos como glicoproteínas son mucho más abundantes en la membrana plasmática, preferentemente localizados en la monocapa externa. Los glúcidos de las membranas se ensamblan principalmente en el aparato de Golgi, aunque su síntesis se inicia en el retículo endoplasmático. Figura 1. Esquema de algunas moléculas glicosidadas de la membrana plasmática. Los glicolípidos son principalmente esfingolípidos con diferente composición de glúcidos. Algunos proteoglicanos tienen su parte proteica insertada entre las cadenas de ácidos grasos. Numerosos glúcidos de la membrana forman parte de las glicoproteínas, formando enlaces tipo O (con los aminoácidos serina) o tipo N (con los aminoácidos asparragina) (Modificado de Fuster y Esko, 2005). Hay tres tipos de glicolípidos: los glicoesfingolípidos, que son los más abundantes en las células animales, los gliceroglicolípidos y los glicosilfosfatidilinositoles. Los gliceroglicolípidos son típicos de las membranas plasmáticas de las plantas. Sin embargo, la mayoría de los glúcidos de membrana se encuentran asociados a las proteínas, denominadas glicoproteínas. Mientras que prácticamente todas las proteínas contienen sacáridos unidos, sólo un 5 % de los lípidos los poseen. Al conjunto de glúcidos localizados en la membrana plasmática se le denomina glicocálix. En algunos tipos celulares la cantidad de glúcidos que se encuentran en la superficie celular es tan grande que puede observarse con el microscopio electrónico. En algunas células como en los enterocitos el glicocálix se puede extender más de 1 µm desde la membrana celular. La célula queda así recubierta por una envuelta de glúcidos que representa entre el 2 y el 10 % del peso de la membrana plasmática. El grado de desarrollo del glicocálix depende del tipo celular. Los glúcidos sirven básicamente para dos cosas: lugares de reconocimiento y unión, bien por moléculas propias (reconocimiento célula-célula) o externas (patógenos, toxinas, aglutininas) y también tienen un papel estructural y de barrera física. Por ejemplo, los grupos sanguíneos vienen determinados por glúcidos de la membrana, lo que implica que tienen capacidad de respuesta inmunitaria. Cuando se produce una infección, las células endoteliales próximas exponen una serie de proteínas llamadas selectinas que reconocen y unen sacáridos de los linfocitos circulantes en el torrente sanguíneo y permiten su adhesión y el cruce del propio endotelio para dirigirse hacia la zona infectada. El reconocimiento celular mediado por los glúcidos es también muy importante durante el desarrollo embrionario. En determinadas circunstancias los carbohidratos de superficie se modifican. Por ejemplo, en las células cancerosas hay un repertorio distinto de glúcidos de membrana, siendo algunos específicos de determinados tipos de cáncer. Por ejemplo, repeticiones en tándem de ácido siálico potencian las propiedades malignas del cáncer, tales como la proliferación, invasión celular, migración, adhesión y metástasis. Probablemente estos glúcidos modifiquen la capacidad de recibir e interpretar señales por parte de la célula. El gangliósido GD3 se ha asociado con melanomas y es una diana para tratamientos terapéuticos. Los glúcidos de membrana son unos de los principales lugares de reconocimiento por parte de los patógenos para unirse e infectar a las células. Los virus como el de la gripe, bacterias como las E. coli patógenas y protozoos patógenos deben adherirse a la superficie celular para infectar, de otra manera serán barridos por los mecanismos de limpieza del organismo. Estos organismos patógenos poseen unas proteínas de membrana denominadas lectinas que tienen afinidad por determinados azúcares o cadenas de azúcares y por tanto sólo reconocerán a las células que los posean. La selectividad en la infección de determinados tipos celulares depende de la composición de azúcares de su glicocálix. Curiosamente, algunos patógenos son capaces de "vestirse" con glúcidos superficiales similares a los de las células del hospedador, de manera que pueden pasar desapercibidos. Existen diferencias entre los glúcidos de la membranas de vertebrados, invertebrados y protozoos. Bibliografía ↷ Bibliografía Fuster MM, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. Nature reviews cancer. 2005. 5(7):526-542. Page 4 El citosol es la parte del citoplasma sin los orgánulos y sin el núcleo, mientras que el citoplasma es todo el contenido celular, excepto el núcleo. El citosol es una sustancia acuosa semifluida que rodea a los orgánulos y núcleo, pudiendo representar más de la mitad del volumen celular en las células animales, mientras que en las células vegetales maduras la mayor parte del volumen celular está ocupado por las vacuolas. El citosol está formado en su mayor parte por agua en la que se encuentran disueltas una gran cantidad de moléculas e iones. Tan grande puede llegar a ser la concentración de moléculas e iones que en muchas ocasiones se llega a densidades relativamente viscosas. En comparación con el medio extracelular, el citosol tiene una alta concentración de potasio y una baja concentración de sodio y calcio. Es un medio tamponado con pHs que van normalmente entre 7 y 7.4. Es el medio en el que desarrolla una enorme actividad molecular: muchas reacciones metabólicas como la glicolisis, la traducción de las proteínas en los ribosomas libres, cascadas de señalización resultado de la comunicación celular y de la comunicación entre orgánulos, etcétera. Es el medio por el que difunden los iones y segundos mensajeros, y por él se mueven las moléculas y las vesículas que comunican las diferentes partes de la célula. También en el citosol se encuentra el citoesqueleto, el cual es el esqueleto y los músculos de la células, formado por filamentos proteicos altamente versátiles y plásticos. En el citosol también se acumulan moléculas de reserva en forma de gotas de lípidos y de glucógeno. Aunque las células eucariotas tienen muchos y variados compartimentos internos delimitados por membrana, también hay otros espacios celulares en el citosol que no están rodeados por membrana y que se pueden comportar como compartimentos especiales a modo de orgánulos. Por eso se llaman orgánulos sin membrana (MLO en inglés "membraneless organelles"). Estos "orgánulos" se generan por la propiedad de segración de fase líquido-líquido, que consiste en que dos componentes con propidades diferentes se autosegregan en espacios diferentes (como ocurre, por ejemplo, con la segregación del agua y el aceite). En el citosol y en el nucleoplasma hay moléculas como proteínas, lípidos y ácidos nucleicos que se asocian a otras moléculas para formar una fase líquida más densa diferente del resto. Estas asociaciones están presentes tanto en eucariotas y como en procariotas. Por ejemplo, en el núcleo un MLO es el nucléolo. En el citosol están los gránulos de estrés, gránulos transportadores de ARN y los cuerpos P. Las funciones de los MLOs son variadas. Por ejemplo, los gránulos transportadores de ARN y los gránulos de estrés acumulan ARN evitando su traducción, mientras otros aceleran reacciones químicas por concentración de los elementos necesarios para ellas. Hay mecanismos para modular estos MLOs, como fosforilación, metilación, acetilación, adición de poli-ribosas. El citosol de las céluas animales es un componente propio de cada célula y su contenido no se suele compartir con otra células vecinas. A veces, sin embargo, existe comunicación directa citosol-citosol entre células vecinas gracias a las uniones en hendura, canales proteicos que se forman en las membranas plasmáticas y que establecen conductos de comunicación. Por estos canales pasan sólo moléculas de bajo peso molecular y los iones. Por otra parte, en las plantas es muy frecuente que el citosol de células vecinas esten conectados a través de los plasmodesmos, que son túneles en la pared celular que

permiten esta comunicación. Tanto es así que las membranas plasmáticas de las células vecinas son continuas. [illegible]

formed in the interior of the progenitor cell that divides the cytoplasm in two parts (Figure 4). In plants, the first evidence of this new cell wall formation is the phragmoplast. Phragmoplast is a complex structure composed of some microtubules from the mitotic spindle and vesicles delibered by the Golgi apparatus. Vesicles coming from the Golgi apparatus are moved to this central area by microtubules and motor associated proteins. Vesicles fuse between each other and start to form the cell membranes of the new cells, whereas their content forms the medial lamina of the new cell wall. In plants, the cell wall grows centrifugally, i.e. fom the inner part of the cell toward the periphery. In fungi cells, which have no phragmoplat, it is in the other way around, the more external components are the more recently synthesized. A division furrow is not observed in plant cells, but it is present in fungi cells. In plants, the orientation and position of the division plane is established under the influence of the nucleus during the late G2 phase. Microtubule bundles are oriented around the cell nucleus before the M phase starts, and this scaffold, known as preprophase band (PPB), leaves a trace in the cortical cytoplasm. The bundle of microtubules around the nucleus disappears at the beginning of mitosis, but the modifications they made in the cortical cytoplasm affect the formation of the pragmoplast. Figure 4. Stages of mitosis, from metaphase (left) to telophase (right) in an onion root meristem. The formation of a new cell wall happens at an equidistant position from the two sets of chromatids, which form the two nuclei of daughter cells. This initial structure that evolves into the new cell wall is the phragmoplast (arrow). The process is also observed in this image. Bibliography Pardo M. 2005. Citoquinesis en células eucariotas. Investigación y ciencia 346:40-49. Wanke C, Kutay Uz. 2013. Enclosing chromatin: reassembly of the nucleus after open mitosis. Cell 152: 1222-1225. Page 11 G1 phase (G stands for gap) spans from the end of mitosis to the start of S phase. During G1 phase, cells check the external environment and the intracellular state, and decide if they continue with the cell cycle or not. In multicellular organisms, the cell cycle progress is mainly influenced by extracellular signals, like cell adhesion, or molecules released by other cells, such as trophic factors. There is also internal information about the state of the cell that affects the progress of the cell cycle, like the health condition of the cell, if there is a correct number of cellular components after cell division, or if chromosomes were properly segregated. When external and internal signals are right, proliferating cells grow and get ready to enter S phase. However, cells of multicellular organisms do not proliferate, but quit the cell cycle from G1 phase, either transiently or permanently. Stopping the cell cycle means that cells are going to differentiate, stay quiescent, go through senescence processes, or die by apoptosis (Figure 1). When cells remain in a quiescent state, it is said that they are in G0 phase. From G0 and differentiated states, some cell types are able to restart the cell cycle. Entering in quiescent stage means the expression of a particular set of genes and the repression of those genes that promote differentiation, senescence or apoptosis. Quiescent cells repressed genes that promote the cell cycle. From apoptosis or senescence, cells cannot return to the cell cycle. Thus, these are four possible decisions that cells may take during G1 phase, which depends on molecular complexes known as checkpoints. Cells must pass these checkpoints before entering S phase. If cells do not pass one of them, it is said that a decision has been taken. However, if cells do not stop in any of them, S phase will eventually start. This is the default process. The molecular mechanisms at the checkpoints have to be fast, complete, and irreversible. Figure 1. Decisions that a cell may take during G1 phase. The green color means that cell can return to the cell cycle (modified from Blomen and Boonstra, 2007). Checkpoints are molecular complexes that control the progress of the cell cycle. At the core of checkpoints there are cyclin-dependent kinases (CdKs). Nine different CdKs have being found in eukaryotes. To be active, CdKs need to bind a regulatory protein known as cyclin, and be phosphorylated as well. Once activated, CdKs phosphorylate several substrates, such as inhibitors of the cell cycle, thus allowing the progress of the cell cycle. Cyclins are proteins synthesized periodically during the cell cycle. There are 16 different cyclins in eukaryote cells. Cyclins A, B, D, and E have more impact in the progress of the cell cycle. Cyclins D (there are 3) and E (there are 2) are important for G1 phase progress. CdK4/cyclin D (D/CDK4) and CdK2/cyclin E (E/CDK2) phosphorylate the transcription factor retinoblastoma, which is part of the last checkpoint of the G1 phase. Centrosome and cell cycle. The checkpoint where retinoblastoma is phosphorylated is known as restriction point because, if this checkpoint is passed, cells immediately start the S phase. The concept of restriction point was introduced by A. Pardee in 1974. It is very important because there is no turning-back, once S phase is started, cells will divide and no external signals will stop the cell cycle. To reach the restriction point during G1 phase, cells need extracellular signals like mitogens, but they are not needed once this restriction point is passed. The sequence is as follows. Mitogens activate plasma membrane receptors that stimulate Ras-GTPases, which promote the expression of genes of transcription factors like c-Myc and others. Transcription factors boost the expression of cyclin D that in turn activate CdK4 and 6, which phosphorylate retinoblastome proteins that release the E2F factor (Figure 2). Figura 2. Interactions between retinoblastome (Rb), CDK-cyclins and E2F factor at the begin of the S phase At the core of the restriction point are CdK-cyclin, retinoblastoma and E2F (Figure 2). Retinoblastome is not phosphorylated during early G1 phase. In this form, retinoblastoma is bound to E2F and inhibits the expression of genes that promote the cell cycle progress. Retinoblastome is first phosphorylated by CdK4/cyclin D and then by CdK2/cyclin E, both in G1 phase. Retinoblastome have 16 phosphorylation sites, which indicates how complex is the regulation. Phosphorylation of all sites is successive, and each CdK phosphorylates specific sites. It seems that when 14 sites are phosphorylated, the affinity of retinoblastome for E2F decreases and, therefore, E2F factor may promote the expression of genes that favor the beginning of S phase. In this molecular framework, the signals carrying information about the environment (nutrients, trophic signals, and others), DNA damages, or about the proper size for division are integrated. If everything is correct, the cell passes the restriction point and S phase begins. If something is wrong, there are several types of inhibitors that stop the cell cycle progress. One of them is p53, which is activated in many tumors. When DNA is damaged, or the cell is under stress, or there are changes in the pH, or any other potentially dangerous situations for the cell are present, p53 is over-expressed and activates the p21 gen, which in turn inhibits the phosphorylation of retinoblastoma, so that the cell cycle is arrested and does not start the S phase. As we mentioned above, most cells of a multicellular organism are not in permanent proliferation. This is because there are inhibitors of Cdk/cyclins in G1 phase that make cells quit the cell cycle. Bibliography Blomen VA, Boonstra J. 2007. Cell fate determination during G1 phase progression. Cellular and molecular life sciences. 64:3084-3104. Matson JP, Cook JG. 2017. Cell cycle proliferation decisions: the impact of single cell analyses. The FEBS journal. 284: 362-375. Page 12 The end of the S phase of the cell cycle leads to G2 phase, which in turns leads to the M phase. During G2 phase, there must be an increasing amount of molecules needed for M phase. G2 phase has been traditionally regarded as a transitional phase between S and M phases. However, during G2 phase, it is checked if any error occurred during DNA replication and if the DNA has been completely replicated. If something went wrong during S phase, M phase does not start until the errors are repaired. It is of great importance the detection of errors before M phase starts, otherwise they will be inherited by daughter cells. During G2 phase, cell increases in size, and centrosomes of animal cells, that were duplicated during S phase, are positioned at opposite locations in the cytoplasm. During M phase, centrosomes polymerize the mitotic spindle. Centrosome cycle The moment where G2 phase finishes and M phase starts is not clearly established, and some authors suggest that it is actually in the middle of the mitotic pro-phase. Anyway, the end of G2 phase is mediated by the type I cyclin dependent kinase (CdK) and by B1 cyclin. B1 cyclin is synthesized during the late S phase. This CdK-I/B1 complex, helped by some other kinases and phosphatases, triggers the starting of the M phase, so it is a checkpoint. Until recently, it was thought that G1 phase was the only key element for restricting the progress of the cell cycle because it is where the response to mitogens takes place. However, now we know that there is time window in G2 phase that determines decisions that will be taken in G1 phase. For example, there is a dephosphorylation process in G2 phase needed in G1 for sensing mitogens and takes a decision. Without this dephosphorylation, the cell proliferates even without mitogens during G1 phase. If there is no dephosphorylation during G2, the cell is committed to proliferate and G1 phase is quite short. Mitogens during G2 phase inhibit the dephosphorylation process. Bibliography Matson JP, Cook JG. 2017. Cell cycle proliferation decisions: the impact of single cell analyses. The FEBS journal. 284: 362-375. Page 13 This page index 1. Morphology 2. Organization 3. Models 4. Functions Golgi apparatus was discovered by Camillo Golgi in 1889, when he was studying neurons, but it was no completely accepted as a true organelle for decades. Membrane organization of the Golgi apparatus was described in detail by Dalto and Felix (1954) by using transmission electron microscopy. They proposed the name Golgi apparatus. 1. Morphology In animal cells, the Golgi apparatus is commonly located close to the centrosome, which in turn use to be near the nucleus. This position is determined by the interactions of Golgi membranes with microtubules. Microtubules are radially oriented with their minus ends anchored to the centrosome. Golgi apparatus is made up of flat cisterns piled in several stacks. Each stack is referred as dictyosome (Figures 1 and 2). cisterns are usually thinner in the middle and thicker in the edges (like pizzas), and are more or less bent showing a convex and a concave surface. Several stacks are present in one cell and some cisterns are large enough to be part of two near stacks (Figure 1). The number (normally 3 to 8) and the size of the cisterns in one stack are variable and depend on the cell type, as well as the cellular physiological state. In one cell, the Golgi apparatus includes all the stacks and their lateral connections. Figure 1. In animal cells, Golgi apparatus is made up of several stacks, located close to the centrosome, and therefore close to the nucleus. Some nearby stacks are laterally connected. Figure 2. Transmission electron microscopy image of Golgi apparatus showing several stacks (arrows). In animal cells, there is a fibrous protein matrix where Golgi cisterns are located that may help to maintain the Golgi apparatus structure. However, it has been shown that the structural integrity of the Golgi apparatus mainly relies on microtubule organization (Figure 3). The position of the Golgi apparatus depends on microtubules nucleated from the centrosome, whereas the stack integrity is maintained by microtubules nucleated from the Golgi cisterns. Actin filaments and myosin motor proteins help to fine-tune the final location of Golgi stacks. In addition, the Golgi apparatus structure depends on the vesicular trafficking between the Golgi apparatus and the endoplasmic reticulum. When this trafficking is stopped the Golgi apparatus disappears. Figure 3. Organization of the Golgi apparatus in animal and plant cells. In animal cells, Golgi stacks are located close to the centrosome, near the nucleus, and are dragged mainly along microtubules. In plant cells, cisterns are distributed through the cytoplasm, they are moved by actin filaments. Arrows indicate the movement direction of the vesicles. In plant cells, which do not have centrosomes, there are small stacks of cisterns or even individual cisterns dispersed throughout the cytoplasm (Figure 3). The Golgi apparatus looks like divided in many units distributed through the cytoplasm. Each of those units work independently. Plant Golgi apparatus cisterns are smaller than in animal cells, although the total number in a cell could range from dozens to more than a hundred. In plant cells, ERGIC (see below) has not been observed, but the trans Golgi network (TGN) is well developed, so much that some authors endorse it as a different organelle. Individual cisterns or stacks are moved thanks to actin filaments and are found around the transitional areas, where there is vesicle formation by the endoplasmic reticulum. The stack organization does not change during these movements. Vesicles released from the stacks that are targeted to vacuoles are moved by actin filaments too. One more feature of Golgi apparatus organization in plant cells is that cisterns do not disappear during mitosis, as it occurs in animal cells, because Golgi apparatus is needed for synthesizing the new cell wall that separate the two new cells during cytokinesis. There are differences in the Golgi apparatus organization depending on the cell type and in different species. For instance, the fruit fly cells show a Golgi apparatus organization similar to that of plant cells, although they have centrosome. Furthermore, the lateral connections between adjacent cistern stacks have only been observed in mammalian cells. 2. Organization Golgi apparatus is a polarized organelle. Cistern piles are organized in domains: cis, intermediate and trans domains (Figure 4). Intermediate cisterns locates between cis and trans domains. In the cis domain, there is an ongoing addition of new material coming from the endoplasmic reticulum via the ERGIC (endoplasmic reticulum Golgi intermediate compartment) compartment. In the trans domain, there is a tubulo-vesicular arrangement of the membranes, referred to as TGN (trans Golgi network), where molecules are distributed into vesicles and tubules in their way toward other cell compartments. Thus, molecule journey through the Golgi apparatus starts in the cis cisterns, goes through intermediate cisterns, and ends in the trans domain. The Golgi apparatus is in permanent renewing and its organization and size is affected by the trafficking of molecules. It is particularly well-developed in those cells showing an intense secretory activity. The resident Golgi enzymes involved in modifying the molecules that go through the organelle are selectively distributed in a specific domain (cis, intermediate or trans) according to the chemical reaction they are performing. Figure 4. Golgi apparatus is divided in domains. Cis domain is where ERGIC bodies and vesicles coming from the endoplasmic reticulum fused between each other to form the first Golgi cistern. ERGIC is not a Golgi compartment, but a transient one between the endoplasmic reticulum and Golgi apparatus. cisterns in the middle of stacks are known as intermediate cisterns. Trans domain is where cisterns are transformed in vesicles and tubules containing the molecules to be delivered. TGN (trans Golgi network) is this trans network of vesicles and tubules. Red arrows indicate the main flux of molecules to be processed in the Golgi apparatus. From the lateral part of cisterns (gray arrows), COPI coated vesicles arise and are moved toward the endoplasmic reticulum. This is a recycling pathway. 3. Golgi apparatus models a) Cisternal maturation model (Figure 5). It proposes that ERGIC bodies get fused between each other to form the first cistern at the Golgi cis domain. Then, this cistern moves toward the trans domain, where it is eventually split in vesicles. The internal material of the cistern is progressively processed during this journey, i.e., there is a maturation of the cistern. Currently, this is the most accepted model because it can explain some observations that do not fit in other models. Figure 5. Models for molecular transport (colored in orange) and processing through a Golgi stack. In the cistern maturation model (upper), cisterns are generated in the cis domain and subsequently moved toward the trans domain, while molecules are being processed. In the trans domain, cisterns get split in many vesicles. In the vesicular transport model (lower), molecules are transported from one cistern to the next by vesicles (orange colored vesicles). b) Vesicular transport or stationary cisternae model. In this model, ERGIC and vesicles coming from the endoplasmic reticulum are fused with the cis cistern. From cis cistern, vesicles bud and fuse with the next cistern of the Golgi stack. In this way, the molecules are transported by vesicles from one cistern to the next, while they are specifically processed in each cistern. The cistern of the trans domain does not disappear when molecules are packaged into vesicles. The support for this model has declined nowadays. c) Connection by tubules. Tubular connections between adjoining cisterns have been observed at electron microscopy. These connections are like bridges that appear to be transient and depending on the material to be processed. This proposal is not incompatible with the other models, since tubular connection may happen in both between stable cisterns and between cisterns that move through the stack. Functions a) The Golgi apparatus is the main glycosylation center of the cell. Many carbohydrates that form part of glycoproteins, proteoglycans, glycolipids and other polysaccharides, such as hemicellulose in plants, are added and modified in the Golgi apparatus. Sialic acid is a carbohydrate specifically added in this organelle. Carbohydrates are added through a particular type of binding known as O-type glycosylation, which is the attachment of carbohydrates to the hydroxyl groups of the amino acids, such as serine, threonine and hydroxylysine. Sulfation of proteoglycans also happens in the Golgi apparatus, as well as phosphorylation, palmitoylation, methylation, and other chemical modifications. In plants, the glycosylation function of the Golgi apparatus is essential because many glycoconjugates are part of the cell wall. However, cellulose is synthesized at the cell membrane. Carbohydrates are added by glycosyltransferases and removed by glycosidases. About 200 different types of these enzymes can be found in the Golgi apparatus. Different cisterns have different roles in the carbohydrate processing, which is an ordered process. Evidences suggest that there is a concentration gradient of enzymes, from cis- to trans-domain, so that those enzymes involved in the first steps of glycosylation are concentrated on the cis domain, and those participating in the last steps are mostly found near the trans domain. b) The synthesis of sphingomyelins and glycosphingolipids is accomplished in the Golgi apparatus. Ceramide, synthesized in the endoplasmic reticulum, is the molecular base used by enzymes located in the Golgi apparatus for synthesizing these lipids. VLDL (very low density lipoproteins) are apolipoproteins that are also assembled in the Golgi apparatus. c) Golgi apparatus is a center for shipping molecules coming from the endoplasmic reticulum or synthesized in the Golgi apparatus. Once processed, molecules are distributed into different types of vesicles which are targeted to other cell compartments. In the trans domain, TGN is the structure where this process takes place (see figure). Some vesicles are moved to and fused with the plasma membrane, process known as exocytosis. There are two types of exocytosis: constitutive and regulated (see next page). Furthermore, there are vesicles leaving the trans domain targeted to late endosomes/multivesicular bodies/lysosomes, or to vacuoles in plants. Trans domain is also a target for other vesicles coming from endosomes, where some molecules are recycled. In plants, Golgi apparatus can receive vesicles directly from the plasma membrane. Thus, Golgi apparatus participates in both, endocytosis and exocytosis. A variety of vesicle morphologies arise from tubular expansions of the TGN membrane complex, not just typical round-shape vesicles. Molecules must be selected and included in specific vesicles to be shipped to different targets. Those transmembrane proteins targeted to late endosomes (and basolateral membranes in epithelial cells) are selected by a particular amino acid sequence of the cytosolic domain. However, molecules targeted to plasma membrane (or to the apical membrane of the epithelial cells) are selected by selectins which recognize O and N glycosidic bonds. Cytoskeleton moves vesicles to their target compartment. For example, in animal cells, microtubules transfer vesicles to the plasma membrane, whereas in plant cells, vesicles targeted to vacuoles are propelled along actin filaments. d) Golgi apparatus performs other non-conventional functions. It works as a calcium store, as an intracellular signaling platform, contributes to the cellular levels of sterols, starts the cell response to starvation, and is a microtubule nucleator. Bibliography Farquhar, MG, Palade GE. 1981. The Golgi apparatus (Complex) - (1954-1981) - from artifact to center stage. The journal of cell biology. 91: 77s-173s. Glick, BS, Luni A. 2011. Models for Golgi traffick: a critical assessment. Cold Spring Harbor perspectives in biology. 3: a005215. Vildanova MS, Wang W, Smirnova EA. 2015. Specific organization of Golgi apparatus in plant cells. Biochemistry (Moscow). 79: 894-906. Page 14 Most proteins and lipids leaving the endoplasmic reticulum are packaged in vesicles or incorporated in tubulo-vesicular compartments that are detached from the endoplasmic reticulum membrane (Figure 1). They are shipped to the Golgi apparatus. There are ribosome-free regions distributed along the endoplasmic reticulum membranes, referred as transition zones or ER exit zones, where vesicles and their cargoes are assembled. Transition zones are about 0,5 µm in length and, at least in mammals cells, are quite stable in both time and space. Figure 1. COPII (coat protein II) vesicles are released from the transition zones of the endoplasmic reticulum, are moved away, and fused between each other forming the ERGIC (endoplasmic reticulum-Golgi intermediate compartment), which moves toward the cis face of the Golgi apparatus. In animal cells, vesicles and ERGIC movements are mediated by microtubules and motor proteins. In the cis side of the Golgi apparatus, all the ERGIC bodies and some vesicles from the endoplasmic reticulum coming from different parts of the cell form the cis cisternae of the Golgi apparatus. At the same time, from the ERGIC and cis cisternae, COPI coated vesicles are released and travel toward the endoplasmic reticulum in a recycling process. Transition zones are associated with the Golgi stacks. They are close to each other. This makes sense because the communication is more efficient. Vesicles don't need to travel long distances and the existence of the Golgi apparatus itself depends on a continuous process of vesicle incoming. It has been observed that a new transition zone led quickly to the nearby formation of a new Golgi stack. On the contrary, if a transition zone disappears, the associated Golgi cisternae are also lost. Transition zones can fuse with others and one transition zone can be split in two. Their associated Golgi stacks match this behavior. Vesicles budding from the transition zones are COPII coated vesicles (COPII: coat protein II; Figure 1). Several proteins are involved in the formation of this COPII molecular framework: Sec16, Sar1 GTPases, Sec23/24 and Sec13/31. In this order, they are assembled at the cytosolic surface of the transition zone membranes. Transition zones are the more suitable environments for the assembling of COPII coats by having a particular membrane lipid composition and a higher concentration of the protein Sec16, which recruit Sec23/24 proteins, that in turn recruit Sec13/36. COPII proteins participate in two mechanisms: a) vesicle formation and b) selection of cargoes. a) COPII proteins, mostly the external layer formed by Sec13/31, are involved in the formation of the vesicle. To do this, they induce the bending of the membrane by producing mechanical tensions and folds in the membrane for form the vesicle. Furthermore, COPII proteins are thought to participate in the excision of the vesicle. b) Two types of cargoes are going to be transported: transmembrane cargo and soluble cargo. Soluble cargoes must be "fished" by transmembrana receptors. The cytosolic domains of both, transmembrane cargoes and receptors, are recognized by COPII proteins and included in the vesicle. The recognition is mediated by Sec 24 proteins. Thus, COPII proteins are needed for selecting molecules to be transported in the vesicle. In addition, any protein to be transported has to be properly folded. Otherwise, it will be removed from the endoplasmic reticulum before the recognition. A quality control of proteins is always at work in the endoplasmic reticulum. COPII coated vesicles are released from the transition zones of the endoplasmic reticulum, they partially lose the protein coat, and are fused together to form the ERGIC (endoplasmic reticulum-Golgi intermediate compartment) or tubule vesicular transporter, which is moved by microtubules and motor proteins toward the cis face of the Golgi apparatus. All the ERGIC bodies arriving at the cis face are fused to form the cis cisternae of the Golgi apparatus. COPII coated vesicles is the usual way to travel from the endoplasmic reticulum to the Golgi apparatus. However, there are alternatives, or variations, to this vesicular mechanism. For example, a typical COPII vesicle of about 60-90 nm in size, cannot enclose a pro-collagen molecule, which is 300 to 400 nm long. Likewise, chylomicrons traveling from the reticulum to the Golgi apparatus need larger special vesicles. It looks like that the activity of the Sar1 GTPase is important for modifying the vesicle coat and then produce vesicles larger than 500 nm, where larger molecules can fit. Bidirectional trafficking between endoplasmic reticulum and Golgi apparatus has two roles: getting back resident proteins and keep constant the sizes of both organelles. ERGIC compartment matures during the journey to the Golgi apparatus. This maturation process brings a progressive change of the molecular composition of its membrane that in turn allows the attachment of some cytosolic proteins, such as COPI coat molecules. In this way, COPI proteins are recruited to the ERGIC and Golgi cisterns membranes. The COPI vesicle formation mechanism is similar to that of COPII vesicles, but COPI vesicles are targeted to the endoplasmic reticulum with lipids and endoplasmic reticulum resident proteins. This is a recycling pathway. COPI coated vesicles brings back membrane for replacing the membrane used to form COPII coated vesicles. In addition, they transport v-SNARE and receptors needed for selecting cargoes to be included into the COPII coated vesicles. Resident proteins perform their functions in the endoplasmic reticulum, but not in the Golgi apparatus, and may enter by diffusion into COPII vesicles while budding. The number of COPI vesicles depends on the amount of endoplasmic reticulum resident proteins present in the Golgi apparatus/ERGIC compartment since these proteins stabilize the COPI coat. Another recycling mechanism mediated by tubular structures between endoplasmic reticulum and Golgi apparatus/ERGIC compartment has been suggested. Selection of resident proteins in the ERGIC/Golgi compartments is done in two ways. Resident transmembrane proteins are recognized by COPI molecules, whereas soluble resident molecules are selected by KDEL receptors. Both, transmembrane and soluble resident proteins have particular amino acid sequences. COPI coated proteins are released from the ERGIC/Golgi compartment, moved toward the endoplasmic reticulum, and fused with it, releasing the transported molecules. KDEL receptors commute between the endoplasmic reticulum and the Golgi apparatus. They are able to travel in both COPII and COPI coated vesicles. KDEL receptor recognizes endoplasmic reticulum resident proteins in the ERGIC/Golgi apparatus, and receptor-ligand complex is then included into COPI vesicles. In the endoplasmic reticulum de resident protein is dissociated ,and KDEL receptor is included in COPII coated vesicles. The mechanism for binding the ligand in one compartment and releasing it in another is based on the pH differences between both compartments. Endoplasmic reticulum pH is more basic and the affinity of KDEL receptor for its ligand is very low. However, in the ERGIC/Golgi apparatus compartment the pH decreases, which in turn increases the affinity of KDEL receptors for endoplasmic reticulum resident proteins. Thus, pH is the key element for this recycling mechanism. Bibliography Antonny B, Schekman R. 2001. ER export: public transportation by the COPII coach. Current opinion in cell biology. 13:438-443. Saito K, Maeda M, Katada T. 2017. Regulation of the Sar1 GTPase cycle is necessary for large cargo secretion from the endoplasmic reticulum. Frontiers in cell and development biology. 5: 75. Zanetti G, Pahuja KB, Studer S, Shim S, Schekman R. 2012. COPII and the regulation of protein sorting in mammals. Nature cell biology. 14: 20-28. Page 15 Endoplasmic reticulum is a complex membrane-bound compartment arranged in tubules and flattened cisterns interconnected and sharing the same lumen (inner space) and membrane. Endoplasmic reticulum membrane is also continuous with the outer membrane of the nuclear envelope. Tubules and cisterns are distributed trough the cytoplasm from the nuclear envelope to close to the plasma membrane, so that they can account for half of the total cellular membranes. Endoplasmic reticulum membrane is thinner (around 5 nm in thickness) than other organelle membranes because they have lipids with shorter fatty acid chains. Endoplasmic reticulum membranes are organized in domains or regions that carry out different functions. At transmission electron microscopy, it is easy to distinguish two domains: rough and smooth endoplasmic reticulum (Figure 1). Rough endoplasmic reticulum membranes are arranged forming cisterns and more or less straight tubules, both having many ribosomes associated to their cysotolic membrane surface (that is why the name rough). Smooth endoplasmic reticulum is organized in irregular and convoluted tubules, with no associated ribosomes. The outer membrane of the nuclear envelope may regarded as part of the rough endoplasmic domain because it is physically continuous with the membranes of the rough endoplasmic tubules and it has many associated ribosomes doing translation. Figure 1. Endoplasmic reticulum can be found all over the cytoplasm, from the nuclear envelope to the plasma membrane. Rough and smooth endoplasmic reticulum membranes are continuous. Rough endoplasmic reticulum is organized in cisterns and tubules that show associated ribosomes, whereas smooth endoplasmic reticulum is arranged in tubules with no ribosomes attached. The outer nucelar envelope membrane is continuous with the endoplasmic reticulum membranes Rough and smooth endoplasmic reticulum do not usually share the same cytosolic space. This non overlapping distribution is observed in hepatocytes, neurons and cells synthesizing steroid hormone. However, in some cytosolic regions there is not clear segregation between both domains, and tubules with associated ribosomes are intermingled with naked tubules. The spatial distribution of the endoplasmic reticulum through the cytosol is set by the cytoskeleton, mostly microtubules in animal cells, whereas actin filaments are the major responsible for the endoplasmic reticulum distribution in plant cells. 1. Rough endoplasmic reticulum Rough endoplasmic reticulum is organized in more or less straight tubules and flattened cisternae. Sometimes, cisternae are tidily piled. The name "rough" comes from the electron microscopy images where ribosomes, black particles, are observed coating the endoplasmic reticulum membrane (Figure 2). The density of associated ribosomes influences the rough endoplasmic reticulum membranes organization, so that a high density causes a cistern-like morphology, whereas lower density is found in tubules. Cisternae and tubules coexist in the same cell, but those cells with an intense secretory activity show dense piles of cisternae, which means a highly developed rough endoplasmic reticulum. Fiigure 2. Transmission electron microscopy image. Rough endoplasmic reticulum cisternae are observed, which extend from the nuclear envelope to the proximity of the plasma membrane. Ribosomes are observed as black dots associated to endoplasmic reticulum membranes. Note ribosomes associated to the outer membrane of the nuclear envelope as well. Protein synthesis Protein synthesis is the main function of the rough endoplasmic reticulum. These proteins end up at different places, such as the extracellular space, plasma membrane, or at membranes and lumen of several organelles involved in the vesicular trafficking, such as Golgi apparatus, endosomes, and lysosomes. Many proteins will be part of the plasma membrane, and of other membranes, as transmembrane proteins. Furthermore, rough endoplasmic reticulum needs to synthesized proteins for itself, which are known as resident or constituent proteins. Keep in mind that each protein should "know" its correct destination in the cell. This can be accomplished by a signal peptide (short sequence of amino acids) or specific modifications of the protein, which work as postal addresses. For example, endoplasmic reticulum resident proteins contain a four amino acid sequence close to the end carboxyl group. Almost any protein targeted for secretion or being part of a compartment of the vesicular traffic begins to be synthesized in cytosolic free ribosomes, but the synthesis ends in the endoplasmic reticulum, leaving the protein either free in the lumen or as part of the reticulum membrane. The process of protein synthesis starts when an mRNA, which carries information for a protein of the vesicular trafficking, joins to a small ribosomal subunit, and then to a large ribosomal subunit, to begin the translation (Figure 3). The first translated segment of the mRNA is a sequence of amino acids known as signal peptide, which is about 70 amino acids long. This sequence is recognized by a cytosolic molecule referred as SRP (sequence recognition particle). SRP is a mix of 1 RNA and 6 polypeptides that joins to the signal peptide and slows down the translation process. The mRNA-ribosome-SRP-signal-peptide complex diffuses through the cytosol until it hits a rough endoplasmic reticulum membrane. In these membranes, there are SRP-receptors which recognize the SRP. The whole complex becomes attached to the membrane and then interacts with a translocon, a large transmembrane protein with a channel. Then, SRP and SRP-receptor are released and mRNA-ribosome-signal-peptide attached to the translocon can resume translation, but the nascent polypeptide grows now inside the translocon channel. The signal peptide gets fixed to the channel walls, while the rest of the protein is falling into the lumen of the endoplasmic reticulum cistern. The signal peptide is removed by a peptidase, and once completely translated, the new protein is released and remains free in the lumen, and quickly is folded to get a proper spatial conformation helped by chaperones, another type of proteins. When translation is finished, the ribosome-mRNA is disengaged from the translocon, and the three are free in the cytosol for another round. Figure 3. Synthesis of soluble proteins that are released into the lumen of the endoplasmic reticulum. Transmembrane proteins contain sequences of hydrophobic amino acids. When these sequences are being translated and going through the translocon channel, they can cross the wall of the translocon and be among the fatty acid chains of membrane lipids. The process is rather complex and diverse depending on the protein. For example, some receptors are transmembrane proteins with a chain of amino acids that can cross seven times the cell membrane, by means of alternating hydrophobic and hydrophilic amino acid sequences. There are other proteins spanning just one monolayer of the membrane, and they have to be either in the cytosolic monolayer or in the monolayer facing the lumen. Although it is no common in animal cells, rough endoplasmic reticulum may import some proteins completely synthesized in the cytosol by a process known as posttranslational translocation, which is mediated by chaperones. Proteins in the rough endoplasmic reticulum are modified while they are being synthesized. a) Asparagine amino acids are glycosylated (N-glycosylation) with a molecular complex composed of 15 monosaccharides. This molecular complex is first assembled into a molecule of dolichol phosphate, a membrane lipid, and then is transferred to an asparagine of the nascent protein. In the Golgi apparatus, some terminal monosaccharides of this complex will be lost and another saccharides will be added. b) Some proteins, mostly those targeted to the extracellular matrix, are hydroxylated. In this way, proline and lysine amino acids of the collagen molecules end up being hydroxyproline and hydroxylysine amino acids. c) Some proteins of the plasma membrane are chemically bonded to some membrane lipids. This chemical bond is also established in the rough endoplasmic reticulum. d) Disulfide bonds between polypetide chains are also formed in endoplasmic reticulum. A quality control of synthesized proteins occurs in the rough endoplasmic reticulum, so that those defective proteins are removed from the reticulum and degraded in the cytosol. Proteins known as chaperones, which are needed for a proper folding of newly synthesized proteins, also play an essential role in detecting defective proteins and labelling them for degradation. Other proteins bearing lectin domains are able to detect and recognize wrong arrangements of carbohydrates. Wrong folding of nascent proteins, which may lead to cell damages, is more frequent than one may imagine. 2. Smooth endoplasmic reticulum Smooth endoplasmic reticulum is a network of interconnected tubules, which are continuous with the rough endoplasmic reticulum. There are no ribosomes associated to its membrane, therefore it is named smooth, and the majority of proteins in this compartment comes from the rough endoplasmic reticulum domain. Smooth endoplasmic reticulum is abundant in those cells involved in lipid metabolism or detoxification, and is also an organelle for calcium storage. Salient functions of the smooth endoplasmic reticulum are: Lipid synthesis Most membrane lipids are assembled in the smooth endoplasmic reticulum, including glycerophospholipids and cholesterol. The components of the glycerophospholipids come from other parts of the cytoplasm and assembled in the membranes of the endoplasmic reticulum. Fatty acids are inserted in the cytosolic monolayer of the organelle membrane. Glycerophospholipid heads are then linked to these fatty acids. Most part of the sphingolipid synthesis, however, happens in the Golgi apparatus, but ceramide, the basic component of sphingolipids, is assembled in the smooth endoplasmic reticulum. Once glycerophospholipids and ceramide are complete assembled, they are initially located in the cytosolic monolayer of the endoplasmic reticulum membrane. Since flip-flop movement is nearly forbidden for lipids by the hydrophobic environment of fatty acid chains, lipids need help

to be transferred to the other monolayer (that facing the lumen). There are specialized proteins that can move lipids from one monolayer to the other: flippases, floppases and scramblases. Figure 4. Suggested ways for transferring lipids between cellular membranes: vesicular trafficking, carriers and membrane contacts. Transferring lipids between membranes is done by vesicles, molecular carriers and at membrane physical contac sites (Figure 4). Vesicles, through vesicular trafficking, transport in their membranes lipids synthesized in the endoplasmic reticulum to other membranes. Mitochondria, chloroplasts and peroxisomes are not part of the vesicular traffic, so some membrane lipids must be synthesized locally, but many others are imported from the endoplasmic reticulum by molecular carriers. For example, glycerophospholipids are transported by a cytosolic protein known as glycerophospholipid interchanger. It can take a lipid from the membrane of the smooth endoplasmic reticulum and leave it in the membrane of other organelles. Furthermore, many electron microscopy images show physical contacts between membranes of different organelles, for example between endoplasmic reticulum and mitochondria or peroxisomes. These contacts may facilitate interchange of lipids between different membranes. Chloroplasts can synthesize their own glycerophospholipids and glycolipids. However, endoplasmic reticulum membranes and chloroplast membranes are also observed very close to each other in electron microscopy images. Cholesterol is mostly synthesized in the smooth endoplasmic reticulum. It is an important molecule for membranes, particularly for the plasma membrane. Cholesterol is transported by vesicles and molecular carriers from the endoplasmic reticulum to the plasma membrane (Figure 4). In yeasts, that have ergosterol in their membranes instead of cholesterol, the most important way to move ergosterol from the endoplasmic reticulum to the plasma membrane is a diverse set of transporters. This transport does not need ATP. Triacylglycerols are synthesized in the smooth endoplasmic reticulum. These lipids are stored in the reticulum itself or as lipid droplets. The synthesis of triacylglycerols is intense in adipocytes, the fat storing cells of animals. Glycogen is a polysaccharide of glucose that is stored in cytoplasmic lipid droplets. This fat-storing organelle works as an energy source for the body when needed, and in some species for thermal insulation as well. Triacylglycerides are also part of the lipoproteins, and required for the production of steroid hormones and bile acids. Detoxification Hepatocytes, the liver cells, show a highly developed smooth endoplasmic reticulum. In the smooth endoplasmic reticulum, there is P450 protein family is in charge of removing potentially toxic metabolites, as well as liposoluble toxins incorporated during the digestion. The shape of the smooth endoplasmic reticulum tubules, and the lack of ribosomes, allows a large surface of membrane related to the organelle volume. In addition, it is able to increase the length of the tubules to make room to all these enzymes, which turn in it depends on how many toxic substances the animal body contain. Dephosphorylation of 6-phosphate glucose Glucose is usually stored as glycogen, mainly in the liver. Two hormones regulate the glucose release from the liver to the blood: insulin and glucagon. Catabolism of glycogen produces 6-phosphate glucose, which cannot cross the cell membrane, and hence cannot leave the cell. Glucose 6-phosphatase, which is anchored to the endoplasmic reticulum, removes the phosphate residue allowing glucose to be moved out of the cell. Calcium storage Smooth endoplasmic reticulum also works as a storage compartment for cytosolic calcium. Calcium is transported into the lumen by calcium pumps located in the endoplasmic membrane. Calcium concentration in the smooth endoplasmic reticulum is in millimolar (mM), whereas in the cytosol it is nanomolar (nM). Stored calcium is released to extracellular and intracellular signals acting via second messengers, resulting in a cellular response, for example exocytosis. A remarkable example is the endoplasmic reticulum of the muscle cells (known as sarcoplasmic reticulum) that captures and releases calcium to produce a cycle of muscle cell contraction and relaxation. Bibliography English AR, Zurek N, Voeltz GK. Peripheral ER structure and function. Current opinion in cell biology. 2009. 21:596-602. Daleke DL. Phospholipid Flippases. The journal of biological chemistry. 2007. 282:821-825. Page 16 A eukaryotic cell may resemble a big city with several districts. Each of them is specialized in a particular role, such as energy production, manufacturing products, exportation, importation, communication with other cities, recycling, and so on. To work properly, a rich and complex communication system is needed between districts, which is carried out by carriers that go through multiple pathways. Cellular districts are the intracellular compartments and many of them are the membrane-bound compartments, i.e. organelles. Each organelle is specialized in one or several functions. For example, endoplasmic reticulum is in charge of synthesizing proteins for secretion and lipids for making membranes. Golgi apparatus produces carbohydrates to be attached to proteins and lipids, and is also a distribution center, lysosomes are the main digestion centers, and mitochondria and chloroplasts synthesize ATP. Communication between some organelles is mediated by vesicles, which carry molecules both in the interior and as part of the membrane of the vesicle. Vesicular trafficking includes all the communication pathways mediated by vesicles, as well as the organelles that send or receive vesicles (Figure 1). There are two main roads in this trafficking road map. One, known as secretory pathway, starts in the endoplasmic reticulum, that sends vesicles to the Golgi apparatus, which in turn sends vesicles targeted to the plasma membrane (exocytosis). This pathway releases molecules to the extracellular space, and also carries molecules to the plasma membrane. The other pathway is an importing pathway that begins at the plasma membrane where vesicles and other large compartments are originated by membrane invagination (endocytosis). These vesicles fuse with the endosomes, which end up becoming lysosomes. Lysosomes degrade the endocytoled molecules, both those from the extracellular space and those forming the membrane of the vesicle. It is an importing pathway. There are many other communication pathways mediated by vesicles so that it looks like that all the organelles are connected through vesicles between each other. This has not been probed yet. However, there is this rule saying that the communication by vesicles between two organelles use to be bidirectional, i.e., the organelle A sends vesicles to the organelle B, and at the same time the organelle A receives vesicles from the organelle B. For example, the endoplasmic reticulum sends vesicle to the Golgi apparatus, which in turn sends vesicles back to the endoplasmic reticulum. The same happens between the plasma membrane and endosomes, and between Golgi apparatus and endosomes. Figure 1. Main pathways of communication mediated by vesicles between organelles. Roads and compartments constitute the vesicular traffic. Communication is usually bidirectional. No all the communication paths are depicted. There are organelles, such as mitochondria, chloroplasts and peroxisomes, which are not part of the vesicular traffic because they do not usually send nor receive vesicles. However, these organelles communicate with other organelles by other mechanisms. For example, by physical contacts of their membranes. It is frequently observed the mitochondrial external membrane in close apposition to endoplasmic reticulum membranes. Some authors propose that there is a high transfer of molecules, mostly lipids, through these areas of membrane contacts. There are also transporters that exchange lipids between membrane of different compartments. Page 17 Vacuoles are membrane-bound organelles found in plant cells and fungi, including yeasts. They are critical organelles for plant cell function. 1. Features Vacuoles are usually large compartments that in mature cells may be up to 90 % of the total cell volume (Figures 1 and 2). They are the largest compartment of plant cells. The term vacuole is derived from the Latin word "vacuus", which means empty. This was clearly a misunderstanding because vacuoles are not empty, but filled with a more or less concentrated aqueous solution. The membrane of the vacuole is known as tonoplast, and it is an essential part for the function of this organelle. In plants, there are several types of vacuoles according to the role they carry out. A plant cell may contain different types of vacuoles, and a vacuole can modify its enzyme repertory and then change its function. Figure 1. Drawing of a parenchymatic cell showing a large vacuole Figure 2. Photosynthetic parenchyma cells of Vicia faba leaves. Large vacuoles are stained in purple. Vacuoles are the clear spaces. Nucleus and chloroplasts can be observed. The image on the bottom comes from photosynthetic parenchyma of a pine leaf showing vacuoles stained in purple. Vacuoles are usually rounded, but the final shape is influenced by cell morphology. One large vacuole is often observed in mature plant cells. However, the membrane of the vacuole gets sometimes deeply and profusely folded and forms small compartments that look like many small vacuoles when observed at light microscopy, but they are actually just one vacuole because membrane is continuous. New vacuoles are formed by fusion of vesicles released from the Golgi apparatus. Initially they form a new compartment known as pro-vacuole. A meristematic cell may have hundreds of pro-vacuoles. Then, during cell differentiation, pro-vacuoles fuse between each other into small vacuoles, and the fusion process continues until a large central vacuole is formed. The endoplasmic reticulum might be also involved in the formation and growth of vacuoles in some plant cells, mostly in seeds. Once a large vacuole is present, vesicles from the Golgi apparatus and plasma membrane are still needed for the vacuole to grow by adding and removing membrane. The main vacuole of most plant cells is a large compartment filled with an acidic solution containing salts (sodium, potassium), metabolites (carbohydrates, organic acids) and some pigments. Some of these molecules enter the vacuole from the cytosol against concentration gradient. The normal pH inside the vacuole ranges between 5 and 5.5, although it can be around 2 in the lemon fruit, or even 0.6 in some algae. 2. Function Vacuoles are essential for physiology and homeostasis of plant cells, and perform different functions according to the cell type. The following are some of them: Turgor Cell turgor is the level of hydrostatic pressure against the cell wall of the plant cell. This pressure is under the control of vacuoles, which get different substances inside, including ions, to produce variable inner osmotic environments when compared with those of the cytosol. The different osmolarity at both sides of the vacuole membrane makes the water cross the membrane, either inward or outward. The substances that contribute to the vacuole osmolarity can cross the vacuole membrane by ATP dependent transport mediated by ionic pumps. H(+)-ATPase and H(+)-pyrophosphatase are able to form proton gradients between both sides of the vacuole membrane, and these gradients are used to transport other molecules. The ability to store water inside the vacuole is essential for plant cell grow after mitosis. Plant cells can increase their size 10 to 20 times, which is very useful for the body plant grow and for modifying the shape of plant organs. The grow mediated by hydrostatic pressure saves energy because it is cheaper to increase the amount of water than synthesize new molecules (animal cell growth is based on molecular synthesis). It is safer for plant cells to accumulate water in the vacuole because in this way the cytosolic molecules do not get diluted, which would compromise cell survival. Storage Vacuoles are the last station for some vesicular traffic pathways. In some cells, they are the compartment to store carbohydrates and proteins. This clearly happens in seeds, where vacuoles accumulate proteins needed during germination. Storage vacuoles become lytic vacuoles during cell differentiation. Unlike animals, plants do not have an excretory system, nor they can move to avoid toxic substances. In plants, potentially dangerous substances are stored in vacuoles. In this way, metabolism residues and toxic substances like heavy metals (cadmium, zinc and nickel) are found in vacuoles. In addition, they also store other substances such as pigments (for example, anthocyanins) in the epidermal cells of petals, toxic substances against herbivores, resins, alkaloids like opium, etcetera. Most of the taste of fruits and vegetables is the result of substances stored in vacuoles. Degradation centers Lytic vacuoles can be found in vegetative tissues, so they are also known as vegetative vacuoles. They contain enzymes like proteases and nucleases, as well as a number of proteins involved in the defense against pathogens. Proton pumps inserted in the vacuole membrane enter protons into the vacuole and acidify the interior content. The low pH and the lytic enzymes allow degradation processes. Vacuoles have a similar role to lysosomes of animal cells. Furthermore, like lysosomes, vacuoles participate in autophagy. Vacuolar processing enzymes are proteins also found in vacuoles. They transform molecular precursors arriving to the vacuole as inactive molecules into active molecules. Apoptosis Vacuoles are involved in plant cell apoptosis via a mechanism known as autolysis. In addition, a type of cell death known as hypersensitive cell death occurs in plant cells when the vacuole membrane gets broken. Others There are specialized vacuoles in different plant tissues. For example, in the seed internal tegument, vacuoles accumulate flavonoids for protection against ultraviolet light. Flavonoids are synthesized in the cytosolic surface of the endoplasmic reticulum membranes and then translocated to the interior of vacuoles for a final chemical processing. In the vacuole membrane there are transporters to carry out this translocation. Some plant species, like brassica, have vacuoles in their vegetative tissues for repealing herbivores. These vacuoles store proteins, such as myrosinases. Once released by the herbivore activity, these enzymes degrade molecular compounds of the leaves that become toxic for the animal. Cells storing myrosine are known as myrosine cells and can be found near the vascular bundles of leaves. Plants lack immune system so that each cell has their own defense system. Defense proteins and enzymes can be found in vacuoles. There are two defense mechanisms that vacuoles can perform (Figure 3): vacuole membrane collapses and fusion of membrane vacuole with plasma membrane. Viral infections lead to vacuole membrane breakage and release enzymes into the cytosol, where they can attack viruses. The fusion of vacuole membrane and plasma membrane releases vacuole enzymes to the extracellular space where they can kill pathogens like bacteria. Figure 3. Vacuole defense mechanisms. Vacuole membrane breakage and fusion between vacuole membrane and plasma membrane. Vacuole enzymes are released either into the cytosol or to the extracellular space, respectively. (Adapted from Shimada et al., 2018) 3. Vesicular traffic Vacuoles are part of the vesicular traffic. Actually, they may be regarded as an end-product of the vesicular trafficking since their formation and maintaining depends on the incoming vesicles. Molecules which are going to be stored or degraded, included hydrolytic enzymes, as well as membrane molecules are targeted to vacuoles via vesicles. Vacuoles can follow different vesicular pathways to get to vacuoles: Golgi apparatus→ Vacuole; Golgi apparatus→ pre-vacuolar compartment → vacuole. This is the default pathway to transport hydrolytic enzymes toward vacuoles. Pre-vacuolar compartments are similar to multivesicular bodies/late endosomes of animal cells. Curiously, hydrolytic enzymes are not selected in the Golgi apparatus by 6-phosphate-mannose moieties, but by a sequence of amino acids located in their amino acid chain. There are specific sequences of amino acids for targeting proteins to the hydrolytic vacuoles and other sequences are specific for moving other proteins toward the storing vacuoles. All proteins targeted to vacuoles have a signal sequence, and they need to be specifically recognized by receptors. Endoplasmic reticulum→ vacuole. Molecules may arrive to vacuoles directly from the endoplasmic reticulum. This pathway is prominent in seeds as a pathway for storing. However, in other plant cells, as in leaves, this pathway might be very rare. Vesicles traveling from the endoplasmic reticulum to vacuoles are independent of COP-II coats, which are needed for vesicles targeted to the Golgi apparatus. In the endoplasmic reticulum-vacuole pathway, there are sometimes intermediate compartments, but they are transient trans-Golgi membranes derived vesicles, which are usually not required before they arrive to the vacuole. This vesicular pathway may be favored during seed formation since the Golgi apparatus may not be able to cope with the high rate of synthesis of storage proteins. Golgi apparatus→ vacuole. Proteins and glycoproteins and they need to be targeted to the vacuolar membrane, if they are integral membrane proteins, are trafficked from the Golgi apparatus to the vacuole with no intermediate compartments. Plasma membrane → vacuole. Endocytic vesicles fuse directly with vacuoles, which work like early endosomes. Bibliography Marty F. 1999. Plant vacuoles. Plant cell 11:587-600. Pereira C, Pereira S, Pissarra J. 2014. Delivering of proteins to the plant vacuole--an update. International journal of molecular sciences 15: 7611-762. Shimada T, Takagi J, Ichino T, Shirakawa M, Hara-Nishimura I. 2018. Plant vacuole. Annual review in plant biology. . Taiz L. 1992. The plant vacuole. Journal of experimental biology 172: 113-122. Zhang C, Hicks G R, Raikhel NV. 2014. Plant vacuole morphology and vacuolar trafficking. Frontiers in plant sciences 5: 476. Page 18 The basic vesicular trafficking roadmap of animal cells is also found in plant cells. However, plants show some distinct features (Figure 1). Vacuoles, which communicate with other cellular compartments, are prominent organelles with essential functions for the plant cell. Furthermore, endocytosis and exocytosis are not as intense as in animal cells. Early and recycling endosomes have not been found in plant cells, and their functions are carried out by the TGN (trans Golgi network) of the Golgi apparatus. Figure 1. Main pathways of the vesicular trafficking in plant cells (adapted from Hawes et al. 1999). As in the animal cells, endoplasmic reticulum is the organelle for synthesizing proteins, lipids and carbohydrates that travel through vesicular trafficking. In plant cells, there is also a quality control of protein synthesis in the endoplasmic reticulum. In plant cells, the bidirectional communication between the endoplasmic reticulum and the Golgi apparatus is peculiar because both organelles are very close to each other in the cytoplasm. There is not a central Golgi apparatus in plant cells, but many cistern stacks scattered through the cytoplasm. Some authors suggest that instead of vesicles as transporters, sometimes transient membrane bridges between both organelles allow a direct way for molecules to move from one compartment to the other. Whatever the communication mechanism between both organelles, it is widely accepted that there is no ERGIC compartment in plant cells. In plant cells, besides the Golgi apparatus, the endoplasmic reticulum is able to target vesicles to vacuoles. This vesicular pathway has been suggested because some storing vacuoles may contain a high proportion of non-glycosylated proteins. However, endoplasmic reticulum and vacuoles are not connected directly but by an intermediate organelle known as intermediate vesicles. Furthermore, some experiments suggest that there is a direct vesicular pathway between endoplasmic reticulum and plasma membrane. Vesicles Plasmodesmata Vesicles that move from endoplasmic reticulum to the Golgi apparatus follow the default pathway. The Golgi apparatus has a relevant role in plant cells because most cell wall carbohydrates, but not cellulose, are synthesized in this organelle. From the Golgi apparatus, vesicles are also targeted to prevacuolar compartments, which fuse with vacuoles, both lytic and storage vacuoles. Pre-vacuolar compartment sends back recycling vesicles to the Golgi apparatus, like the multivesicular bodies/late endosomes do in animal cells. In differentiated cells, lytic and storage vacuoles can fuse with each other, which has effects on vesicular traffic pathways. Proteins targeted to vacuoles, either lytic or storage, must have a signal peptide to be recognized in the TGN of the Golgi apparatus. However, it is unknown the recognizing motif for proteins included in vesicles toward the plasma membrane. The default pathway for vesicles departing from the Golgi apparatus is toward the plasma membrane, mainly intended for membrane homeostasis and for releasing cell wall molecules. Endocytic vesicles fuse with pre-vacuolar compartments, with the Golgi apparatus, or directly with vacuoles. In plant cells, the endocytic pathway is poorly known and it seems less prominent than in animal cells. Although plant cells have endocytosis and contain the molecular machinery for that, some proteins are missing. For example, Rab proteins involved in phagosomes, cilia and lysosomes have been probably lost during evolution in plant cell lineage. Caveolin mediated endocytosis is also missing. In plant cells, endocytosis is mediated by several factors; for example, auxin hormone controls the transport of metals and therefore the absorption of metals by roots. In plant cells, primary and recycling endosomes are not as well characterized as in animal cells. It has been suggested that the TGN of the Golgi apparatus may perform the function of these types of endosomes. However, prevacuolar compartment are generated from TGN of the Golgi apparatus and partially from the endoplasmic reticulum. Plant cells do not show typical lysosomes, and degradative processes occur in lytic or digestive vacuoles. Vacuoles are membrane-bound organelles with a variety of functions (digestion, storaging, maintaining hydrostatic pressure, and many others). They also show different sizes and shapes. Although functionally distinct, vacuoles can fuse between each other to form a large vacuole in differentiated cells. The central vacuole is very important because it regulates the hydric balance of the cell. Lytic vacuoles are similar in function to animal cell lysosomes because they are in charge of degradation processes. Storage vacuoles are needed during seed germination and are involved in the response to certain environment changes. Cell wall In plant cells, there is a membrane-bound organelle present just during cell division. It is known as phragmoplast. It is a cell structure made up of microtubules and vesicles that gives rise the two plasma membranes that separate the two new cells during cytokinesis, and it is also in charge of the formation of the middle lamella of the cell wall, which is built between both plasma membranes. Vesicles come from the two Golgi apparatus, one per new cell, are targeted to an intermediate space between the two mitotic nuclei. In this space, vesicles fuse to form the middle lamella. This is the default pathway for vesicles coming out from the Golgi apparatus during cell division. Bibliography Hawes, C.R., Brandizzi, F., Andreeva, A.V. Endomembranes and vesicle trafficking. 1999. Current opinion in plant biology. 2:454-461. Paez-Valencia J, Goodman K, Otegui MA. 2016. Endocytosis and endosomal trafficking in plants. Annual review in plant biology. 67:309-335. Page 19 Plastids are organelles found in plant and algae cells. They are also found in some marine animals. These organelles arose during evolution after an endosymbiosis process, when a bacterium with photosynthetic abilities, similar to current cyanobacteria, was engulfed by an eukaryote cell, and became an endosymbiont instead of being digested. During evolution, most genes of the engulfed cyanobacteria were transferred to the host nucleus, and endosymbiont became plastids, which developed a diverse set of functions: photosynthesis, amino acid and lipid synthesis, storage of lipids, carbohydrates and proteins, providing color to different parts of the body plant, sensing gravity, regulation of stomata behavior, and many others. Plastids are limited by a double membrane and an intermembrane space (Figure 1). Inside, they contain other membrane limited compartments like thylakoids of chloroplasts and tubules of chromoplasts. Like mitochondria, plastids also contain DNA and the molecular machinery to grow and divide, both processes under the control of the cell nuclear genes. Every plastid in every cell comes from another existing plastid, and they are transmitted to the next plant generation included in gametes during fertilization. In this way, all the plastids of a plant are descendant of the embryo plastids, known as proplastids. Proplastids are also found in meristematic cells of adult plants, where they divide before the cell division to ensure a proper number of proplastids in the two new cells. When cell differentiation begins, proplastids also initiate their own differentiation program, so that different types of mature plastids are generated according to the cell type. They can be leucoplastids (elaioplasts, amiloplastids, and proteoplastids), chloroplasts and chromoplasts. Chloroplasts may also differentiate into other types of plastids and other plastids may differentiate into chloroplasts. It is a bidirectional differentiation path (Figure 1). Figure 1. Types of plastids and differentiation paths (adapted from Jarvis and López-Juez, 2013) 1. Proplastids Proplastids are small plastids, about 1 µm in diameter, and less complex at the structural level than other plastids of the plant. They are colorless, can change their morphology and may content a variable amount of tubular-like internal membranous compartments, as well as starch depots. These features are shared by two types of proplastids: germinal and nodule proplastids. Germinal proplastids are found in plant embryos (in seeds) and meristematic cells. By division and differentiation, they give rise to the rest of the plastids of the plant. They may also carry out some metabolic functions such as gibberellic acid synthesis, which is very important for meristematic metabolism. Nodule proplastids, as the name suggests, are found in the nodules of the roots and are involved in the fixation of nitrogen. Etioplasts, another type of plastids, are found in stems, but not in roots. They are a long-lasting intermediate stage in the way of differentiation from proplastids to chloroplasts under very low intensity of light or darkness. Etioplasts restart the differentiation toward chloroplasts as soon as the light is intense enough. 2. Leucoplasts Leucoplasts are colorless plastids (without pigments), that function as storaging organelles. Leucoplasts comprise amyloplasts, elaioplasts (or oleoplasts), and proteinoplasts. They store starch, lipids and proteins, respectively. In plant cells, amyloplasts synthesize starch (Figure 2). All the stored starch in a cell can be found in plastids as starch granules. Amyloplasts are specialized in this role and contain large depots of starch. Apart from storing starch, mayloplasts are gravity sensors in root cells. Starch granules are denser than water so that amyloplasts fall to the bottom of the cell, and this is what cells need to detect the gravity vector. Amyloplasts are also involved in the metabolism of nitrogen. Elaioplasts are small size plastids containing oil and lipids forming fat drops. In plant cells, there are two synthetic pathways for lipids. The eukaryotic pathway depends on the smooth endoplasmic reticulum, whereas the so-called prokaryotic pathway involves elaioplastids. These two pathways produce different types of lipids. Elaioplast are also involved in pollen maturation. Some plants are able to store lipids in other organelles known as elaiosomes, which are derived from endoplasmic reticulum. Proteinoplasts contain a high concentration of proteins, so high that proteins form crystals or very dense amorphous material. However, it is not clear yet if there is a type of plastids exclusively dedicated to protein storaging in plant cells. 3. Chromoplasts Chromoplasts contain carotenoid pigments that give the red, orange and yellow colors to the plant structure where they are present (Figure 2. These plastids are abundant in flowers, fruits, old leaves, and some roots. It is thought that one of their main function is to attract animals for pollination or for spreading the seeds. Chromoplasts are metabolically active, though they contain fewer DNA copies than chloroplasts. Figure 2. Plastids. Chloroplasts (A and B). A) Photosynthetic parenchyma. A stoma is shown in B). Chromoplasts (C and D) from a tomato. Amyloplasts (E and F) from a potato. Chromoplasts contain lipid drops with carotenoids and complex molecules known as fibrils, which also contain a core of carotenoids. Chromoplasts are differentiated from chloroplasts, as well as from proplastids. During differentiation from chloroplasts, the photosynthetic machinery and thylakoids are degraded and carotenoids are synthesized along with compartments where they are going to be included. These compartments, known as plastoglobules, are lipid drops with abundant triacylglycerides located in the stroma of the plastid. Plastoglobules may be also found in other plastids. Carotenoids, mainly xanthophylls, are also stored in other places of the plastid stroma, so concentrated that they can form filaments or crystals. Chromoplasts develop an internal layered membrane system in the outer part of the stroma. These new membranes arise from the invagination of the inner membrane and do not from the old degraded thylakoids. Carotenoids like lutein, beta-carotenoid, and others, may be also associated to these new membranes. Occasionally, the internal membranes show a reticular arrangement. During chromoplast maturation, the concentration of pigments may be so high that they can form crystals, like beta-carotenoids in the carrot root, or lycopene in tomato. Carotenoids can also be concentrated in tubular structures. Chromoplasts may contain other components such as starch granules and protein aggregates. Although chromoplast have been considered an advanced stage of the chloroplast development, it has been observed that they are able to become chloroplasts again. Some root and fruit tissues may become green again. For example, lemon fruits which are left on the tree change the color from yellow to green, and carrot roots turn green when they are exposed to the light. 4. Chloroplasts Chloroplasts are the most abundant and studied plastids. They will be study in the next page. 5. Another types of plastids During aging and death periods, cells contain gerontoplasts, which are differentiated from chloropasts. Muroplasts are found in glaucocystophyta algae, and contain a vestigial wall of peptidoglycan located between the inner and the outer membrane of the organelle. Type S and T plastids have been found in the sieve cells of the phloem, and may respond to wounds. Rhodoplasts are photosynthetic plastids found in red algae, and contain type a chlorophyll, but not b or c chlorophylls. Rhodoplasts contain thylakoids not arranged in stacks, and also molecular aggregates containing red pigments known as phycobilisomes, which are able to capture the light wavelengths that reach long distances under the sea water. Finally, some animals eat algae and chloroplasts are incorporated into the animal cells instead of being digested. These plastids are able to photosynthesize during months, feeding in this way the animal (for example, Elysia chlorotica). These plastids are known as cleptoplasts. Furthermore, some parasites, like Plasmodium, may contain plastids known as apicoplasts. Bibliography Jarvis P, López-Juez E. 2013. Biogenesis and homeostasis of chloroplasts and other plastids. Nature reviews in molecular and cell biology. 14: 787-802. Ljubesic N, Wrischer M, Devidé Z. 1991. Chromoplasts--the last stages in plastid development. International journal of development biology. 35: 251-258. Wise RR. 2006. The diversity of plastid form and function. In The structure and function of plastids. Springer Netherlands. p. 3-26. Page 20 Chloroplasts are usually large organelles (1 to 10 µm) found in plant cells. A cell from a leaf may contain from 20 to 100 chloroplasts (Figure 1). The shape of chloroplasts is variable, from round to ellipsoid, or much more complex. Chloroplasts are members of the plastids family and contain DNA with around 250 genes derived from the prokaryotic bacterial ancestor. These genes code for ribosomal RNA, transference RNA and messenger RNA. Messenger RNAs are translated in the chloroplast and give proteins needed for organelle growth and division, and for photosynthesis. Chloroplasts produce chlorophyll for catching the light energy. Fogure 1. Chloroplasts of a photosynthetic parenchyma (A), and in the cells of a stoma (B). 1. Morphology Chloroplasts are made up of several compartments (Figure 2). In the periphery, there are an outer membrane, an inner membrane and an intermembrane space between them. Unlike mitochondria, the inner membrane has no folding. Inside chloroplasts there are membrane sacs known as thylakoids, which are usually arranged in stacks referred as granum. Thylakoids of different grana (plural of granum) may have continuous membranes. Proteins in charge of photosynthesis are located in the thylakoid membranes. The space between thylakoids and inner membrane is known as stroma, where DNA, enzymes, ribosomes, and other types of molecules are found. Figure 2. Chloroplasts are composed of outer membrane, inner membrane, intermembrane space between them. The inner space is known as stroma and contains many membrane sacs known as thylakoids, arranged in stacks. 2. Division and movement Chloroplasts need to divide in proliferating cells for getting a right number of chloroplasts and perform a proper photosynthetic activity. Chloroplast division may be synchronized with cell division. This happens in some alga species with cells having just one chloroplast. Chloroplast division usually happens during S phase of the cell cycle, where replication of the DNA takes place. The process is less known in plant species with cells containing many chloroplasts. In some cells, the number of chloroplasts is not related to cell division. For example, in parenchyma cells of leaves, chloroplasts divide to increase their number although the cell is not going to divide anymore. Curiously, the number of chloroplasts is related to the surface of the leaf. In leaf parenchyma cells, it is though that the number of chloroplasts is regulated by the size of the cell. In proliferating cells, the chloroplast population of the mother cell should be split in two more or less equal populations for the two daughter cells. Chloroplasts distribution during mitosis is mediated by actin filaments. The division of chloroplast to increase their number in a cell depends on some proteins synthesized by the nucleus and other synthesized by the chloroplast itself (all proteins involved in mitochondria division are coming from nuclear genes). The division starts with two protein rings, one inner ring composed of chloroplast proteins and an outer ring containing dynamin related proteins coded by nuclear genes. Both rings are connected through transmembrane proteins. These two rings drive the chloroplast division. Changing the position of chloroplasts in the cytoplasm is a cell strategy to get adapted to variable light conditions (Figure 3). The movement of chloroplasts is slow, around 1 µm/min. A high intensity of light may be harmful for chloroplasts and a faint light decreases the photosynthesis activity. Cells of the parenchyma of leaves can move the chloroplasts from the periclinal walls (parallel to the leave surface) to the anticlinal walls (perpendicular to the leave surface). Light intensity is detected by receptors located in the outer chloroplast membrane, but there are plasma membrane photoreceptors as well. There are two types of movements. Under low light intensity, chloroplasts are moved to the periclinal walls, and under high light intensity they are moved to the anticlinal walls (Figure 3). Photoreceptors in plasma membrane triggers the first movement, and those located in the chloroplast are responsible for the second movement. Figure 3. Organization of chloroplasts in the cell of a leaf of A. thaliana (adapted from de Wada and Kong, 2018). The motor drive that move chloroplasts is provided by actin filaments and myosin motor proteins, which are found around the chloroplast in a more or less complex organization. Actin filaments may also be involved in anchoring chloroplasts to the plasma membrane. 3. Functions Photosynthesis Photosynthesis is the main role of chloroplast. It transforms the electromagnetic energy of light into chemical bonds thanks to chlorophyll, ATP synthase (Figure 4), and ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). Photosynthesis is divided in two stages: a light-dependent part, where the energy of light is transformed in a gradient of protons used for producing ATP and NADPH, and a light-independent part (it does not need light but the products generated during the light-dependent stage) where carbon from CO_2 is fixed on phosphorylated carbohydrates by RUBISCO. The first stage of the photosynthesis takes place in the thylakoidal membranes and the second stage in the stroma. Figure 4. Summary of the main steps during the light-dependent stage of photosynthesis. All proteins are transmembrane or associated to the thylakoidal membrane. Protons are moved to the interior of the thylakoidal sac, whereas ATP and NADPH are synthesized toward the stroma. The split of water contributes to the proton gradient by increasing the amount of protons in the interior of the thylakoid. Photosynthesis consists of several steps. a) Photosystem II splits two molecules of water that yield one O_2 molecule and four protons. This reaction releases four electrons that are moved to the chlorophyll molecules of the photosystem II. There, the energy of light raises the energy of these electrons and so they are released from the photosystem II. b) The electrons are caught by a plastoquinone and quickly donated to cytochrome b6/f complex that uses the energy of electrons to enter four protons into the thylakoid. c) Cytochrome b6/f complex gives the electrons to plastocyanin that transfers them to the photosystem I. Here, again, thanks to the energy of light, chlorophyll raises the energy of electrons. Ferredoxin-NADP reductase is associated with the photosystem I and transforms molecules of NADP+ into NADPH, which remains in the stroma. The protons which are removed from the stroma and those that are produced or entered into the thylakoid create altogether a proton gradient across the thylakoidal membrane. This gradient is used by the ATP synthase to produce ATP. Since the catalytic center of the ATP synthase is facing the stroma, the new ATP molecules are synthesized into the stroma. Both NADPH and ATP are eventually used in the Calvin cycle, which is a metabolic chain of reactions where CO_2, ribulose-1,5-bisphosphate, and RUBISCO make possible the fixation of carbon into phosphoglycerate. Another functions Besides photosynthesis, chloroplasts carry out many other salient functions, such as the synthesis of amino acids, nucleotides and fatty acids, the production of hormones, vitamins and other secondary metabolites. They are also involved in the metabolism of nitrogen and sulfur. Nitrate is the main source of nitrogen for plants. The last step to get assimilated nitrogen from nitrate is transforming nitrite into ammonium, which happens in chloroplasts by the nitrite reductase. Nitrite is formed from nitrate. Nitrite and ammonium may be toxic for the cell above a concentration, but not nitrate, which can be transiently stored in vacuoles. Some metabolites produced by chloroplasts are involved in protecting against pathogens and in the adaptation of plants to stress, excess of water, and extreme heat. By releasing hormones, chloroplasts may also influence cell found far away in the body plant. Chloroplasts are in permanent communication with other compartments of the cell, either by sending molecular signals or through direct contact between their membranes. It is relatively frequent to observe physical contacts between the chloroplast membrane and the membranes of the endoplasmic reticulum and mitochondria. However, the most intense communication is with the nucleus, because it contains many genes for proteins that must work inside the chloroplast, some of them needed for photosynthesis. In this regard, nucleus and chloroplast must be well coordinated because they have to work together. Bibliography Wada M, Kong S-G. 2018. Actin-mediated movement of chloroplasts. Journal of cell science. 131. doi: 10.1242/jcs.210310. Page 21 Cells adhere to other cells and to the extracellular matrix by means of adhesion transmembrane proteins. Integrins, cadherins, selectins and immunoglobulins stand out. Sometimes many adhesion proteins join to eacg other, and to other proteins, to form large macromolecular adhesion complexes, generally known as cell junctions. These molecular scaffolds are essential to maintain the integrity of many tissues, such as epithelial, muscular, and nervous tissues. Cell junctions are classified according to their morphology, the type of adhesion molecules they contain, the structures to which they adhere to, and their interactions with the cytoskeleton. Cell junctions were first observed with electron microscopes and were named according to their morphology, but it was the molecular biology techniques that uncover their complex molecular organization. 1. Tight junctions Tight junctions (Figure 1), also known as zonula occludens, are found in the more apical part of the epithelial cells, in cardiac muscle, in the endothelium of the central nervous system, and hepatocytes. They make so strong and tight contacts between adjoining cells that the intercellular space is nearly occluded. Figure 1. Organization of epithelial tight junctions in the intestine. This molecular organization is similar in other epithelia, as well as in the cardiac muscle (modified from Niessen, 2007). In the epithelial cells, tight junction work as a zipper around the cell perimeter. Apart from the strong adhesion, tight junctions carry out other functions. For example, in the intestine epithelium, tight junctions do not allow molecules diffusing through the intercellular space between enterocytes, and are forced to cross through the enterocyte, a much more selective pathway. Furthermore, tight junctions maintain the polarity of the epithelial cells because they form a physical barrier, so that they prevent lateral diffusion of membrane molecules, both proteins and lipids. It is a physical barrier for lateral diffusion in the plasma membrane. Thus, an apical plasma membrane domain is established with a particular set of molecules, different from the laterobasal domain. This is essential to properly direct molecules from the intestine lumen toward the blood vessels: enter the enterocyte through the apical domain and exit through the basolateral domain. Tight junctions are composed of more than 40 types of proteins. Transmembrane proteins are the claudins, a family of proteins known as occludins, and the JAM proteins (Junctional Adhesion Molecules). Claudins are transmembrane proteins in charge of making the cell-cell adhesion, and between the adhesion points there is a very narrow extracellular space (about 1 nm) that allows ions to travel between the cells. There are twenty types of claudins that form passages with different sizes. Cells can change the expression of claudin types, thus regulating the permeability through the intercellular space. Occludins are not really necessary for tight junctions, but they provide stability to the cell junction and help to create a better barrier. JAM proteins establish intercellular connections, but they appear to be more important in stabilizing the macromolecular scaffold. The intracellular domain of these proteins is associated with other molecules known as ZO (zonula occludens), which in turn are connected to actin filaments and other cytosolic proteins. Depending on the adhesion state of the cell, these intracellular molecular interactions may trigger signaling pathways that affect the cell physiology. An interesting observation is that the occurrence of tight junction in cells may need the presence of adherent junctions. 2. Adherent junctions Adherens junctions (zonula adherens) are cell junctions between epithelial cells. They are found in the apical part of the cell, just below tight junctions. During development, adherent junctions are the first cell junction to appear in epithelia, before than tight junctions. They are thought to be involved in morphogenetic processes. Like tight junctions, adherent junctions form a belt-like structure all around the cell. The extracellular domains of E-Cadherins and nectins are involved in the cell-cell contacts, whereas their intracellular domains are linked to several molecules like β- y α-catenins, catenin p120 and afadin. These proteins are intermediaries between adhesion molecules and actin filaments. Furthermore, β-catenin may enter the nucleus and change gene expression. Adherens junctions are sequentially assembled. First, junctions mediated by nectins are established, which form rather weak adhesions, and then cadherins are recruited to form stronger and more stable contacts between cells. In some cellular types, adherent junctions initiate the formation of tight junctions. Occludins, together with their protein scaffold, are first recruited by the adherent junctions, and ZO proteins may have a relevant role in this process. Tight junctions, adherent junctions and desmosomes are needed for the integrity of the epithelial layer. But only the adherent junctions are needed for the coordinated movements of groups of epithelial cells through the epithelial layer, which is a relatively frequent movement. The cell-cell adhesions made by adherent junctions build a connected net of actin filament bundles from different cells that join together a cell population. These connected cells behave coordinately, for example, to cover a wounded region. 3. Desmosomes Desmosomes (macula adherens) make spot-like cell-cell adhesions, like rivets (Figure 2). They are very abundant in epithelial and muscle tissues, but can also be found in virtually every tissue, for example in the nervous tissue. Cadherins (desmoglein and desmocollin) are the adhesion proteins in desmosomes. The intracellular domains of desmosome cadherins are associated to intermediate filaments through intermediary proteins. Figure 2. Organization and composition of desmosomes (modified from Huber, 2003). 4. Hemidesmosomes Hemidesmosomes and focal adhesions make adhesion contacts between cells and the extracellular matrix (Figure 3). Both contain integrins as the adhesion molecule. Hemidesmosomes are junctions between epitelial cells and the basal lamina (a type of extracellular matrix). Intracellularly, the cystolic domain of these integrins are associated to intermediate filaments. Although hemidesmosomes look like one half of a desmosome, they are compose of different molecules. Focal adhesions are smaller in size and make junctions between cells and extracellular matrix by means of integrins, which are attached intracellularly to actin filaments. Figure 3. Organization of a hemisdesmosome (modified from Hahn 2001) It is common to include gap junctions within the cell junctions group. Gap junctions are molecular channels located in the plasma membrane that allow the direct communication between the cytoplasm of adjoining cells. Every channel, known as connexon, is made up of 6 connexins, and makes possible the diffusion of small molecules between both cytoplasms. That is why gap junctions should be regarded as cell-cell communication molecular complexes instead of involved in cell-cell adhesion. Gap junctions Bibliography Campbell HK, Maiers JL, DeMali KA. (2017). Interplay between tight junctions and adherent junctions. Experimental cell research 358: 39-44. Hahn B-S, Labouesse M. (2001). Tissue integrity: Hemidesmosomes and resistance to stress. Current biology 11:R858-R861. Huber O. (2003). Structure and function of desmosomal proteins and their role in development and disease. Cell and molecular life science. 60:1872-1890. Ladoux B, Mege RM. (2017). Mechanobiology of collective cell behaviours. Nature reviews in molecular cell biology. 18:743-757. Niessen CM. (2007). Tight junctions/adherent junctions: basic structure and function. Journal of investigative dermatology. 127:2525-2532. Page 22 The nucleus is the characteristic structure of eukaryotic cells. It contains DNA and all the molecular machinery to produce RNA, by a process known as transcription. Typically, cells contain only one nucleus, but there are cells with more than one, such as osteoclasts, and some cells may contain many of them as the skeletal muscle fibers or the epithelial cells of some invertebrates. The shape of the nucleus is variable and is usually adapted to the morphology of the cell (Figure 1). Typically, the nucleus is rounded, but other forms are also found, as in neutrophils, which contain multi-lobulated nucleus. The location of the nucleus is normally in the center of the cell, but it can also be found in peripheral locations. For example, secretory cells have their nucleus situated in the basal part, and skeletal muscle cells have their nuclei close to the plasma membrane. Figure 1. Different nuclear shapes. A. Epithelial cells from human gall bladder with rounded to elongated nuclei . B. Monocyte from blood smear showing a kidney-shaped nucleus. C. Neutrophils from blood smear containing a multi-lobulated nuclei. D. Longitudinal view of a skeletal muscle fiber with many nuclei, all of them located toward the periphery (arrows). Figure 2. The size and shape of the nucleus depends on the cell type, despite that nearly all the nuclei of an individual contain the same amount of DNA. Neurons and glial cells are shown in this picture. Nuclei of neurons contain more loose chromatin, whereas glia nuclei have highly condensed chromatin, and therefore the size is much smaller. Although the amount of DNA is almost the same in all the cell nuclei of an organism, the size of the nucleus is variable depending on the cell type (Figure 2). Furthermore, cells from species with different amount of DNA may show a similar nuclear size. These data indicate that the size of the nucleus is adapted to the size and the physiology of the cell, but does not depend closely on the amount of DNA. There are two nuclear compartments: nuclear envelope and nucleoplasm (Figure 3). Nuclear envelope is the peripheral part of the nucleus that separates the interior of the nucleus, known as nucleoplasm, from the cytoplasm, and consists of an outer membrane, an inner membrane and a perinuclear space (between both membranes). Nucleoplasm-cytoplasm communication is regulated by the nuclear pore complexes, which are molecular scaffolds inserted in the nuclear envelope. The nuclear lamina is a layer of proteins lining the inner surface of the inner membrane of the nuclear envelope, which provides mechanical support to the nucleus. Figure 3. Components of the nucleus. In the nucleoplasm, DNA and associated proteins form the chromatin, which is referred as heterochromatin if it is highly condensed or as euchromatin if it is loosely packaged. Decondensation of chromosomes after mitosis results in chromatin, and each chromosome decondensates in specific regions of the nucleoplasm. The nucleolus, a distinct chromatin domain, is also found in the nucleoplasm, as well as other dense structures known as nuclear bodies, which are composed of chromatin and other proteins. In this section of the atlas, we could go into all the molecular processes involved in DNA transcription and replication, as well as gene regulation. In the future, a new section may be opened to deal with these topics. We will be focused in some features of the nuclear morphology and physiology, and encourage the reader to visit other excellent sites related to genetics. Bibliography Edens LJ, White KH, Jevtic P, Li X, Levy DL. 2013. Nuclear size regulation: from single cells to development and disease. Trends in cell biology 23:151-159. Guo T, Fang Y. . 2014. Functional organization and dynamics of the cell nucleus. Frontiers in plant biology. vol 5: 378. doi: 10.3389/fpls.2014.00378 . Page 23 This page content 1. Components 2. Function 3. Mitosis 4. Nuclear position In the late XIX century, a barrier limiting the nucleus was suggested, which was later confirmed by electron microscopy (Figure 1). The nuclear envelope is composed of two membranes performing a number of functions: a) it is a physical barrier that separates nucleoplasm (chromatin and the rest of the molecular content of the nucleus) from cytoplasm; b) it controls the communication between both, that is the movement of molecules between the nucleoplasm and cytoplasm; c) it is in charge of the nuclear morphology; d) it contributes to the inner organization of the nucleus providing the anchoring points where chromatin is attached. Nuclear envelope is connected to the cytoskeleton, microtubules and actin filaments, to set the position of the nucleus in the cell. Figure 1. Electron microscopy image of the nuclear envelope. 1. Components Figure 2. Organization of the nuclear envelope. It is composed of outer membrane, intermembrane space (perinuclear space), inner membrane, and nuclear lamina. The outer membrane is continuous with the endoplasmic reticulum membrane. Nuclear pores are another component of the nuclear envelope. The nuclear envelope is composed of two membranes, outer and inner membranes, and an intermembrane space (25-40 nm in height) between them, also known as perinuclear space. All together, they form the so-called perinuclear cisternae (Figure 2). The outer membrane is continuous with the membrane of the endoplasmic reticulum. This membrane continuity communicates the lumen of endoplasmic reticulum and the lumen of perinuclear cisternae. Thus, the nuclear envelope may work as a calcium-storing compartment, along with the endoplasmic reticulum. Attached ribosomes to the outer membrane are observed. The inner membrane has a distinct molecular composition. For example, there are transmembrane proteins linked to chromatin and to the nuclear lamina, another component of the nuclear envelope (see below). The inner and outer membranes are continuous at the nuclear pore complexes insertion places. How is it possible that outer and inner membranes have different molecular composition? A selective retaining molecular mechanism in the inner membrane has been suggested. Transmembrane proteins are synthesized in the outer membrane of the nuclear envelope or in the rough endoplasmic reticulum, and arrive to the inner membrane by lateral diffusion (thanks to the membrane continuity), but those that become linked to chromatin or to the nuclear lamina are retained in the inner membrane of the nuclear envelope. In animal cells, the nuclear lamina is a molecular scaffold located between the inner membrane and chromatin. In mammals, nuclear lamina is around 20 - 25 nm thick. The main components of nuclear lamina are proteins known as laminas, with two isoforms: type A (A and C laminas, which are the result of the alternative splicing of the same mRNA, i.e., the same gen) and type B (B1 and B2/B3 laminas). All of them are members of the intermediate filaments family. They are organized in a net lining the inner surface of the inner membrane of the nuclear envelope, linking the inner membrane and the chromatin. The attachment between the inner membrane and the nuclear lamina is mediated by at least 20 different types of proteins inserted in the inner membrane. Nuclear lamina performs multiple functions. It contributes to keep the organization of the nuclear envelope, and therefore the shape and size of the nucleus. The nuclear morphology changes when the expression of proteins of the nuclear lamina is altered, which can be observed during embryo development, cell differentiation and some cell pathologies. Nuclear lamina is also a place for anchoring the nucleus to cytoskeleton, allowing to place the nucleus in a precise location within the cell, as well as moving the nucleus from one place to another. This connection is mediated by proteins inserted in the membranes of the nuclear envelope. The spatial distribution of nuclear pore complexes in the nuclear envelope is also influenced by the nuclear lamina. Another function of nuclear lamina is to provide physical support for chromatin, which affects gene expression. For example, chromatin anchored to nuclear lamina is not usually transcribed. These chromatin anchored regions are different depending on the cell type and differentiation state of the cell. It is suggested that nuclear lamina-chromatin interactions are regulatory elements of gene expression. During mitosis, the nuclear envelope should be disassembled and assembled again. This process is mediated by enzymatic action (phosphorylation) over the laminas that causes breakdown of nuclear lamina, so that microtubules can contact with chromosomes. Pathological alterations of laminas result in the so-called laminopathies causing nuclear disorganization, weaker nuclear envelope structure, and eventually cell death. Nuclear pores are inserted in the nuclear envelope. They are in charge of the trafficking between the cytoplasm and nucleoplasm (see next page). 2. Function Since it involves quite amount of resources, why do eukaryotic cells need to separate the DNA from cytoplasm? Some of the reasons are the following: a) Gene stability. Confining the genome inside a compartment helps to maintain the stability of genes, which is higher than in prokaryotes; it should keep in mind that there is a huge amount of DNA. b) Gene regulation. Separation of the genome from the cytoplasm allows gene regulation at a level that prokaryotes will never reach. For example, it prevents or allows the access of transcription factors to DNA. Transcription factors are proteins synthesized in the cytoplasm that regulate gene expression. They must cross the nuclear envelope to work on the DNA. The molecular mechanisms that allow a transcription factor to enter the nucleus is usually the result of a chain of molecules, which may start with the activation of a receptor located in the plasma membrane. If some step of this chain is stopped, the gene will not be expressed. c) Eukaryotic genes contain exons and introns, meaning that a maturation process (cutting and splicing) of the primary mRNA is needed. It is dangerous to translate an unprocessed mRNA because it will produce malformed proteins, which may even cause pathologies. This mRNA processing takes place in the nucleoplasm and only mature mRNA is allowed to cross the nuclear envelope. d) Transcription and translation take place in separate compartments (nucleoplasm and cytosol, respectively) and provide an additional tool for regulating the flux of information from DNA to proteins. In this way, the transcription of a gene into mRNA does not mean immediate translation. For example, preventing a particular mRNA to cross the nuclear envelope means that the cell will not get the protein right away. However, when needed, the synthesis of this protein will be very quick because the mRNA was already synthesized and to cross the nuclear envelope is the only remaining step for the mRNA to be translated in the ribosomes. 3. During mitosis In most eukaryotic cells, the nuclear envelope breaks in little vesicles during the mitotic prophase. It is known as open mitosis because cytosolic microtubules can gain access and make contact with chromosomes. Once chromosomes are segregated, nuclear envelope is assembled again during telophase from the membranes of the endoplasmic reticulum to form the nuclei of the two new cells (Figure 3). In yeasts, however, the integrity of the nuclear envelope is maintained and new nuclei are formed by strangulation, as it occurs during cytokinesis. This is because yeasts are able to build a mitotic spindle inside the nucleus. This known as closed mitosis. Figure 3. Assembling of the nuclear envelope during telophase. Membranes of the nuclear envelope are derived from membranas of the endoplasmic reticulum. Chromatin and inner membrane of the nuclear envelope are linked through transmembrane proteins (modified from Wanke and Kutay, 2013). 4. Nuclear position The nucleus can be found in different places of the cytoplasm depending on the cell type, and on the cell activity and physiology. Sometimes the nucleus is passively displaced by other components of the cell like large lipid droplets of adipocytes and myofibrils in skeletal muscle cells, both cell types having the nucleus close to the plasma membrane. In general, the nucleus is actively placed in a specific region of the cytoplasm by the interaction of the cytoskeleton with the nuclear envelope, largely by actin filaments and microtubules, but intermediate filaments can participate as well. In animal cells, the nuclear envelope may be connected with the centrosome, which drags the nucleus when is moved along the microtubules. Microtubules can also contact directly with the nuclear envelope. Movement is the result of the activity of motor proteins associated to the cytoskeleton, although short distant movements may be a consequence of the polymerization and depolymerization of cytoskeletal filaments. Proteins located in the membranes of the nuclear envelope are intermediaries between cytoskeleton and the nuclear lamina. Speeds ranging from 0.1 and 1 µm/min have been recorded. The faster movements, 10 µm/min, are those of the pronuclei of zygotes after fertilization. There are several protein complexes linking the nuclear lamina to the cytoskeleton. They include transmembrane proteins in the outer membrane, KASH proteins (nesprins in mammals), and in the inner membrane, SUN proteins, of the nuclear envelope. There may be more proteins involved in making these cytoskeleton-nuclear envelope bridges (Figure 4). Figure 4. Interactions between the nuclear envelope and cytoskeleton that affect the movement, position, and size of the nucleus in the cytoplasm (modified from Starr and Fridolfsson, 2010, and Wilhelmsen et al., 2006). In mammals, there are 5 genes coding for KASH proteins, some of them showing mRNA alternative splicing that can generate quite long protein isoforms exceeding 800 kDa. KASH proteins have very long molecular processes that extend into the cytosol and contact the cytoskeleton. SUN proteins are linked to KASH proteins in the perinuclear space (between the two membranes of the nuclear envelope), and to laminas (components of the nuclear lamina) by their nucleoplasmic molecular domain. Bibliography Gundersen GG, Worman HJ. 2013. Nuclear positioning. Cell. 152: 1376-1389. Guo T, Fang Y. 2014. Functional organization and dynamics of the cell nucleus. Frontiers in plant biology. 5: 378. doi: 10.3389/fpls.2014.00378 Rowat AC, Lammerding J, Herrmann H, Aebi U. 2008. Towards and integrated understanding of the structure and mechanics of the cell nucleus. BioEssays 30: 226-236. Starr DA, Fridolfsson HN. 2010. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. Annual review of cell developmental biology. 26: 421-444. Wanke C, Kutay U. 2013. Enclosing chromatin: reassembly of the nucleus after open mitosis. Cell 152: 1222-1225. Wilhelmsen K, Ketema M, Truong H, Sonnenberg A. 2006. KASH-domain proteins in nuclear migration, anchorage and other processes. Journal of cell science 119: 5021-5029. Page 24 This page contents 1. Regions 2. rRNA 3. Ribosomal subunits Nucleolus is a distinct structure observed at light microscopy after standard histological staining (Figure 1). It is a nuclear compartment containing highly dense chromatin and a number of associated proteins. In the nucleolus, ribosomal RNA (rRNA) is synthesized and ribosomal subunits are assembled. It was described by F. Fontana in 1781. Cells usually have more than one nucleolus, and the total number depends on the cell type, differentiation state and physiology of the cell. Mammal cells may contain from 1 to 5 nucleoli. The size of the nucleolus may change along with the cell activity, sometimes becoming larger than 1 µm in diameter. During interphase, several nucleoli may join together to form a larger one. In general, cells having intense protein synthesis show larger nucleoli. It is also larger in large cells and in growing cells. However, in some cells, like sperm, the nucleolus is not visible. The nucleolus disappears during mitosis, allowing chromatin to be packaged into chromosomes. Although the nucleolus is not always visible, and disappears in some cell cycle phases, a cell without nucleolus is regarded as dead or is dying. Figure 1. Motoneurons in the rhombencephalon of a lamprey. Nucleolus is observed as a dark dot in the nucleoplasm (arrows). The nucleolus disappears during the mitotic prophase, allowing the reorganization of chromatin to make up the chromosomes. Both, nucleolar chromatin and proteins are distributed and packaged in different chromosomes. During telophase, nucleolar chromatin decondensates and gather nucleolar proteins to form new nucleoli. For a nucleolus to be formed, besides gathering chromatin and proteins, nucleolar activity must be initiated: transcription and splicing of pre-45S ribosomal RNA, and ribosomal subunits assembling. 1. Regions Figure 2. Fibrillar center is where the genes for pre-rRNA 45S are being expressed. Dense fibrillar component is where pre rRNA 45S is located and processed. Granular component is where ribosomal proteins and the rRNAs, result from the processing of the pre-45S rRNA, are assembled into ribosomal subunits. At electron microscopy, several regions can be morphologically distinguished in the nucleolus: fibrillar center, dense fibrillar component, which surround the fibrillar center, and the granular component (Figure 2). The fibrillar center, or nucleolar organizer, is not present in all eukaryotes and its function is no fully understood. It contains DNA segments with many copies of the gen for pre-45S RNA, the primary ribosomal RNA transcript that gives 3 of the 4 rRNA for constituting the ribosomal subunits. The fibrillar center also contains many associated proteins. It has been suggested that the transcription process occurs at the transition between the fibrillar center and the dense fibrillar component area. In the fibrillar component area, the pre-45S rRNA is cut into small pieces. In the granular center the resulting rRNA segments are further processed and assembled into the ribosomal subunits. 2. Ribosomal RNA Genes for pre-45S rRNA are repeated in some regions of different chromosomes. These regions are known as NOR (nucleolar organizer centers) and are located in heterochromatic regions (condensed chromatin) (Figure 3). Nucleolus is formed by the activity of these regions. The number of copies of the gene for pre-45S rRNA varies. In yeasts, there are from 100 to 300 repeated sequences, while amphibians and plants may have thousands of copies. Humans and mice have 200 copies. However, only part of these genes are usually transcribed to pre-45S rRNA (about 50 % of them in humans) at the same time. The proportion of copies of the pre-45S rRNA gene simultaneously transcribed is higher when cells are in need for high amount of proteins. Figure 3. Regions of the nucleolus. NOR regions of the 5 human chromosomes are depicted in the upper part. 28S, 18S and 5.5S rRNAs result from 45S rRNA processing. 5S rRNA comes from other region of the nucleus.. Why so many copies of the pre-45S rRNA are needed? Most proteins are synthesized from only one single gene. This is the case for erythrocyte hemoglobin and myoglobin of muscles. These proteins are abundant because many mRNAs are transcribed from the same gene, and each mRNA is translated many times into proteins. More than 1000 proteins can be synthesized from translations of one single molecule of mRNA. So, we have two amplification steps, transcription (many copies of mRNA from one gene) and translation (many proteins from one mRNA molecule). When the final product of the expression of a gene is not a protein, but an RNA molecule, the second amplification step is missing. A single eukaryote cell may contain a huge amount of ribosomes and all of them are made up of rRNAs and therefore it needs many rRNAs. One copy of a gene for all the rRNAs may be not enough. Cells solve this problem by having many copies of the same gene. Figure 4. Processing of pre-45S rRNA, transcribed from NOR regions, in the nucleolus. There are two genes that code for rRNAs: one for pre-45S rRNA and another for 5S rRNA. In humans, the 200 copies of the gene for pre-45S rRNA are distributed in 5 chromosomes. RNA polymerase I transcribes these copies. It has a high affinity for the promoters that helps to increase the number of pre-45S rRNAs. The copies of the gene for pre-45S rRNA are gathered together at some places in the nucleoplasm to form the fibrillar center of the nucleolus (Figure 4). 5S rRNA is part of the large subunit of the ribosome. There are around 2000 copies of the gene for 5S rRNA, which are transcribed by type III RNA polymerase. However, the gene copies for 5S rRNA are not part of the nucleolus. pre-45S rRNAs have to be cut and trimmed into other smaller rRNAs that will be components of the ribosomal subunits (Figure 4). This processing ends up with three new rRNAs: 18S rRNA, 28S rRNA, and 5.8S rRNA. As it was mentioned earlier, 5S rRNA is synthesized in other region of the nucleoplasm. The long pre-45S rRNA molecules are continuously produced and processed in the nucleolus. The large ribosomal subunit contains 5.8S, 28S, and 5S rRNAs, and the small ribosomal subunit only contains 18S rRNA. 3. Assembling ribosomal subunits Assembling the ribosomal subunits is a striking process. First, the transcription of genes for ribosomal proteins occurs outside the nucleolar region (Figure 5). Then, the mRNAs cross the nuclear envelope thorough the pore complexes and are translated by free ribosomes in the cytoplasm. These new ribosomal proteins enter into the nucleus and reach the nucleolus, where they are associated with the rRNAs to form a complete ribosomal subunit, either the small or the large subunit. Once assembled, ribosomal subunits cross the nuclear envelope toward the cytoplasm, where they start working in translation. In this way, the nucleolus is a consequence of the presence of many molecules and processes going on at the same place and at the same time: ribosomal genes being transcribed to produce pre-45S rRNA, proteins involved in the processing of these transcripts, ribosomal proteins to form ribosomal subunits, plus proteins involved in subunit assembly. Altogether, it is estimated that around 690 different proteins are more or less permanently associated with the nucleolus. Figure 5. Trafficking of ribosomal components between the cytoplasm and the nucleoplasm. There are non-permanent proteins in the nucleolus. Many proteins remain in the nucleolus for short periods of time and then can spread through the rest of the nucleoplasm. However, they stay longer in the nucleolus. Since not all these proteins are related to the synthesis of ribosomes, it is thought that the nucleolus develops additional functions. For example, there are proteins in the nucleolus involved in processing other non-ribosomal RNAs such as small nuclear RNAs, some participate in several steps of the tRNA processing, there are also kinases and there are also proteins for repairing the DNA. Bibliography Kressler D, Hurt E, Baßler J. 2017. A puzzle of life: crafting ribosomal subunits. Trends in biochemical sciences. 42: 640-654. Mangan H, Gail MO, McStay B. 2017. Integrating the genomic architecture of human nucleolar organizer regions with the biophysical properties of nucleoli. The FEBS journal. 284: 3977-3985. Tsekrekou M, Stratigi K, Chatzinikolaou G. 2017. The nucleolus: in genome maintenance and repair. International journal of molecular sciences. 118: 1411. Page 25 This page contents 1. Components 2. Transport 3. Chromatin The nuclear envelope consists of and outer membrane, an inner membrane, and a perinuclear space between them. At some points, the inner and the outer membranes are fused in a way that form open channels that make possible a direct communication between cytoplasm and nucleoplasm. Nuclear pore complexes are located in these channels of the nuclear envelope. They are very large protein aggregates, so big that can be observed with the electron microscope (Figure 1). They are the communication gates between nucleoplasm and cytoplasm since all the molecular trafficking between these two compartments occurs through the nuclear pore complexes. Controlling this traffic is vital for the cell. For example, the entrance of

transcription factors into the nucleus influences the expression of particular genes, and the exit of mRNA makes it possible the synthesis of proteins. Thus, nuclear pores are a key element during cell physiology. Figure 1. Transmission electron microscopy image of the nuclear envelope. The two constrictions of the nuclear envelope are nuclear pore complexes. Nuclear pore complexes are very abundant in cells showing an intense trafficking between nucleoplasm and cytoplasm, as in differentiating cells. It is estimated around 11 nuclear pore complexes per µm2 of nuclear envelope, which means around 3000 to 4000 nuclear pores in a single cell. In the cell cycle, new nuclear pore complexes are synthesized and assembled during interphase, previously to mitosis. However, they are also synthesized after cell division. In open mitosis, where the nuclear envelope is disorganized, the nuclear pore complexes are also disorganized and their proteins are freed in the cytoplasm. After mitosis, these nuclear pore proteins are grouped again in new nuclear pore complexes while the nuclear envelope is assembled at the same time. 1. Components Proteins of the nuclear pore complexes are known as nucleoporins. In yeasts, 30 different nucleoporins have been found, whereas in metazoa may be more than 40 different nucleoporins. There are several copies of each nucleoporin in the same nuclear pore complex. Thus, in mammals, a nuclear pore complex contains around 500 to 1000 nucleoporins. The total structure is 100 to 150 nm wide, 50 to 70 nm high, and contains an inner hydrophilic passage of around 40 nm. Nuclear pore complexes are among the largest protein structures of the cell, about 125000 kDa. It is remarkable that nucleoporins show a great stability, sometimes as long as the whole life of the cell, whereas the lifetime of a common protein may last a few days. Nucleoporins are grouped in 8 blocks, which are organized as a regular octagon forming ring-like structures (Figure 2). The cytoplasmic ring faces the cytoplasm, the radial ring is in the channel of the nuclear envelope and anchors the nuclear pore complex to the nuclear envelope, and the nuclear ring is facing the nucleoplasm. Furthermore, there are fibrils extending from each of the 8 blocks: cytoplasmic fibrils and intranuclear fibrils. The intranuclear fibrils are connected to the nuclear ring by one of their ends and to other proteins that form another intranuclear ring known as distal ring. Intranuclear fibrils and distal ring together form the nuclear basket, also known as nuclear cage. Figure 2. Protein organization in a nuclear pore complex (modified from Beck et al., 2007) Besides the structural role, nucleoporins are classified according to their function. There are transmembrane proteins for anchoring the whole complex to the membrane of the nuclear envelope. The scaffold proteins form the rings, and inner proteins that form the hydrophilic passage and regulate the molecular trafficking through the nuclear pore complex. Those proteins that form the fibrils and nuclear cage recognize the molecules that are then allowed to cross the nuclear passage. It should be noticed that molecules coming in and going out of the nucleus do no need to cross any membrane, but just the hydrophilic channel of the nuclear pores. 2. Nucleo-cytoplasm transport The nuclear pore complex contains a hydrophilic passage ranging from 80 to 90 nm in diameter. When a nuclear pore complex is at rest (no trafficking) the usable space is about 45 to 50 nm in diameter, and it can be increased when some molecule is being transported. Small molecules (less than 60 kDa) like salts, nucleotides, small molecules and short polypeptides can cross freely through the hydrophilic channel, but other larger molecules with physiological roles are not allowed to go across freely. Even some molecules smaller than 20-30 kDa such as histones, tRNAs and small mRNAs may need the involvement of nucleoporins to cross the nuclear envelope. The selective transport, mediated by nucleoporins, is known as passive facilitated transport. Energy is not needed for it. The nuclear pore complex does not determine the direction of the transport, getting in or out the nucleus, but molecules travel according to a gradient of the Ran proteins (Figure 3). The energy is spent in generating this Ran gradient. Figure 3. Ran gradient between the cytoplasm and nucleoplasm. In the cytoplasm, the energy needed to create this gradient is supplied by ATP, transforming Ran-GDP in Ran-GTP. Thus, the nucleoplasm is a sink of Ran-GDP and a source of Ran-GTP. In the cytoplasm, Ran-GTP is converted into Ran-GDP. Thus, the cytoplasm is a source of Ran-GDP and a sink of Ran-GTP. In this way two gradients are created, Ran-GDP and Ran-GTP. The size of the icons in the figure depicts levels of concentration. Trafficking through a nuclear pore complex is high, with more than 1000 translocations per second. This movement of molecules across the nuclear envelope is regulated by the gradient of Ran proteins. Ran are involved in both importing and exporting molecules between the nucleus and the cytoplasm. They generate the Ran-GTP/Ran-GDP needed for the transport, and the generation of these gradients consumes ATP. Ran molecules can be in three states: Ran-GTP, Ran-GDP and Ran. The state of a Ran molecule depends on several enzymes. In the nucleoplasm, Ran-GTP is more abundant, whereas Ran-GDP is concentrated in the cytoplasm (Figure 3). Karyopherins are a family of proteins, divided in two subfamilies: importins and exportins, which are responsible for selecting the molecules that can cross the nuclear pore complex. Proteins that need to be imported into the nucleus have a particular amino acid sequence, known as entrance signal peptide, and those that need to be exported to the cytoplasm have an exit signal peptide. These short peptide sequences are not identical for all proteins, but similar. The entrance and exit signal peptides are recognized by importins and exportins, respectively. There are members of importins and exportins with different affinity for the import and exit sequences. Nucleoporins do not interact directly with the transported molecules, but with importins and exportins. Importin and exportin use the Ran-GTP/Ran-GDP gradients for transporting the cargoes in a specific direction. In this way, importins spontaneously joins to their cargoes in the cytosol, but once in the nucleoplasm cargoes are released by Ran-GTP, which is abundant inside the nucleus. On the other hand, exportins need Ran-GTP to join to their cargoes in the nucleoplasm, but once they (exportin-cargo-Ran-GTP) are translocated to the cytoplasm, Ran-GTP is converted into Ran-GDP, which breaks the complex, and then exportin, cargo and Ran-GDP are detached from each other becoming free in the cytosol (Figure 4). Figure 4. Molecular trafficking across the nuclear pore complex mediated by karyopherins: importins and exportins. Molecules to be specifically transported across the nuclear envelope need to have export or import sequences, but it is not enough. These sequences need to be accessible to exportins and importins. Conformational changes or chemical modifications of the molecules having these sequences may prevent their recognition by karyopherins, so they remain in the nucleoplasm or cytoplasm until the sequence is properly exposed. This mechanism adds a new step of regulation to nucleus-cytoplasm trafficking. Besides proteins, other molecules need to cross the nuclear envelope. Different types of RNA are transported by different mechanisms, but there are always proteins involved. mRNA is not transported naked. Once the mRNA transcript is synthesized, it is quickly associated with different nuclear proteins forming a large size molecular complex known as ribonucleoprotein. Before mRNA is transported, a quality control checks if it has been correctly processed. If there are any errors, the mRNA is degraded and it does not reach the cytoplasm. If there are no flaws, the ribonucleoprotein is transferred to the nuclear basket of the nuclear pore complex and gets associated with the proteins Nxf1-Nxt1, which facilitate its transport through the hydrophilic channel. This transport does not use the Ran-GTP gradient. However, it needs the hydrolysis of ATP, which is consumed when the macromolecular association (ribonucleoprotein-Nxf1-Nxt1) needs to be disengaged in the cytosolic face of the nuclear pore complex. Then, ribonucleoprotein is free from Nxf1-Nxt1 and cannot get back to the nucleus. Some mRNA molecules uses the protein CRM1 to be transported. In this case, the Ran-GTP gradient is needed. Transfer RNA (tRNA) is recognized by a type of exportin known as exportin-t, which uses the Ran-GTP gradient to get the tRNA out from the nucleus. Small nuclear RNA (snRNA), another type or RNA, is transported by CRM1 protein and Ran-GTP gradient. The exporting mechanism for ribosomal subunits, which are assembled in the nucleolus, is a big challenge for nuclear pore complexes because they are really large protein-rRNA complexes. The molecular changes happening in the nuclear pore complex allowing the ribosomal subunits to get to the cytoplasm is not known so far. Finally, it has been shown that some proteins are able to bind directly to nucleoporins, so they do not need karyopherins. 3. Chromatin interactions Nuclear pore complexes are involved in additional functions that help in the regulation of the nucleus-cytoplasm communication. In this context, they are thought to participate in DNA repairing and in the regulation of transcription, processing and quality control of mRNA. At transmission electron microscopy, it is observed that heterochromatin is not distributed near the nuclear pore complexes (Figure 1). Thus, they are regarded as spots of permissive gene expression of many genes. This would be an advantageous position for those genes since their transcribed mRNAs are released close to the gateway. The lack of heterochromatin near the nuclear pore complexes may be a consequence of direct interactions between nucleoporins and chromatin. Nucleoporins are evolutively related to COPI and COPII proteins, which form the coated vesicles coming from the Golgi apparatus and endoplasmic reticulum, respectively. It makes sense because the nuclear envelope and endoplasmic reticulum membranes are continuous, and Golgi apparatus membranes are formed from vesicles coming from the endoplasmic reticulum. Bibliography Beck M, Lucic V, Forster F, Baumeister W, Medalia O. 2007. Snapshots of nuclear pore complexes in action captured by cryo-electron tomography. Nature 449:611-615. Cautain B, Hill R, de Pedro N, Link W. 2015. Components and regulation of nuclear transport processes. FEBS journal. 282: 445–462. Carmody SR, Wente SR. 2009. mRNA nuclear export at a glance. Journal of cell science. 122:1933-1937. Guo T, Fang Y. 2014. Functional organization and dynamics of the cell nucleus. Frontiers in plant biology. 5: 378. doi: 10.3389/fpls.2014.00378. Kabachinski G, Schwartz TS. 2015. The nuclear pore complex – structure and function at a glance. Journal of cell sciences. 128, 423–429. Page 26 Proteins are responsible for many of the functions carried out by membranes. The protein-lipid ratio may vary in different membranes, but it is roughly 1:40 in number of molecules and 40 % in weight, which means that membranes usually have a lot of proteins. The proportion of proteins may be even higher in those membranes with strong metabolic involvement such as the inner membrane of mitochondria. About 1/3 of our genes code for membrane proteins. Cell membrane models Membrane models depicting the molecular organization have been modified to reflect the importance of proteins as essential elements for the membrane structure and function. The basal model to build from is the fluid mosaic by Singer and Nicholson. In this model, proteins are scattered in the membrane and can move freely. However, nowadays, the interactions of proteins with both intra- and extra-membrane molecules that restrict the lateral movements have been included in the model. Furthermore, data from atomic force microscopes suggest changes in the models of membranes to include the proportion of proteins and the physical space they occupy (Figure 1). Figure 1. Membrane model after atomic force microscopy (modified from Zhao et al., 2014) Proteins are numerous and diverse, specifically distributed through the cell membranes. They can be classified in different ways. Regarding their function, they can be receptors, enzymes, adhesion molecules, channels, transporters, pumps, membrane handlers, and many others. Some proteins may be included in more than one category. Regarding on how proteins are arranged in membranes, there are two main groups of proteins: integral and associated. 1. Integral proteins Integral proteins are permanent components of the membrane. They may be transmembrane proteins, proteins spanning just one monolayer, or proteins that are chemically linked to a membrane molecule. Transmembrane Figure 2. Types of transmembrane proteins. There are transmembrane proteins, such as glycophorin, with an amino acid chain that spans once the cell membrane, whereas others, such as many receptors, can have several crosses. In these examples, the amino acid chain intermingled among the fatty acids shows an alpha helix arrangement. Aquaporin is also a transmembrane protein forming a channel with several crosses, but the amino acid chain among fatty acid shows a beta organization (modified from Pollard et al., 2007). Transmembrane proteins show three molecular domains: two hydrophylic and one hydrophobic (Figure 2). For instance, plasma membrane proteins have an extracellular, an intra-membrane and a cytosolic domains. The intra-membrane domain contains sequences of hydrophobic amino acids that are located among the fatty acid chains of the membrane lipids, whereas the extra and intracellular domains contain hydrophilic amino acids. Transmembrane proteins proteins are mostly synthesized in the endoplasmic reticulum and distributed to other cell membranes by vesicular traffic. Some transmembrane proteins have amino acid sequences that span the membrane one time, whereas others do it more times, up to 7 crosses. Although most transmembrane proteins work alone, many of them need to be associated to other transmembrane proteins to perform their functions working as oligomeric groups. The functions of transmembrane proteins are diverse. a) Integrins, cadherins, and selectins are adhesion proteins. b) Pumps and ion channels generate and modify ion gradients between both sides of the membrane. For example, to produce ATP, or to exchange ions, such as calcium, sodium, and potassium. c) Transporters make possible large molecules to cross the membrane. For instance, they allow glucose to get get into the cell crossing the plasma membrane. d) Receptors enable cell communication by binding specific ligands, such as hormones, growth factors, neurotransmitters, and others, and transducing these signals into intracellular downstream molecular events. This can be done because transmembrane proteins have two hydrophilic domains, located at either sides of the membrane, which are connected by one or several intramembrane domains. For example, after the recognition of an extracellular signal, a molecular rearrangement occurs in the transmembrane protein that is transmitted to the intracellular domain, which in turn causes a cytosolic molecular process, sometimes ending up with changes in gene expression. Included or linked to one monolayer Figure 3. Main types of peripheral membrane proteins: integral and associated. From left to the right: proteins with a part of the amino acid chain spanning just one monolayer, proteins linked to glycolipids, proteins linked to fatty acid chains inserted among the membrane lipids, proteins associated to the membrane lipid heads by electric affinity and proteins associated to hydrophilic domain of transmembrane proteins (modified from Alberts et al., 2002). There are integral proteins which are not transmembrane proteins. Some of them have part of the amino acid sequence inserted in only one of the monolayers of the membrane, so they have one intramembrane domain and one external domain (Figure 3). If they are in the plasma membrane, depending on the monolayer where they are integrated. The external domain may be cytosolic or extracellular. Another type of integral proteins includes those chemically anchored to other membrane molecules, mostly glycolipids or fatty acids inserted among the membrane lipids. In these cases, the entire protein is either cytosolic or extracellular. Most commonly, they are bound to GPI (glycosylphosphatidylinositol). These proteins were discovered in the seventies of the XXth century and today we know hundreds of them in all eukaryotic cells studied so far. They include, for example, enzymes, adhesion molecules, and components of cellular layers in protozoas. It is interesting that in some some cases, like the adhesion protein N-CAM, the same protein may be transmembrane or GPI-linked protein depending on mRNA differential splicing. The non-trasnmembrane protein may also be known as peripheral proteins, because they are related to only one monolayer. Peripheral proteins also include other proteins referrred as associated protein (see below) 2. Associated proteins Associated proteins are not permanently joined to membranes, i.e. they are not integral proteins. Instead of chemical bonds, electrical interactions, such as van der Waals forces, keep these proteins stuck to the hydrophilic surfaces of membranes (Figure 3). These forces are weak and proteins may switch between be attached to or detached from membranes. Associated proteins are hydrosoluble. Bibliography Zhao W, Tian Y, Cai M, Wang F, Wu J, Gao J, Liu S, Jiang J, Jiang S, Wang H. 2014. Studying the nucleated mammalian cell membrane by single molecule approaches. PLOSone. 9 (5):e91595.

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