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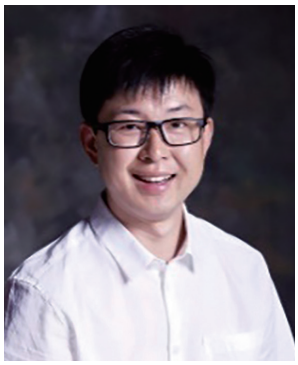
http://www.genelab-bch.com.cn/



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溶酶体相关细胞器: 生物发生与功能

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摘要 溶酶体相关细胞器是真核动物细胞中功能特异的细胞器, 其装配、成熟及运输过程需要借助内体–溶酶体运输途经。该文总结了具有代表性的四种溶酶体相关细胞器(黑素小体、血小板致密颗粒、大致密核心颗粒及Weibel-Palade小体)的结构、功能、生物发生的分子细胞机制, 为更深入了解溶酶体相关细胞器的生理和病理意义提供参考。

关键词 溶酶体相关细胞器; 白化病; 内体–溶酶体运输; 囊泡运输

Lysosome Related Organelles: Biogenesis and Functions

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Abstract LROs (lysosome-related organelles) are a class of organelles with specific functions in eukaryotic cells, and their assembly, maturation, and trafficking processes take advantage of the endo-lysosomal pathways. This review summarizes the molecular cellular mechanisms of the structure, function, and biogenesis of four representative LROs (melanosomes, platelet dense granules, large dense-core vesicles, and Weibel-Palade bodies), providing some cues for a better understanding of the pathophysiological roles of LROs.

Keywords lysosome-related organelles; albinism; endo-lysosomal trafficking; vesicle trafficking

溶酶体是真核细胞中由单层生物膜包被的细胞器, 主要功能是降解生物合成途径及细胞内吞作用摄入的大分子[1-2]。溶酶体作为最终降解场所, 依靠内部的酸性环境以及多种水解酶执行大分子的水解功能。溶酶体大小、形态、密度、运动、酶类组成多样, 并处于同其他细胞器互作的动态变化之中。因此, 溶酶体通常被定义为包含酸性水解酶、溶酶体相关蛋白(lysosomal-associated membrane proteins, LAMPs), 并缺乏甘露糖-6-磷酸受体(mannose-6-phosphate receptors, MPRs)的酸性细胞器[2-3]。ZHU等[4]利用细胞器膜片钳技术对膨大的溶酶体进行代谢组学鉴定, 并根据代谢相关内容物的种类将溶酶体分为五个类群, 其中包括负责降解外源物质的内吞溶酶体(endo-lysosome)以及降解细胞内物质的自噬溶酶体(autolysosome)。此外, 多数细胞中还有具有分泌功能的溶酶体, 其又称分泌型溶酶体(secretory lysosome), 甚至形态上也不容易与降解型溶酶体区分[5]。由此可见, 精确定义处于连续变化过程中的溶酶体十分困难。

值得关注的是, 真核细胞中存在很多种具有细胞特异性的细胞器(表1~表3)。它们既享有内体和溶酶体的诸多特性, 又具有独特的形态特征与内容物, 以此实现特异的生物学功能[6]。无论从形态学还是细胞影像学角度, 这些细胞器都容易辨认。这些在眼睛和皮肤的色素细胞、血细胞、血管内皮细胞、免疫细胞、肺泡上皮细胞、精子、破骨细胞等特殊细胞中形成的细胞器, 绝大多数会被分泌到胞外或者递送到邻近细胞发挥功能[6]。传统观点认为这些细胞器来源于早期或晚期内体(early or late endosome), 即与溶酶体生成有着相似的分选途经, 因此得名溶酶体相关细胞器(lysosome-related organelles, LROs)[3]。这一概念沿用了近20年, 但是随着对多种LRO研究的深入, 基于对LRO的超微结构多样性、生物发生、成熟和分泌途经多样性的认识, 逐渐揭示了LRO复杂的生物学本质, LRO的概念也开始被重新审视[6]。

本文旨在总结目前研究较多的四种LRO[包括色素合成细胞中的黑素小体(melanosome)、血小板及巨核细胞中的致密颗粒(platelet dense granule, PDG)、神经细胞及分泌细胞中的大致密核心颗粒(large dense-core vesicle, LDCV)、血管内皮细胞中的Weibel-Palade小体(Weibel-Palade body, WPB)]的基本生物学特征(图1)。我们将从结构特征和生物学功能入手, 介绍LRO发生、分选、成熟的生物学过程及其与相关疾病发生的关系。在本文的最后, 我们对新出现的研究方法及LRO研究的关键科学问题及研究方向进行总结和展望。

这四种LRO的装配过程可以根据发生的起源不同分为两类: 黑素小体和DGs被认为主要来源于多囊泡体(multivesicular body, MVB); 而WPBs和LDCV被认为主要来源于高尔基体反面膜囊(*trans*-Golgi network, TGN)[7](图2)。但无论起源于何种细胞器, LRO前体总能通过膜泡融合(vesicle fusion)机制获得成熟过程所需的膜组分(膜蛋白及膜脂)以及可溶性组分, 即货物(cargo), 同时通过膜泡分裂(vesicle fission)机制排出需要分选(sorting)、回收(recycling)或者降解(degradation)的组分。LRO前体与内体–溶酶体(endo-lysosomal)运输途径相互作用, 使LRO获得运输以及分泌相关的关键蛋白复合物。这种货物的分选及运输机制通常由多条途径介导, 确保不同的货物可以到达同一个LRO。另外货物间的先后顺序与协同作用对于LRO的成熟也是至关重

表1 已发现的在脊椎动物存在的LROs[6]

Table 1 Identified LROs in vertebrates[6]

脊椎动物LRO Vertebrates LRO	细胞类型 Cell type	Rab27	CD63	相关疾病 Related diseases
Melanosomes	Melanocytes or melanophores in skin, retinal pigment epithelia and choroid	√	√	HPS, CHS, GS
Weibel palade bodies	Endothelial cells	√	√	HPS
Cytolytic granules	Cytotoxic T cells, natural killer cells	√	√	HPS, CHS, GS, FHL
Dense granules	Platelets, megakaryocytes	√	√	HPS, FHL
Basophilic secretory granules	Mast cells, basophils	√	√	CHS
Lamellar bodies	Alveolar type II cells	√	√	HPS
Phagosomes	Macrophages, neutrophils, dendritic cells	√	√	HPS
MHC class II compartments	Dendritic cells, B lymphocytes, macrophages, langerhans cells	?	√	CHS
Alpha granules	Platelets, megakaryocytes		√	GPS, ARC
Azurophil (primary) granules	Neutrophils, eosinophils	√	?	HPS, CHS
$NOX2^+$ inhibitory lysosomes	Dendritic cells	√	?	GS
Acrosomes	Sperm	√	?	GS
Large dense-core vesicles	Specialized secretory cells (e.g. adrenal chromaffin cells)	√	?	HPS, FHL
IRF7 signaling lysosomes	Plasmacytoid dendritic cells	?	?	HPS
Notochord vacuoles	Notochord inner cell	?	?	HPS

HPS: Hermansky-Pudlak综合征; CHS: Chediak-Higashi综合征; GS: Griscelli综合征; FHL: 家族性噬血细胞性淋巴组织细胞增生症; √: 已确定; ?: 未确定。

HPS: Hermansky-Pudlak syndrome; CHS: Chediak-Higashi syndrome; GS: Griscelli syndrome; FHL: familial haemophagocytic lymphohistiocytosis; √: identified; ?: unidentified.

表2 已发现的在脊椎动物潜在的LROs[6]

Table 2 Putative LROs in vertebrates[6]

潜在的LRO Putative LRO	细胞类型 Cell type	Rab27	CD63	相关疾病 Related diseases
Melanocore-containing organelle	Epidermal keratinocytes	?	√	?
Secretory MVEs	Most cell types, model organisms	√	?	?
Fusiform vesicle	Urothelium	√	?	?
Osteoclast secretory lysosome	Osteoclast	√	?	?
Specific (secondary) granules	Neutrophils	√	?	?
Gelatinase (tertiary) granules	Neutrophils	√	?	?
Lamellar bodies lamellar granules	Epidermal keratinocytes	?	?	ARC
Surfactant production and storage organelles	Teleost swim bladder epithelium	?	?	HPS
Presynaptic vesicles	Neuron synaptic cleft	√	?	?
Pathogen-containing phagosomes or vacuoles	Various host cells	?	?	HPS ?
Non-acidic late endosomes	Neurons (axons, dendrites)	?	√	?

HPS: Hermansky-Pudlak综合征; CHS: Chediak-Higashi综合征; GS: Griscelli综合征; FHL: 家族性噬血细胞性淋巴组织细胞增生症; √: 已确定; ?: 未确定。

HPS: Hermansky-Pudlak syndrome; CHS: Chediak-Higashi syndrome; GS: Griscelli syndrome; FHL: familial haemophagocytic lymphohistiocytosis; √: identified; ?: unidentified.

要的[8]。

有关LRO生成的研究多来自对HPS综合征(Hermansky-Pudlak syndrome)和CHS综合征(Chediak-Higashi syndrome)的机制研究[9-10]。目前已经发现11种人和鼠共有的*HPS*致病基因以及另外4种小鼠*HPS*基因[10]。针对这15个基因的功能研究发现，他们的编码产物参与装配了多个货物运输必需的复合物: 衔接蛋白复合体(adaptor protein complex,

表3 已发现的在无脊椎动物中存在的LROs[6]

Table 3 Identified LROs in invertebrates[6]

无脊椎动物LRO Invertebrates LRO	对应的组织 Organism	疾病模型 Disease model
Pigment granules	*Drosophila* melanogaster retinal cells	HPS
Zinc storage granules	*Drosophila melanogaster* Malpighian tubule epithelial cells	HPS
Gut granules	*Caenorhabditis elegans* intestinal cells	HPS
Post-lysosomes	*Dictyostelium discoideum*	CHS
Mucocysts	*Tetrahymena thermophila*	HPS
Riboflavin granules	*Bombyx mori* Malpighian tubules	HPS
Integument urate granules	*Bombyx mori* epidermal cells	HPS

HPS: Hermansky-Pudlak综合征; CHS: Chediak-Higashi综合征; GS: Griscelli综合征; FHL: 家族性噬血细胞性淋巴组织细胞增生症; √: 已确定; ?: 未确定。

HPS: Hermansky-Pudlak syndrome; CHS: Chediak-Higashi syndrome; GS: Griscelli syndrome; FHL: familial haemophagocytic lymphohistiocytosis; √: identified; ?: unidentified.

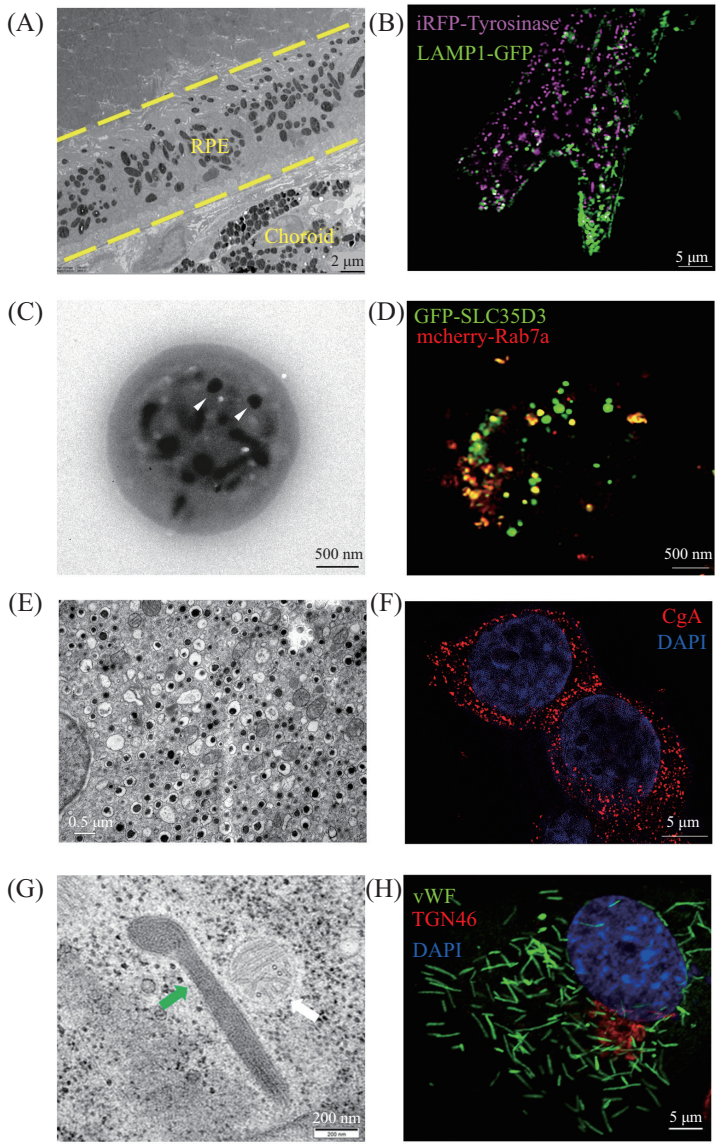
APC)、溶酶体相关细胞器发生复合体(biogenesis of lysosome-related organelles complex, BLOC)、同型融合蛋白分选复合物(homotypic fusion and protein sorting complex, HOPS)。APC是一类异源四聚体接头蛋白复合物, 他们能够识别和结合货物蛋白在胞质结构域的分选信号, 双亮氨酸基序(dileucine motif)和酪氨酸依赖的分选信号(tyrosine-based sorting signals), 使货物在特定膜区域内聚集, 同时招募外被蛋白(coat protein, CP)或其他辅助蛋白来产生运输载体, 促进货物运送至目的地[11]。APC共分5种: 其中只有AP-1、AP-2和部分AP-3使用网格蛋白(clathrin)作为涂层; AP-2主要参与细胞质膜上clathrin介导的内吞作用; 其他的AP介导TGN和内体–溶酶体之间的蛋白分选。多物种的遗传学分析表明, AP-3在LRO的货物蛋白分选中发挥重要作用。AP-3是稳定的异源四聚体, 由δ、β3、μ3和σ3等4个亚基组成, 编码这些亚基的基因在真核生物进化过程中高度保守; 在哺乳动物的不同组织中β3、μ3和σ3亚基存在变体: β3A、μ3A和σ3A亚基在大多数组织和细胞中普遍表达, β3B、μ3B和σ3B亚基的表达局限于神经元细胞并参与突触囊泡内的货物蛋白分选[12]。BLOC包含BLOC-1、BLOC-2、BLOC-3等3种复合物, 每种复合物包含不同的亚基: 其中BLOC-1含有BLOS1、BLOS2、BLOS3、cappucino、muted、pallidin、snapin和dysbindin等8个亚基; BLOC-2含有HPS3、HPS5和HPS6等3个亚基; BLOC-3含有的2个亚基分别是HPS1和HPS4[9]。HOPS的VPS33A亚基调控SNARE复合物形成并驱动膜融合, 可能介导了含有货物蛋白的运输囊泡与MVB的融合。

1 4种代表性LRO的结构、功能与生物发生

1.1 黑素小体的结构与功能

黑素小体是黑色素细胞装配的一种LRO, 用以合成、贮存以及运输黑色素(melanin)[13-14]。古脊椎动物的黑素小体可以追溯到石炭纪至上新世。圆口动物、鱼类、蛙类、蜥蜴、鱼龙类、蛇颈龙类、龟类、翼龙、被羽和非被羽类恐龙、鸟类以及哺乳类脊椎动物化石中均可发现黑素小体[15-16]。与现存的脊椎动物相似, 古脊椎动物的黑素小体存在于上皮、毛囊、腹内膜、耳蜗、虹膜、间脑以及其他组织[17]。因此, 黑素小体具有保守且多样的生物学功能。黑素小体的形态具有较强的物种特异性以及组织特异性[18]。人和小鼠皮肤中的黑素小体从前黑素小体(premelanosome)到成熟的黑素小体(mature melanosome)要经过四个时期, 最终会形成装载了黑色素的橄榄形细胞器(长×短≈0.5 μm×0.2 μm), 进而被转运到角质细胞(keratinocyte)中发挥功能[14,19-20]。

人的黑色素细胞的主要功能是通过装配黑素小体, 合成真黑素(eumelanin, Eu)及褐色素(pheomelanin, Pheo)两类色素[14]。由于黑色素对紫外线的吸收作用, 角质形成细胞内的黑色素可以保护细胞免于紫外线灼伤以及DNA突变; 而视网膜色素上皮(retinal pigmented epithelium, RPE)细胞中的黑色素使视网膜免于光氧化毒性的伤害。黑素小体数量、体积、排列方式以及Eu的含量对皮肤、毛发以及虹膜颜色有决定性作用。黑色毛发中的毛囊黑色素细



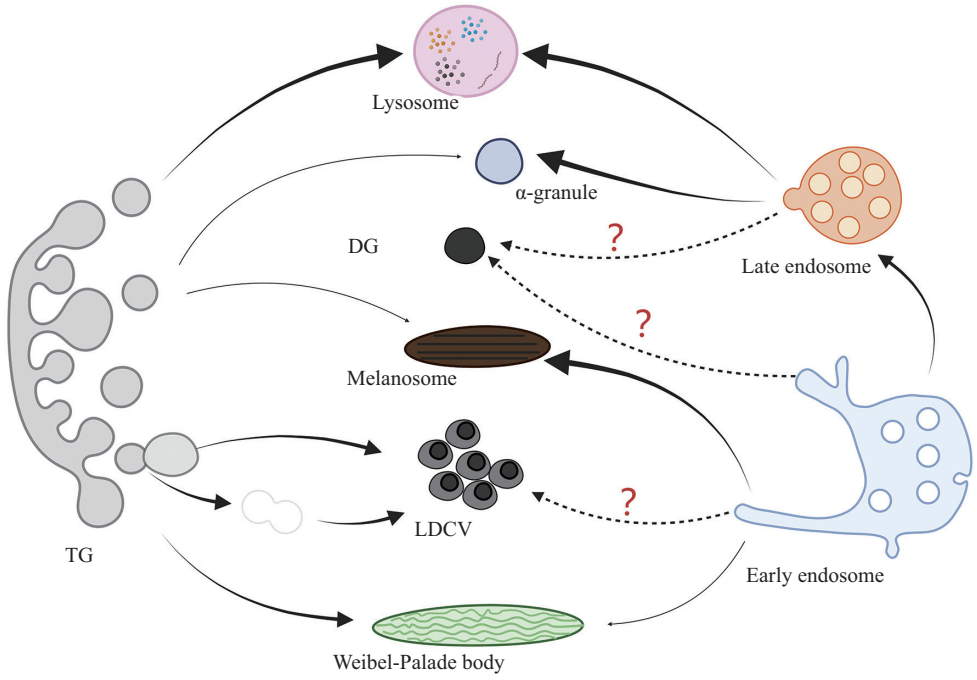
A: 小鼠视网膜(RPE)及脉络膜(choroid)中黑素小体的电镜图; B: 小鼠黑色素瘤细胞B16中远红外荧光蛋白标记的酪氨酸酶(iRFP-Tyrosinase)以及绿色荧光蛋白标记的溶酶体相关膜蛋白(LAMP1-GFP)的定位; C: 小鼠血小板whole mount电镜图; D: 分化后的人巨核细胞MEG-01中远红外荧光蛋白标记的Rab7a(endo-lysosome定位)以及绿色荧光蛋白标记的SLC35D3(DG定位)的细胞器定位; E: 肾上腺髓质嗜铬细胞瘤的细胞中大致密核心颗粒透射电镜照片; F: 大鼠PC-12细胞经免疫荧光标记CgA(红色)及DAPI标记的细胞核(蓝色); G: 人脐带静脉内皮细胞HUVECs中成熟WPB(绿色箭头)和未成熟WPB(白色箭头)的电镜图; H: HUVECs经免疫荧光标记vWF(绿色)、TGN(红色), 和DAPI标记的细胞核(蓝色)。

A: electron microscopic images of melanosomes in the mouse retina (RPE) and choroid; B: localization of far-infrared fluorescent protein labeled tyrosinase (iRFP-Tyrosinase) and green fluorescent protein labeled lysosomal associated membrane protein (LAMP1-GFP) in mouse melanoma cell B16; C: whole mount electron microscopy of mouse platelets; D: Rab7a (endo-lysosome localization) labeled with far-infrared fluorescent protein and SLC35D3 (DG localization) labeled with green fluorescent protein in differentiated human megakaryocytes MEG-01; E: electron microscopy images of large dense core particles of adrenal medullary pheochromocytoma cells; F: rat PC-12 cells were immune-labeled with CgA antibody (red) with the nucleus stained with DAPI (blue); G: electron microscopy of mature WPB (green arrow) and immature WPB (white arrow) in human umbilical vein endothelial cells (HUVECs); H: HUVECs were immunofluorescence stained with vWF (green), TGN (red), and DAPI stained with nuclei (blue).

图1 四种代表性溶酶体相关细胞器的透射电镜(TEM)及结构化照明显微图像(SIM)

Fig.1 Transmission electron microscopy images (TEM) and structural illumination microscopy (SIM) images of four typical LRO

胞拥有数量最多、Eu含量最高的黑素小体; 棕色毛发黑素小体体积较小, 但也以Eu为主; 金色毛发真黑素合成不足; 而红色毛发主要积累Pheo, 且呈现不规则簇状分布[17,21-23]。昆虫、鱼类、两栖类以及鸟类的外被颜色一方面由不同的色素种类(化学色)决定, 另一方面还由色素体的排列方式(结构色)决定, 以实现包括颜色呈现与求偶、警戒与保护色、保暖功能在内的生物学行为[16]。黑色素能够结合金属离子,



溶酶体的发生过程既需要高尔基体反面膜囊(TGN)也需要晚期内体(late endosome)的物质运输。而LRO的组分来源各有偏好, 例如α颗粒主要来自晚期内体, 而黑素小体的发生主要来自早期内体(early endosome), 致密颗粒来源可能兼而有之。黑素小体的成熟还需要TGN的物质运输。图中粗箭头显示的是主要生成途经, 小箭头显示的是次要生成途经, 但两者均必不可少。问号及虚线箭头代表未确定。大致密核心颗粒主要来自TGN, 其在TGN膨大处发生, 后结合分选膜泡的同型融合机制才能成熟。Weible-Palade小体也是主要来自TGN, 但成熟过程还需要早期内体递送物质。

The process of lysosome formation requires materials transported from both the TGN (*trans* Golgi network) and the late endosomes. The assembly of LRO have different routes, such as α-granules mainly come from late endosomes, melanosomes mainly come from early endosomes, and dense particles may come from both. The maturation of melanosomes also requires the transport of TGN cargos. The thick arrow in the figure shows the main biogenesis path, while the small arrow shows the secondary biogenesis path. Note that both pathways are essential. The question mark represents unconfirmed pathways. On the other side, large dense core vesicles mainly come from TGN, which occurs at the budding part of TGN, undergoes homotypic fusion mechanism of sorting membrane bubbles and becomes mature. The Weible-Palade bodies derive mainly from TGN, but their mature process also requires delivery of cargoes from the early endosomes.

图2 四种代表性溶酶体相关细胞器生物发生模式图

Fig.2 Biogenesis models for four representative LROs

具有维持金属离子稳态及免疫调节功能[24-25]。与体表的黑色素不同, 体内细胞中合成的黑色素, 例如在黑质致密部(substantia nigra pars compacta, SNpc)的儿茶酚胺能神经元(catecholaminergic neurons)中合成的神经黑色素(neuromelanin, NM), 是由Eu和Pheo混合组合而成的。NM的主要作用体现在两个方面, 一是在NM合成过程中会消耗氧化多巴胺, 起到保护神经元的作用, 二是NM通过螯合铁离子调节细胞内氧化还原状态, 防止氧化应激反应以及铁死亡发生[26-27]。黑质致密部神经元的丢失将会导致帕金森病(Parkinson's disease, PD)的发生。

色素合成的减少和丧失会导致白化现象(如咖啡色熊猫七仔、白虎、白鳄鱼、白色乌鸦等)的出现。包括高加索人在内的白色人种也是一种色素降低的现象, 以适应人类从低海拔区域向高海拔地区迁徙过程中日照强度降低的环境改变。现在的观点认为如果白化累及视觉功能则可被诊断为白化病, 如眼皮肤白化病(oculocutaneous albinism, OCA)、眼白化病(ocular albinism, OA)以及综合征型白化病[HPS(Hermansky-Pudlak syndrome)及CHS(Chediak-Higashi syndrome)][28-29]。此外, 黑色素细胞异常增殖会导致黑色素瘤(melanoma), 黑色素在黑色素瘤增殖时增加而在发生转移时减少。值得注意的是, 具有黑色素瘤家族史的人群患帕金森病的风险要比无家族史的人群高2倍, 而且帕金森病人群患恶性黑色素瘤的几率较非帕金森患者高0.5~2.0倍。白人种患此两种疾病的概率要比其他有色人种高[30-32]。暗示这两种既往看似不相关的疾病在色素代谢环节可

能存在某种内在联系。已报道黑色素瘤与帕金森病的关联基因存在诸多交集，但二者与色素合成主要信号通路的关系尚未建立[33]。因此，探寻色素合成的分子细胞机制对于我们了解白化病这种罕见病的发生具有重要意义，并且通过深入探究白化病的致病机制将可能有助于我们理解黑色素瘤与帕金森病等疾病的发生机制。

1.2 黑素小体的发生与成熟机制

黑素小体是研究的最早且最为透彻的LRO[34-35]。黑素小体的生物发生过程可以依据形态学分为四个时期：I期黑素小体类似早期内体系统中的MVB，包含一些内部的小泡(intraluminal vesicle, ILV)，但在一侧或者双侧包被了致密的网格蛋白。这个被称作平板晶格(planar lattices)的胞质侧平板结构表现出内陷曲度，且不具备出芽(budding)的能力。与MVB不同的是，I期黑素小体内会积累弱碱类物质，表明其内部具有弱酸性[36]。ILV为I期黑素小体向II期转变提供起始装配位点，跨膜糖蛋白PMEL在经过分选途径递送到I期黑素小体后，被Ca^{2+}依赖的酸性水解酶切为由二硫键连接的两个部分。二硫键打开前，成熟的PMEL与ILV结合，构成纤维状条纹结构的装配起始位点。而二硫键打开后，C末端疏水区可能发生降解，留下N末端部分继续参与条纹状结构的装配。纤维条纹是PMEL通过β折叠形成的淀粉样蛋白聚集体(amyloid)，也是II期黑素小体的标志性结构[37-40]。条纹状结构既是合成黑色素的场所，又是使黑素小体获得橄榄形结构的支撑骨架。PMEL敲除小鼠Eu合成减少，II期黑素小体中的条纹状结构消失，且成熟的黑素小体不再是橄榄形，而是近球形[41]。因此，PMEL也被认为是黑素小体发生的驱动力(driving force)。I期黑素小体在GRP143(G protein-coupled receptor 143)的协助下分选II期黑素小体组分以及晚期内体组分。而GPR143突变导致眼白化病1型(OA-1)，这也是目前唯一已知的眼白化病类型。GPR143是一个非常特殊的G蛋白偶联受体，主要定位在晚期内体以及黑素小体上。GPR143的功能一方面是抑制PMEL等货物向溶酶体的转运，另一方面是通过与转运必需内体分选复合体(endosomal sorting complex required for transport, ESCRT)相互作用，促进货物向MVB转运[42-43]。GPR143缺失或突变会导致黑素小体数量减少、体积增大、细胞内分布改变[44]。

黑素小体的成熟过程除了PMEL形成的纤维骨架、关键的合成酶以及转运复合物之外，还需要离子通道和转运体蛋白、磷脂合成酶与脂蛋白、小G蛋白家族、可溶性NSF连接蛋白受体(soluble NSF attachment protein receptor, SNARE)蛋白家族，以及运输相关蛋白复合体。这些组分的鉴定工作得力于眼皮肤白化病、LRO相关疾病分子机制的研究。例如，酪氨酸酶(tyrosinase)突变导致OCA-1，氯离子通道OCA2突变导致OCA-2，酪氨酸酶相关蛋白1(tyrosinase-related protein-1, TYRP1)突变导致OCA-3，H^+/糖转运蛋白SLC45A2突变导致OCA-4，K^+-依赖Na^+/Ca^{2+}反向转运蛋白NCKX5/SLC24A5突变导致OCA-6，多巴色素异构酶(DOPAchrome tautomerase, DCT)突变导致OCA-8。OCA5的致病基因至今没有克隆，OCA7(LRMDA)被证明是一个跨膜蛋白，并参与PMEL加工以及黑素小体的pH调节[45]。目前已知的8种眼皮肤白化病亚型中有4个基因产物直接参与黑素小体内离子稳态调节。我们最近的研究显示，定位于黑素小体的双孔通道2(two pore channel 2, *TPC2*)发生突变(R210C)，导致Ca^{2+}/Na^+通透能力增强，使得黑素小体pH下降，抑制色素的生成[46]。*TPC2*-R210C有别于已知的隐性遗传方式的OCA与OA，携带该突变的小鼠表现为显性遗传，并呈现明显的剂量效应。携带该突变的患者由于并未表现出眼睛结构性改变与视觉功能减退，因此无法被确诊为OCA或OA，我们提出*TPC2*-R210C是皮肤白化病(cutaneous albinism, CA)的致病基因。TPC2多态性及功能增强与皮肤毛发颜色减退存在相关性[47]，但是作为致病基因的R210C目前只有我们一例临床报道。另外，TPC2的小分子激动剂可以恢复神经细胞溶酶体贮积症的表型，而TPC2缺失突变的黑色素瘤细胞增殖、迁徙与浸润的能力都会减弱，提示TPC2可能成为探索色素合成、神经退行性疾病以及肿瘤发生内在关系的切入点[48-49]。

当II期黑素小体内的条纹结构成熟后，黑色素的合成就开始了。因此，在III期黑素小体中会发现变粗、变黑的条纹结构。III、IV期黑素小体内离子成分会发生变化。酪氨酸酶需要结合Cu^{2+}和Zn^{2+}才能发挥功能，这就需要Cu^{2+}和Zn^{2+}的转运蛋白被提前运输到黑素小体上。另外，黑素小体内pH会逐步升高，以适应酪氨酸酶最适酶活性的要求。排出黑素小体腔内的H^+存在多种方式，质子泵(V-ATPase)以及多种离子/底物转运体对黑素小体腔内pH具

有的调节作用, 在此不再赘述[50]。离子转运蛋白如OCA2以及SLC45A2均是在III期开始才被递送到黑素小体的, 说明这些蛋白的分选机制不同于PMEL。现在认为这些货物主要依靠I期黑素小体分裂出的内体组分, 通过出芽形成的管状和球状管状循环内体定向投送到III期黑素小体[51]; 另外一种分选途径来自TGN, 例如带有DCT以及MART-1(melanoma antigen recognized by T cell-1)的囊泡从TGN出芽后直接递送到III期黑素小体[52]。这三种分选递送途径存在先后关系, 且运送不同货物的囊泡之间需要隔离机制。

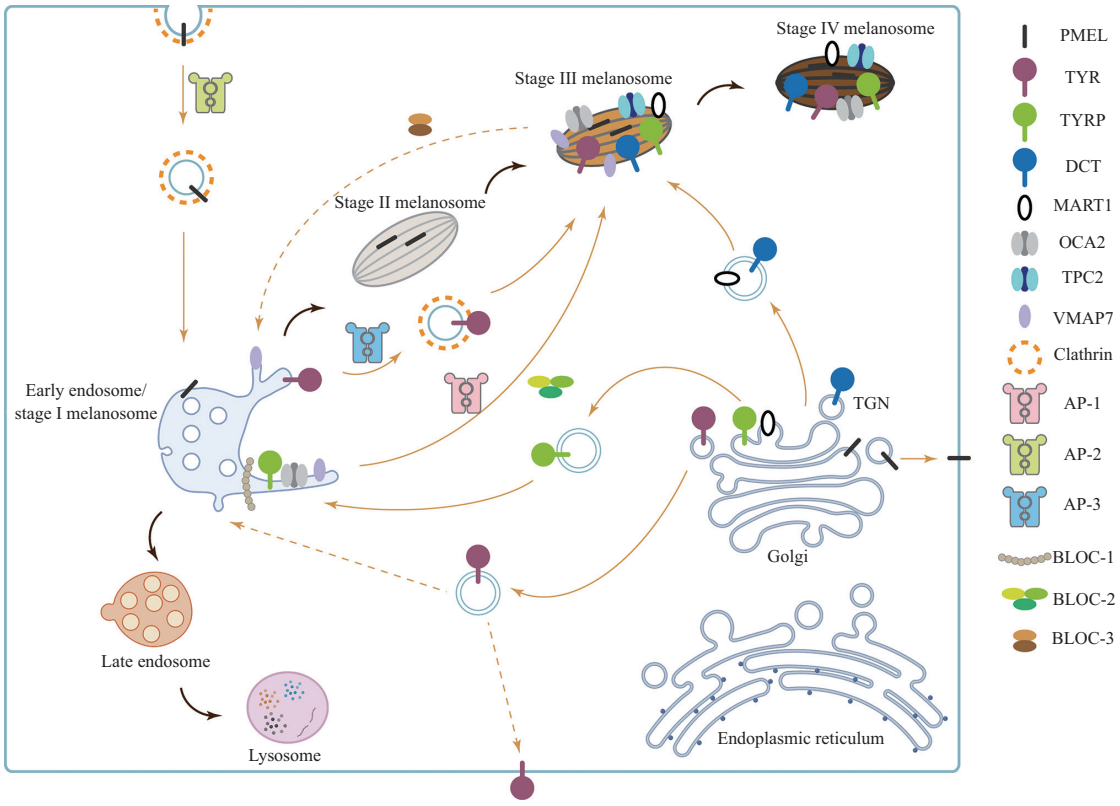
HPS相关蛋白对这一过程进行了精密地调控, 确保黑色素在特定的时间和地点合成, 避免由于氧化中间产物泄露造成的细胞毒性。黑素小体成熟过程中大部分的货物分选是由APC完成的。五种APC中的三种, AP-1、AP-2、AP-3参与黑素小体的分选。其中AP-2负责分选细胞质膜上的货物到内体上, 而AP-1和AP-3主要对TGN和内体上的货物进行分选[19]。BLOC-1被认为参与管状循环内体的形成, BLOC-1复合物的八个亚基中的任何一个发生突变都会导致整个复合物的崩解[6], 从而导致TYRP1、OCA2、ATP7A等货物在I期黑素小体中储积, 最终造成色素合成减少。BLOC-2复合物被认为参与管状循环内体到III期黑素小体的递送作用。BLOC-2的三个亚基突变对应HPS3、5、6三个亚型, 突变后会导致TYRP1错误定位到TGN、内体以及细胞质膜上。另外, BLOC-2与BLOC-1多个亚基存在相互作用, 而且二者在靠近III期黑素小体的循环内体上共存, 提示BLOC-2可能是BLOC-1的下游事件, 但具体的分子作用机制目前尚不明确[35,53]。BLOC-3参与黑素小体生物发生的作用机制的研究中, BLOC-3作为Rab32/38的鸟苷酸交换因子, 主要参与VAMP7从黑素小体到循环小体的回收。BLOC-3突变后, 因VAMP7的回收障碍, 使得黑素小体体积变大, 发育成熟受阻, 进而导致色素生成减少[54]。黑素小体发生与成熟的关键事件与重要蛋白的功能总结于图3。

1.3 血小板致密颗粒(DGs)的结构和功能

血小板是由骨髓巨核细胞产生的圆盘形无核血细胞。巨核细胞经过细胞器、细胞核倍增作用以及细胞质扩增后, 在微管的调控作用下形成血小板前体, 进而形成成熟的血小板[55]。血小板直径只有2~3 μm, 是血液中最小的细胞, 甚至一度被认为是无功能的细胞碎片。在血管发生破损时, 血小板会被激活, 随后血小板中的颗粒与血小板膜融合, 将内容物释放到周围环境中, 促进凝血过程[56]。血小板中至少具有三种颗粒——α颗粒(α-granules, AGs)、致密颗粒(dense-granules, DGs)以及溶酶体, 并依赖分泌此类颗粒发挥其生物学功能, 即脱颗粒作用。CHEN等[57]对AGs与DGs的区别进行过详尽地总结, 在此不再赘述。血小板中含有5~8个DGs, 透射电子显微镜下观测的直径约为150 nm, 且电子密度极高, 呈现“致密”的状态, 致密颗粒由此得名[58-59]。DGs内储存多种内容物, 这些内容物的释放能够加速血小板活化, 与α颗粒内容物共同作用, 调节血小板介导的凝血和血栓形成过程[60-61]。DGs囊泡内含有高浓度的Ca^{2+}(约2.2 mol/L)、5-羟色胺(400~600 ng/每个血小板)、多聚磷酸(约130 mmol/L)以及核苷酸(ATP和ADP的浓度分别为0.6 mmol/L和0.4 mmol/L)[59]。除了止血和愈伤功能外, 血小板还具有消炎, 调节生长发育、血管生成以及肿瘤转移等功能[62]。

1.4 DG的发生与成熟机制

DG与黑素小体均起源于MVB。很多伴随眼皮肤白化表型的疾病都表现出血小板功能障碍, 但是由于DG研究的诸多条件限制, 对于DG的发生与成熟相关的机制研究并不透彻[10]。已知很多能够定位于DG的整合蛋白在其胞质端结构域均含有基于双亮氨酸和赖氨酸依赖的分选基序, 这些基序是保证货物蛋白被AP-3识别并最终能够运输、定位在DG的关键[7,58]。研究发现, 定位于DG的蛋白LAMP2和VMAT2在AP-3的识别位点突变后异常定位于细胞质膜, 证明其是AP-3的货物蛋白[63]。在HPS小鼠模型*pearl*[64]和*mocha*[65]以及HPS-2[66]和HPS-10[67]病人中, 分别发现了AP-3的β3A和δ亚基突变, 均产生血小板DG缺陷、出血时间延长的表型, 提示AP-3参与了DG货物蛋白的分选过程。在AP-3 β3A亚基突变的*pearl*小鼠血小板中, DG重要的组成型膜蛋白SLC35D3[68]和TMEM163[69]的表达水平显著降低, 提示AP-3能够调控其转运至DG膜上。SLC35D3被认为是核苷糖装运蛋白家族的一员, 负责转运UDP-glucose[70], 该蛋白在血小板中存在与否对DG的生物发生至关重要[63,71]; TMEM163是我们近期发现的影响血小板DG生物发生的锌离子转运蛋白, *Tmem163*敲除小鼠的DG缺乏, 出现出血时间延长的表型[69]。



黑素小体所有组分均在内质网中合成, 并通过高尔基体成熟, 其中一些还需要借助细胞质膜的胞吐和胞吞作用。早期内体或I期黑素体具有典型的多囊结构, 通过有选择性地连续装载不同的货物, 发展成溶酶体或成熟的黑素小体。PMEL首先被运输到细胞质膜, 然后再通过内胞吞途径导入I期黑素体。在内腔囊泡中, PMEL经过加工和成熟, 形成条状的淀粉样纤维。这种纤维结构是黑色素合成的支架, 也是II期黑素体的特征。OA1和其他因子对此过程起到贡献。黑色素合成始于III期黑素小体, 其中包含维持酪氨酸酶活性所需的所有组分, 包括TYR、TYRP1、OCA2、DCT、MART1等。这些参与者可以分为三组。首先, TYRP1可绕过细胞质膜直接传递到I期黑素小体。其次, DCT和MART1可以直接从TGN运输到III期黑素小体。再次, 大多数组分需要经过细胞质膜并通过内胞吞进入I期黑素小体。I期黑素体上的货物有三种方式转运至IV期黑素小体。首先, 像PMEL和OA1一样, 只需顺势即可进入II期黑素小体。其次, 像TYR一样, 利用AP-3复合物, 被分选到III期黑素小体。再次, 像TYRP1一样, 通过AP-1、BLOC-1和BLOC-2在管状结构上进行分选, 朝着III期黑素小体运输, 而这两个细胞器之间的融合需要VAMP7。此外, VAMP7可以通过BLOC-3的帮助, 向I期黑素小体逆向运输。图中已知的运输过程由实线表示, 未确定的过程由虚线表示。关键分子和复合物在图片右侧进行标注。

All the melanosomalcomponents are synthesized in the ER and matured through Golgi apparatus, with some of them take the route of exocytosis and endocytosis though plasma membrane. The early endosome or stage I melanosome, characterized by the multi-vesicle-contained structure develops into lysosomes or mature melanosomes by selectively harboring different cargos in a sequential manner. PMEL is transported to plasma membrane first and then imported into stage I melanosome via endocytic pathway. PMEL gets processed and mature in the intraluminal vesicles, where it forms into elongated amyloid fibrils. The fibril structure is the scaffold for melanin synthesis and also the feature of stage II melanosomes. OA1 and other factors contribute to this process. Melanin synthesis begins from stage III melanosome, where all the components essential to sustain tyrosinase activity have been prepared, including TYR, TYRP1, OCA2, DCT, MART1 and others. These players can be sub-grouped into three teams. First, TYRP1 is directly delivered to stage I melanosomes, by passing the plasma membrane. Second, DCT and MART1 are transported directly from TGN to stage III melanosomes. Third, as most players take, go to plasma membrane and then endocytosed into stage I melanosomes. Cargos on stage I melanosomes take three ways toward stage IV melanosomes. First, like PMEL and OA1, just follow the trends and get into stage II melanosomes. Second, like TYR, take advantage of AP-3 complex and be sorted into stage III melanosomes. Third, like TYRP1, is sorted by AP-1, BLOC-1 and BLOC-2 on a tubule structure toward stage III melanosomes, where the fusion between the two organelles needs VAMP7. Laterally, VAMP7 can retrograde to stage I melanosomes with the help of BLOC-3. Known transport processes are indicated by solid lines and undetermined ones by dashed lines. Key molecules and complex are diagramed with annotations on the right.

图3　黑素小体生成过程中的关键分子和细胞内事件(根据参考文献[6,35]进行修改)

Fig.3　Key molecules and intracellular events during melanosome biogenesis (modified from the references [6,35])

BLOC复合体调节DG生物发生的机制研究非常有限, 但其向成熟的黑素小体的运输功能非常清楚, 推断这一复合物可能也影响DG的发生过程。BLOC-1复合物dysbindin亚基突变的*sdy*小鼠血小板5-羟色胺显著降低[72]; pallidin亚基突变的病人血小板中DG缺乏[73]; 证明其对DG的生物发生很重要。BLOC-2

可以与Rab38和Rab32这两个小GTPase相互作用, 在巨核细胞中, Rab32和Rab38参与了AP-3介导的DG从内体到成熟的货物蛋白转运过程, 间接表明BLOC-2也会影响DG的成熟[63]。BLOC-3在DG生物发生中的作用尚不清楚, 但HPS1、HPS4缺陷的人类患者或小鼠中发现有DG缺乏[9-74], 有研究显示BLOC-3帮助MRP4(multidrug resistance protein 4)/ABCC4(ATP-binding cassette subfamily C member 4)转运到DG并储积ADP[75]。之后我们发现在*pa*和*sdy*小鼠(两者均为BLOC-1缺陷)血小板中, TMEM163蛋白表达水平显著降低, 这一现象也出现在HPS6亚基突变的*ru*小鼠(BLOC-2缺陷)中, 但在HPS1亚基突变的*ep*小鼠(BLOC-3缺陷)中未发现此现象。这一结果表明, BLOC不同复合物在DG发生过程中可能都参与了货物蛋白运输, 但这三种复合物在影响DG发生过程中也存在功能差异。另一个可能参与DG形成的因素是同型融合蛋白分选复合物HOPS, 它的VPS33A亚基调控SNARE复合物形成并驱动膜融合, 可能介导了含有DG货物蛋白的运输囊泡与MVB的融合, VPS33A突变的*bf*小鼠出血时间延长, ATP分泌减少, DG数量减少, 证明HOPS也可以影响DG的生物学发生过程[76]。

在对DG导致凝血障碍的致病机制研究中, 主要关注方向是LRO特异性货物蛋白的分选过程和调节机制。因DG中含有磷酸、ADP、ATP、5-羟色胺等小分子以及多种离子, 提示有离子通道、转运蛋白参与了DG囊泡内小分子的富集和调控。在黑素小体的生物学发生过程中, Na^+、Ca^{2+}和Cu^{2+}对于其稳态维持和功能发挥至关重要。我们有理由相信, 与黑素小体发生机制相似的DG可能也需要特定的离子通道参与功能调节。

有研究证明, TPC2也定位在DG上, 参与调节DG腔内的pH值和Ca^{2+}信号[77]; 囊泡单胺转运蛋白(vesicular monoamine transporter 2, VMAT2)向DG转运5-羟色胺时, 依赖H^+-ATP酶产生的质子电化学梯度[58]; 这些结果表明DG的离子转运调节对其功能十分重要。我们近期的研究发现, Zn^{2+}转运蛋白TMEM163可以定位在DG上, *Tmem163*基因敲除小鼠DG缺乏, DG前体中锌离子堆积, 导致DG发生障碍[69]。虽然研究十分有限, 但这些结果证明DG正常功能的发挥需要离子通道的参与和囊泡内离子稳态的维持, Ca^{2+}、Na^+、Zn^{2+}、H^+等都在其中发挥重要作用, 因此DG定位的离子通道和转运蛋白未来将受到更多关注。本实验室发现UDP-glucose转运蛋白SLC35D3可以定位在DG上, 因此我们推测糖代谢与核酸代谢的重要中间产物可能参与调控DG的生物发生。

1.5 大致密核心颗粒(LDCV)的结构和功能

LDCV是神经内分泌细胞及外分泌细胞的标志性细胞器[78-80]。早在1941年, BENNETT[81]首次在肾上腺嗜铬细胞(chromaffin cell)中描述了LDCV的发生。1953年, 研究人员从嗜铬细胞中分离出LDCV[82], 不久又在电镜下观察到LDCV是一种由单层膜包裹, 含黑色致密核心的细胞器[83]。嗜铬细胞LDCV内腔含有大量的粒蛋白(granin)、神经肽(neuropeptide)、酶和蛋白酶抑制剂, 并贮存儿茶酚胺类神经递质(catecholamine neurotransmitters)、ATP、抗坏血酸(ascorbic acid)和Ca^{2+}等[84]。在分泌细胞受到刺激时, LDCVs通过调节型分泌作用(regulated secretion), 使其贮存的生物活性物质通过血液循环或局部扩散的方式来调节其他器官的功能, 对机体的发育、代谢、行为和神经突触可塑性等都具有重要的调控作用。已发现LDCV在脑、胰岛、垂体、肾、小肠等多种器官中分布, 颗粒直径也从数百纳米到数微米不等。

1.6 LDCV的生物发生及成熟

LDCV的发生起始于TGN内腔膨大, 之后衍生出与TGN的管状网络不同的膨大部。膨大部囊泡大小不一, 形状不规则, 可能是由颗粒组分和非颗粒组分所组成的混合体[85], 这些混合体进一步与TGN分离, 形成未成熟颗粒(immature secretory granules, ISGs)。TGN处的膨大部及刚脱离的未成熟颗粒膜上均有网格蛋白包被。网格蛋白通过APCs参与货物蛋白的分选和招募, 维持新形成囊泡的形状, 并在颗粒成熟前解体脱落。放射性标记的颗粒蛋白从TGN进入ISGs需15 min, ISGs转变为成熟颗粒(mature secretory granules, MSGs)的半衰期约为45 min[86]。体外培养的嗜铬细胞中LDCV的寿命是15~18天[87]。非颗粒蛋白或膜成分通过网格蛋白包裹的小泡从ISGs上脱离。在ISGs成熟过程中, ISGs之间可发生同型融合, 并在融合之后发生膜重塑及货物的进一步分选, 有研究称HID-1介导了在胰岛素颗粒的成熟过程中ISGs之间的同型融合[88]。MSGs不含网格蛋白衣被, 形态规则, 电子致密性比较均一。MSGs和ISGs都可

以响应高钾刺激, ISGs甚至优先释放[86]。

LDCV发生过程中蛋白的分选机制主要有“前置分选”(“sorting for entry”)和“后置(滞留)分选”(“sorting by retention”)两种假说[89]。“前置分选”理论认为TGN作为主要的分选平台, 分泌蛋白在TGN中发生聚集, 之后与TGN膜或膜上的受体结合, 进而通过分选闸门, 并将其他成分排斥在外。“后置(滞留)分选”模型认为分泌蛋白并非在TGN闸门处进行分选, 而是随机进入TGN衍生的所有囊泡, 高度聚集的调节型分泌蛋白滞留在ISGs, 非颗粒成分以受体介导的方式或仅仅伴随液态物质从ISGs排出。LDCVs发生过程中的蛋白分选方式可概括为三类: (1) 内腔蛋白分选, 高度聚集的颗粒内含蛋白(如粒蛋白家族); (2) 膜蛋白分选, 与颗粒内含蛋白直接或间接结合的膜蛋白; (3) 膜外结合蛋白分选, 如衔接蛋白、衣被蛋白及其他胞质蛋白。

LDCVs内腔蛋白的聚集是最早的分选事件。LDCVs内腔含有丰富的结构蛋白–粒蛋白, 包括粒蛋白A(chromogranin A, CgA)、CgB、分泌颗粒素II~IV(secretogranin II-IV, SgII-IV)等[90]。在Ca^{2+}存在的情况下, 垂体和肾上腺髓质中CgA在pH6.5时开始形成沉淀, 沉淀随pH值降低逐渐增多。这说明CgA通过在酸性环境中发生聚集沉淀实现与其他分泌蛋白分离[91]。LIN等[92]发现, LDCV中的基质蛋白SgII高聚体可以被Ca^{2+}诱导发生液–液相分离(liquid-liquid phase separation, LLPS), 进而调控LDCV的大小。粒蛋白聚集体为ISGs在TGN处的出芽提供了动力。在不含LDCVs的成纤维细胞中表达CgA[93]和CgB[94]均可产生具有分泌功能的LDCV样结构, 且该结构具有LDCVs的分泌功能。反之, 在PC12细胞中下调CgA和CgB的表达严重影响LDCVs的形成[94]。随后的研究表明, 在成纤维细胞中分别过表达抗利尿激素原(pro-vasopressin)、催产素原(pro-oxytocin)和阿黑皮素原前体(pro-opiomelanocortin, POMC)均可诱导出LDCV样囊泡[95], 说明粒蛋白和激素原都具有在TGN处诱导出芽形成ISGs的作用。LDCV内腔蛋白除了通过相分离方式进行分选外, 还可以借助与膜蛋白互作实现共分选。已知分选受体羧肽酶E(carboxypeptidase, CPE)的跨膜结构域可以与TGN和LDCVs膜上的脂筏结合[96], 从而介导POMC、proenkephalin和proinsulin等神经肽前体在TGN和LDCVs内腔的分选[97-98]。颗粒膜蛋白之间也存在着互相的识别和互作。膜蛋白phogrin为定位在LDCVs上的I型整合跨膜蛋白, 以130 kDa前体的形式合成, 在进入调节型分泌途径的过程中水解加工为60/64 kDa的跨膜成熟体[99]。WASMEIER等[100]发现, phogrin的N-端内腔结构域可以进入内腔, 并以可溶性蛋白的形式存在, 说明phogrin的分选可能由其内腔结构域来介导。CPE特异结合phogrin全长及phogrin内腔结构域, 在pH5.5和Ca^{2+}存在环境中互作增强。在*CPE*敲除的AtT-20细胞中, phogrin中间体在Golgi/TGN组分堆积, 提示phogrin从TGN向外的运输受阻。phogrin反过来也会影响CPE在LDCVs的定位, 在敲降phogrin的AtT-20细胞中, CPE错误转运到溶酶体降解, 说明phogrin有助于CPE在胞内的正确运输[101]。

分泌细胞中Clathrin分布在TGN膨大部和ISGs处, 提示Clathrin及其他衔接蛋白可能参与LDCV蛋白的分选[85]。已有实验表明AP-1复合体[102-104]和AP-3复合体[105-106]可能参与其中。免疫电镜实验发现, MPRs在ISGs上出芽的衣被小泡上与AP-1共定位。随着颗粒的成熟, MPRs和AP-1含量减少90%, 而膜蛋白phogrin含量没有改变, 说明MPR和AP-1从颗粒上的移除是特异性的[103]。MPRs和AP-1是否参与膜蛋白到颗粒的招募还需要进一步阐明。在AP-3功能缺陷的*mocha*小鼠肾上腺组织及AP-3敲降的PC12细胞中粒蛋白CgA和SgII的含量都显著降低[105]。放射性标记和梯度离心实验进一步表明, 在AP-3功能缺陷的PC12细胞中, SgII进入组成型分泌途径的比例增多, 说明AP-3介导SgII在TGN或/和ISGs中的分选和招募[106], 阻止其到组成型分泌途径的运输。HAO等[107]发现, Muted缺失的细胞中单个LDCV中的CgA增加, 提示Muted缺陷导致CgA在LDCV的滞留, 说明BLOC-1参与ISG成熟过程中过多CgA的外排, 从而参与LDCV的发生。YU等[108]发现, 在BLOC-3复合体的亚基HPS1缺陷的*ep*小鼠小肠潘氏细胞中, 潘氏颗粒(另外一种LDCV)膜蛋白VAMP7在循环内体的分布减少, 在高尔基处的分布增加, 提示BLOC-3可能参与VAMP7的循环回收。衔接蛋白复合体也参与颗粒内可溶蛋白的运输。

1.7 Weibel-Palade小体(WPB)的结构和功能

WPB是一种血管内皮细胞中特异的LRO。1964年, Ewald WEIBEL和George PALADE[109]在大鼠和人的血管内皮细胞中发现了一种棒状的细胞器。随

后的研究发现此种细胞器在多种脊椎动物中都存在[110]。该细胞器被命名为Weibel-Palade小体。在电子显微镜下观察, WPB呈经典的“雪茄状”, 直径为0.1~0.3 μm, 长度为1~5 μm, 表面包被单层膜结构, 内部为平行于长轴规则排列的管状结构。1982年, WANGER等[111]发现WPB中含有一种促凝血蛋白vWF(von Willebrand factor), 在电子显微镜下观察到的WPB内部的管状结构即由vWF的多聚体构成[110,112]。随后又发现WPB中还包含组织型纤溶酶原激活剂(tissue-type plasminogen activator, t-PA)、P-选择素(P-selectin)、白细胞介素-8(interleukin-8)、嗜酸性粒细胞活化趋化因子-3(eotaxin-3)、血管生成素-2(angiopoietin-2)、骨保护素(osteoprotegerin)、内皮素-1(endothelin-1)等多种生物活性分子[113]。这些WPB的内容物可以响应某些信号分子或机械应力刺激而被释放到胞外, 参与调节血管舒张及血管通透性, 并在止血、炎症、血管生成、血栓形成及创伤愈合等多种生理及病理过程中发挥重要作用。vWF是WPB中的主要组分, 可分泌到血液中参与凝血过程。如果血液中vWF缺失、减少或聚合程度低会导致假性血友病(von Willebrand disease, VWD)发生[114], 相反, vWF水平过高则与血栓形成相关, vWF聚合程度过高或者不能从血液中被清除可能会引发血栓性血小板减少性紫癜(thrombotic thrombocytopenia purpura, TTP)[115]。血浆中vWF水平升高还与冠心病、缺血性中风、动静脉血栓形成等有关[116-117]。另外, vWF还与动脉粥样硬化直接相关[118], 在vWF缺失或有障碍的动物中, 动脉粥样硬化的发生率显著降低[119-120]。

1.8 WPB的生物发生及成熟

WPB的发生也起始于TGN内腔膨大。在HEK293细胞、AtT20或犬主动脉内皮细胞中, 表达全长vWF可以形成WPB样细胞器。这说明vWF的多聚化和自我组装是WPB生物发生的主要驱动力[121]。vWF前体蛋白包括一个信号肽(22个氨基酸残基)和350 kDa的具有保守结构域的provWF(D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK)。在内质网中, 两个provWF通过它们C末端的CK结构域连接形成二聚体[122]。到达TGN以后, 该二聚体通过D3结构域之间的连接而形成多聚体, 之后D1-D2结构域(100 kDa, 被称为proregion)被切割掉, 形成成熟的vWF多聚体[123-124]。vWF的多聚体化程度依赖于从内质网(pH7.2)到TGN(pH6.2)的pH值的降低[124]。在TGN中, vWF多聚体以螺旋的方式组装成管状结构, 在proregion的帮助下, 将新的vWF二聚体共价添加到生长螺旋的末端, 这种组装使长链共价连接的vWF存储时不发生缠结。vWF多聚体的组装对于WPB的生物发生以及发挥生理功能都非常重要。有研究表明, WPB腔内的H^+和Ca^{2+}在vWF多聚体的组装过程中发挥重要作用[125]。增加WPB腔内的pH值, 会破坏vWF多聚体的组装, 从而导致WPB丧失其经典的杆状形状[125-126]。vWF多聚体这种组装形式不但可以大大增加WPB内部的储存量, 还可以保证vWF多聚体在分泌时可以迅速有序地解聚形成长纤维丝[127], 释放到血液中去结合血小板共同参与凝血。

初步组装的vWF以依赖于clathrin/AP-1衣被的方式从TGN出芽[128]。而后经过膜分裂的过程, WPB脱离TGN形成未成熟的WPB。YAMAZAKI等[129]发现, 已知的膜分裂调节因子PKD(protein kinase D)以及V-ATPase V0a1参与WPB的膜分裂过程。脱离TGN之后, 未成熟的WPB电子密度较低, 且停留在核周。随着vWF二聚体的持续添加、膜成分及内容物的不断招募, 以及同型融合(homotypic fusion)作用, 未成熟的WPB的大小会不断增加, 内部管状结构排列更加有序, 电子密度变大, 逐渐趋向成熟[121,130-132]。

WPB内含物和膜成分的招募及转运均受到内体–溶酶体运输系统的精密调控。有些组成成分(例如t-PA、angiopoietin-2、osteoprotegerin和P-selectin)是在TGN出芽时与vWF一起招募进入未成熟的WPB[117]。在成熟WPB中没有clathrin/AP-1包被, 说明在WPB的成熟过程中, 可能有clathrin包被的小泡从未成熟的WPB出芽而带走某些特定蛋白。同时, 在成熟的WPB中也可检测到许多不存在于未成熟WPB中的蛋白, 包括CD63、Rab27A、RalA和Rab3D等, 这些组成成分是在WPB脱离TGN之后被转运进入WPB的[133]。例如AP-3将CD63转运到WPB上[134]。也有研究表明AP-3同样介导了VAMP8到WPB上的转运。MADD(MAP kinase-activating death domain)是内皮细胞中Rab27A、Rab3B和Rab3D的一种鸟嘌呤核苷酸交换因子。敲降MADD后, WPB上的Rab27A、Rab3B和Rab3D含量显著减少, 表明MADD驱动这些Rabs募集到WPB的膜上[135]。另

外, BLOC-2复合体亚基HPS6参与了V-ATPase亚基V0D1到WPB的转运过程, 对于WPB的酸化和vWF的正常分泌至关重要[126,136]。此外, 我们近期的研究结果还表明, 线粒体在WPB发生早期(结果待发表)和晚期[137]阶段发挥重要的调节作用。

在整个成熟过程中, 未成熟WPB沿细胞骨架向质膜方向运动, 腔内pH值持续降低, 成熟WPB腔内的pH值通常可达到5.4左右, 且内部管状结构排列更为致密有序。最终成熟的WPB锚定分布在质膜附近, 等待刺激后释放[132-133,138]。已知多种促分泌素能够刺激WPB的释放, 它们主要通过G蛋白偶联的受体刺激细胞内两种主要的信号通路。凝血酶、组胺等一些促分泌素通过依赖磷脂酶C的机制, 提高细胞内游离Ca^{2+}的浓度; 而另外一些促分泌素, 例如肾上腺素和加压素, 会增加细胞内cAMP的水平[139-140]。WPB响应不同的刺激时, 会启动不同的分泌机制, 从而精确地调控所释放的分子, 产生恰当的生理反应[141]。

DG、LDCV和WPB的生成过程及关键分子总结如图4所示。

2 结束语

LRO的生物学特点可以概括为“三多”, 即种类多、功能多和未解之谜多。随着越来越多的LRO被鉴定, 以及LRO相关的致病基因被发现, LRO领域会逐渐成为未来细胞生物学领域的研究热点与新兴增长点。细胞生物学百年的经验积累会在这个领域内形成交叉, 内体–溶酶体途经经典的理论会在LRO领域被重新审视, 以深刻理解LRO特异的结构与功能所代表的生理与病理意义。LRO随生命进化过程发展成为高度特化的细胞器, 以适应其专属的生物学功能。破解其中的奥秘有助于了解生命进化的密码。对LRO病理机制的研究的深入, 也有助于我们对疾病的精准诊疗, 例如在白化病、出血性疾病的治疗中, 可以利用LRO作为靶向治疗的目标, 对色素生成和出凝血功能实现有效把控, 达到精准治疗或干预的目的。

从以上四个代表性LRO的研究中, 我们了解到LRO的合成是多步骤的复杂过程, 涉及到多种货物与膜泡的分选、蛋白与脂质的相变, 细胞器之间以及细胞器与骨架的相互作用, 以及物质跨膜运输等诸多前沿科学问题, 这里机遇与挑战并存。其中亟待解决的科学问题包括但不局限于: LRO内容物聚集体与相变复合物的形成及调控过程; LRO多离子稳态建立机制、偶联关系解析与生理意义; 多细胞器互作网络对LRO生成的调控作用。

但是, 随着新技术、新设备以及新理论的不断出现, 这些问题必然会迎刃而解。近些年出现的超高分辨率超高速成像技术、LRO膜片钳技术、邻近标记技术、光电联用技术、冷冻电镜技术、细胞器快速分离技术以及微量质谱技术等研究手段的兴起以及广泛应用, 正在逐步增加我们解决LRO相关问题的能力。这些在内体–溶酶体研究领域已经被广泛应用的研究技术向LRO领域植入的过程必然会遇到很多困难, 但是也定会取得更多重要的发现。重要的是, LRO的研究多来自临床罕见病, 每一个突变的发现既是一个悲惨的故事也是一缕照亮他人的光亮, 充分挖掘LRO罕见病人的遗传学及生物学价值, 在分子细胞水平上搞清楚LRO相关疾病的机制, 是对每个病人最大的尊重与关爱。我们利用全基因组测序以及全外显子测序手段, 从3 000多人的白化病队列中成功鉴定出若干色素调控基因, 并对这些基因编码的蛋白质功能进行深入地研究。阐明OCA6/NCKX5通过调控线粒体离子稳态控制色素合成的机制, 并提出“线粒体型眼皮肤白化病”这一概念[142]。随后又发现, TPC2-R210C功能增强突变导致皮肤与毛发白化的分子细胞机制, 进而提出“皮肤型白化病”的概念[46]。目前我们团队正在从多基因–多突变的角度理解LRO生物学发生的过程。利用LRO特有的生物学特性, 比如黑色素的黑白特性、DG和LDCV的致密核心以及WPB的雪茄形外观等, 开展高通量筛选, 利用多组学的方法对LRO调节基因以及信号通路的解析是开辟新范式、找到新机制的重要途径。近期, BAJPAI等[143]利用黑色素的散射特性开展全基因组筛查并发现若干调控色素合成的新的色素调控基因, 我们目前正在从生理及病理角度探究这些色素调控基因的分子细胞机制。

LRO和溶酶体的关系并不像我们原来认为的那么密切, 未来LRO可能成为细胞器生物学研究的重要分支领域。

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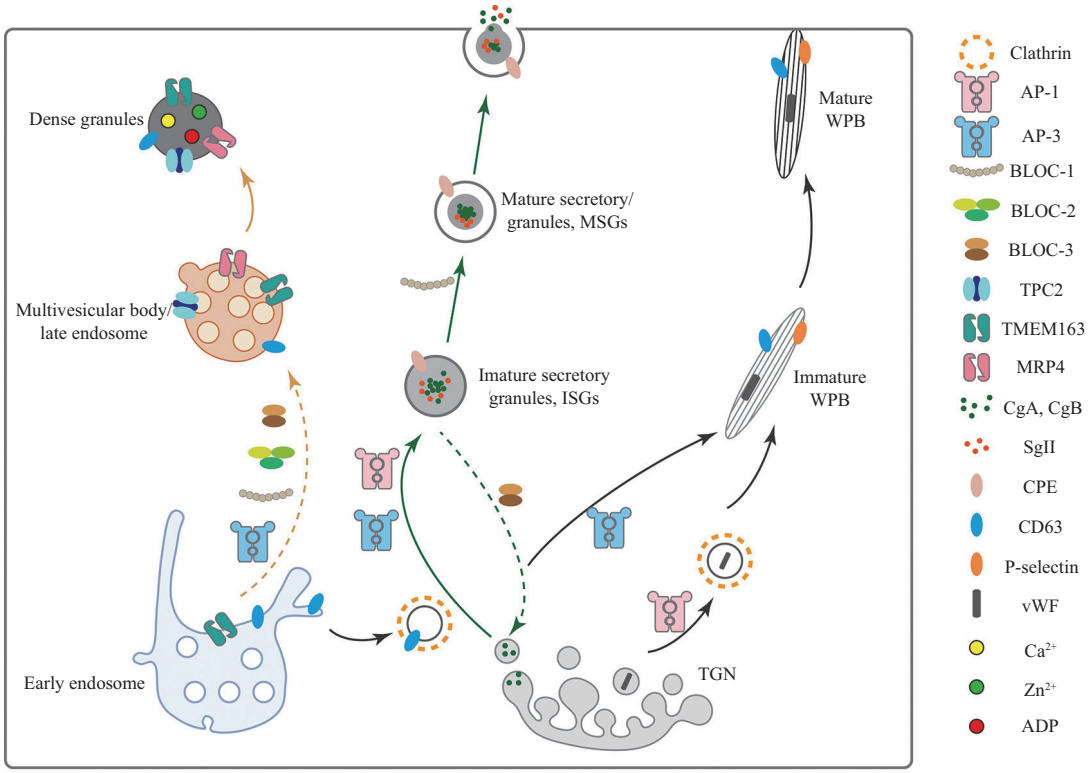

DG(左线)、LDCV(中线)和WPB(右线)的生物发生机制与内吞作用和生物合成途径相关, 涉及的细胞器包括早期内体、高尔基/TGN、多囊体/晚期内体和溶酶体。图中的实线和虚线分别表示已证实和潜在的转运途径。与黑素体成熟类似, DG从早期内体中形成, 可能通过AP-3/BLOC-1/BLOC-2/BLOC-3包被的囊泡分选, 成熟为MVB/晚期内体。TPC2、MRP4和TMEM163存在于MVB/晚期内体中, 作为转运复合物的货物介导Ca^{2+}、ADP和Zn^{2+}的跨膜转运。这些物质在DG成熟和DG离子稳态维持中至关重要, 包括腔内pH和渗透压。DG的成熟还需要小GTP酶, 如Rab32和Rab38(图中未显示), 以及上游的BLOC-3鸟苷酸交换因子发挥作用。LDCV和WPB从TGN开始形成, 它们的特定内容由AP-1和AP-3分选, 逐渐发展为不成熟形式。在ISG中, 蛋白质内容物在Ca^{2+}和H^{+}调控下发生凝聚作用和相分离, 逐步形成致密核心。随着更多的货物的分选进出, ISG逐渐转变为MSG, 并准备释放。同样, vWF在TGN中形成管状物, 然后与P-selectin和IGFBP7等货物一起脱落形成不成熟WPB。其他货物, 如CD63, 可以通过AP-3传递到不成熟的WPB, 而一些其他货物则从成熟的WPB中分选出来, 如clathrin和AP-1。在图片的右侧标注了文本中讨论的关键货物分子。此图根据参考文献[8]中的信息整合加工完成。

Scheme of biogenesis of DG (left thread), LDCV (mid thread) and WPBs (right thread) relative to the endocytic and biosynthetic pathways including early endosomes, Golgi/TGN, MVBs/late endosomes, and lysosomes. Solid lines and dashed lines indicate proved and putative trafficking pathways separately. Similar to melanosomal maturation, DGs emerge from early endosomes, which matured into MVB/late endosomes possibly through sorting AP-3/BLOC-1/BLOC-2/BLOC-3 coated vesicles. TPC2, MRP4 and TMEM163 are present in MVB/late endosomes, delivered as cargos of this complex to transport Ca^{2+}, ADP and Zn^{2+} across the DG membrane. These materials are essential in maturation of DG and pivotal in maintaining homeostasis of DG ionic environment, including luminal pH and osmolarity. The maturation of DGs also required small GTPases, such as Rab32 and Rab38 (not presented in the scheme), down-streaming BLOC-3 which acts as guanine nucleotide exchange factor. LDCV and WPB oriented from TGN, with their specific contents sorted by AP-1 and AP-3, to develop into the immature forms. In the immature SGs, dense core starts to form by the protein condensation and phase separation of Cgs and SgII in the presence of Ca^{2+} and H^{+}. With more cargoes are sorted in and out, the ISGs turn into MSGs and ready to be released. Likewise, vWF forms tubules in the TGN, that then bud off perhaps together with cargoes such as P-selectin and IGFBP7 to form immature WPBs. Other cargoes, such as CD63, could be delivered to immature WPBs via AP-3, while some other cargoes are sorted out of mature WPB, such as clathrin and AP-1. Key cargo molecules discussed in the text are noted on the right side of the panel. The drawing was completed based on the information reviewed in the references [8].

图4 DG、LDCV和WPB生成过程中的关键分子和细胞内事件

Fig.4 Key molecules and intracellular events during biogenesis of DG, LDCV and WPB

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