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评 述 细胞器互作网络及其功能研究专辑

Exocyst复合体的结构与功能研究进展

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摘要 囊泡运输是真核细胞内细胞器间物质交流的重要手段, 主要包括出芽、转运、拴系及膜融合四个环节. 拴系因子调控运输囊泡与靶膜的初始接触, 建立两者间的连接, 并能够促进SNARE介导的膜融合过程. Exocyst是一个保守的八亚基拴系复合体, 主要在胞吐过程中介导囊泡与细胞质膜的拴系过程. 本文主要介绍exocyst复合体的结构和组装机制, exocyst复合体发挥拴系功能和促进SNARE介导膜融合过程的分子机制, 并阐述exocyst复合体的几种主要细胞功能, 为了解exocyst复合体的结构功能及其研究进展提供参考.

关键词 囊泡运输, 囊泡拴系, 膜融合, 拴系因子, exocyst复合体, SNARE

真核细胞内部含有很多由磷脂双分子层包裹的细胞器, 从而使特定的生物化学反应在相对独立的空间里准确高效地进行. 这些细胞器之间的物质交流需要囊泡运输来介导. 囊泡运输(vesicle trafficking)是一个精密且受到复杂调控的过程, 主要包括四个环节: “出芽”(vesicle budding), “转运”(transportation), “拴系”(tethering)和“膜融合”(membrane fusion)[1]. 每个环节由一类或几类特定的蛋白介导完成. 其中, 拴系发生在运输囊泡到达靶膜后与靶膜接触的初期, 由拴系因子介导完成, 而可溶性*N*-乙基马来酰亚胺敏感因子(*N*-ethylmaleimide-sensitive factor, NSF)附着蛋白受体(soluble NSF attachment protein receptors, SNARE)蛋白间的相互作用介导随后的膜融合过程[2,3]. 目前, 已报道的拴系因子分为两大类: 卷曲螺旋状蛋白(coiled-coil tethers)和多亚基拴系复合体(multisubunit tethering complexes, MTCs), 后者主要包括HOPS, CORVET, GARP, EARP, COG, TRAPP I, TRAPP II, TRAPP III, exocyst和Dsl1这10个复合体[4~7].

Exocyst复合体是一个进化保守的八亚基蛋白复合体, 最早发现于酿酒酵母, 由Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70和Exo84八个亚基组成, 主要参与反式高尔基体来源的分泌囊泡与细胞质膜的拴系过程[8,9]. Exocyst复合体通过与多种膜定位的小G蛋白(small GTPases)和膜磷脂的相互作用与囊泡和质膜结合, 并通过复合体的组装在囊泡和质膜之间建立连接. Exocyst复合体的形成使得囊泡与质膜相互靠近, 有助于拉近囊泡上的v-SNARE(vesicle-SNARE)与质膜的t-SNARE(target-SNARE)蛋白间的距离, 促进SNARE复合体的形成, 从而促进膜融合[3,10,11]. Exocyst复合体除参与胞吐过程, 还可以行使多种其他细胞功能, 如细

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胞极化、细胞迁移和侵袭、胞质分裂、初级纤毛发生和细胞自噬等[12,13].

目前，随着对exocyst亚基及复合体结构的解析以及对其动态组装的研究，人们对exocyst复合体的组装和功能机制有了更新的认识. 同时，随着exocyst在各个领域的广泛研究，其越来越多的细胞功能被报道. 本文主要综述exocyst复合体的结构及其功能研究进展, 旨在为全面了解其结构与功能提供参考.

1 Exocyst复合体的结构及其组装机制

Exocyst复合体介导分泌囊泡与质膜的拴系过程, 进而促进SNARE介导的膜融合. 解析exocyst复合体及其各亚基的三维结构，揭示其组装以及解聚机制是理解exocyst复合体如何发挥拴系功能的关键.

20世纪90年代末，人们通过冷冻蚀刻电子显微镜首次观察到了哺乳动物exocyst复合体的大致形状. 在使用戊二醛交联后，exocyst复合体呈“T型”或“Y型”; 天然状态下，复合体呈含有4~6个花瓣的花朵形状[14]. 随后利用X射线衍射和核磁共振(nuclear magnetic resonance, NMR)技术解析了多个exocyst亚基的结构, 包括酵母Sec3的N端结构域(71~241 aa，PDB ID: 3HIE)[15]，近全长的酵母Exo70(67~623 aa，PDB ID: 2B1E)[16]，酵母Sec6的C端结构域(411~805 aa，PDB ID: 2FJI)[17]，酵母Exo84的C端结构域(525~753 aa, PDB ID: 2D2S)[16]，哺乳动物Sec5(1~99 aa，PDB ID: 1HK6)[18]和Exo84(167~279 aa，PDB ID: 1ZC4)[19]的RalA结合结构域，近全长的小鼠Exo70(85~653 aa，PDB ID: 2PFT)[20]，果蝇Sec15的C端结构域(382~699 aa，PDB ID: 2A2F)[21]，近全长的拟南芥Exo70(75~629 aa，PDB ID: 4RL5)[22]以及斑马鱼Sec10(195~708 aa, PDB ID: 5H11)[23]. 尽管各亚基序列同源性低至10%以下，但它们的三维结构相似度却很高, 都含有之前未曾发现的长棒状螺旋束结构(图1). 虽然这些研究解析了多个exocyst亚基的部分结构，但是无法解释exocyst的组装机制.

2018年，Mei等人[24]使用单颗粒冷冻电子显微镜技术解析了exocyst复合体4.4 Å的结构，结合化学交联质谱、分子对接和从头建模的方法，搭建了一个完整组装的酵母exocyst复合体的结构模型. 该模型由几乎

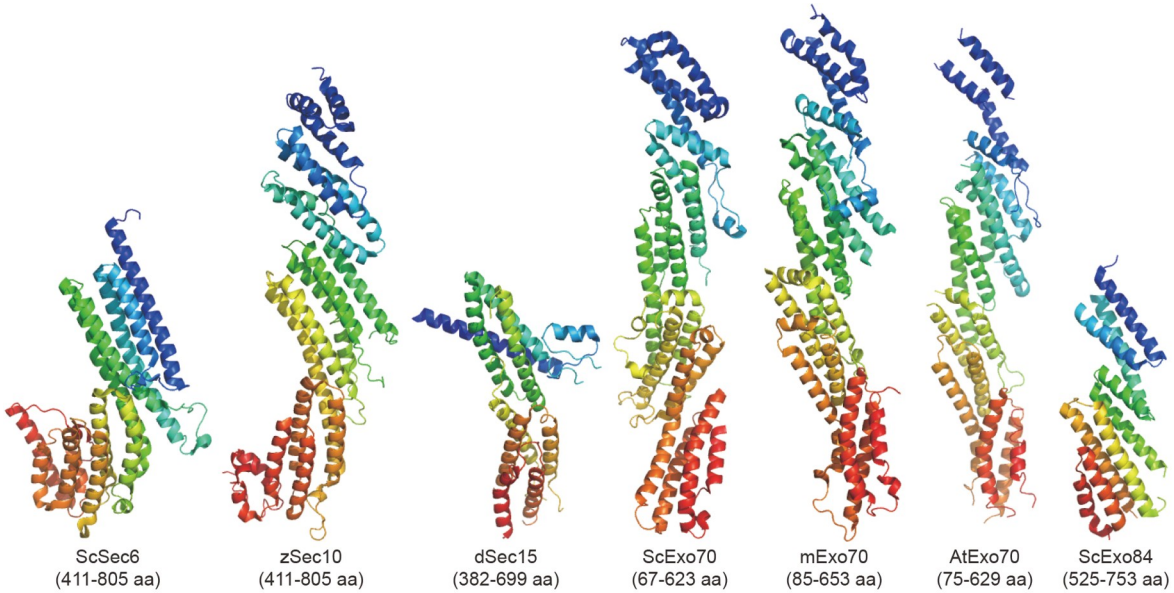

图 1 Exocyst亚基保守的螺旋束结构. 不同物种中exocyst亚基C端结构域的晶体结构显示出保守的螺旋束结构. 各结构从蓝色到红色表示N端到C端. PDB ID: ScSec6 2FJI, zSec10 5H11, dSec15 2A2F, ScExo70 2B1E, mExo70 2PFT, AtExo70 4RL5, ScExo84 2D2S. Sc, 酿酒酵母; z, 斑马鱼; d, 果蝇; m, 小鼠; At, 拟南芥

Figure 1 The conserved helical bundle structures of exocyst subunits. The crystal structures of C-terminal domains of exocyst subunits from different species show conserved helical bundle structures. The blue to red color in each structure indicates the sequence from N-terminus to C-terminus. PDB ID: ScSec6 2FJI, zSec10 5H11, dSec15 2A2F, ScExo70 2B1E, mExo70 2PFT, AtExo70 4RL5, ScExo84 2D2S. Sc, *Saccharomyces cerevisiae*; z, zebra fish; d, *Drosophila*; m, mouse; At, *Arabidopsis thaliana*

全长形式的Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, Exo84以及Sec3的C端一半结构组成. Exocyst复合体的结构以层级方式组装. 每个exocyst亚基的N端以及Sec3的中部都含有一个长度约70个氨基酸的coiled-coil结构, 即CorEx基序. 通过CorEx基序的反向平行配对, Sec3-Sec5, Sec6-Sec8, Sec10-Sec15和Exo70-Exo84形成四个异二聚体. 然后异二聚体Sec3-Sec5和Sec6-Sec8的CorEx基序对相互交织成四股螺旋束, 从而形成Subcomplex I; Sec10-Sec15和Exo70-Exo84以相同的方式组装成Subcomplex II. 最后两个四聚体Subcomplex I和Subcomplex II以叠加的方式形成完整复合体[24](图2). 最近, Zhai等人[25]通过负染电子显微镜三维重构技术对人源exocyst复合体的结构进行了研究, 发现其结构与酵母exocyst结构相似, 提示不同物种中的exocyst复合体的组装方式很可能相同. 高速全内反射荧光显微镜(total internal reflection fluorescence, TIRF)实时荧光成像技术对小鼠exocyst复合体动态组装的研究与这一推测相符[26]. 在哺乳动物细胞中, exocyst复合体由两个四聚体亚复合物SC1(Sec3, Sec5, Sec6和Sec8)和SC2(Sec10, Sec15, Exo70和Exo84)组装而成, SC1和SC2可以独立地与囊泡和质膜结合, 并与八聚体和单体处于动态平衡状态. 值得注意的是, Sec3与SC1中其余亚基的结合相对较弱, 约40%的Sec3处于游离状态, 提示部分SC1以三聚体形式存在[26].

目前, 虽然对于exocyst复合体结构和组装机制的研究取得了重大进展, 但是仍有许多问题还不清楚. 例如, 当前已解析的exocyst复合体的结构仍处于近原子分辨率水平, 无法在原子水平揭示exocyst复合体的组装机制. 虽然人源与酵母的exocyst复合体结构和组装机制相似, 但是其动态机制又存在差异[27], 是什么

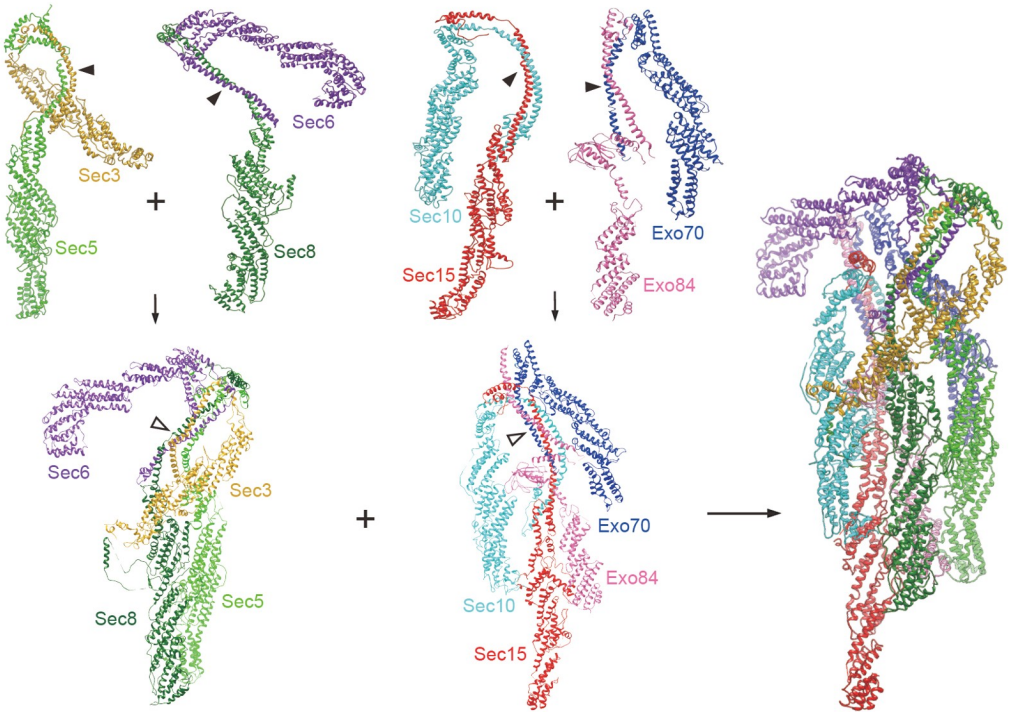

图 2　Exocyst复合体的层级组装. Sec3-Sec5, Sec6-Sec8, Sec10-Sec15和Exo70-Exo84通过各亚基N端CorEx基序间的反向平行配对形成二元复合物, 然后Sec3-Sec5-Sec6-Sec8和Sec10-Sec15-Exo70-Exo84通过CorEx基序形成四股螺旋束从而形成四元亚复合物SC1和SC2, 最后SC1和SC2结合形成八元exocyst复合体. 图中各亚基分别用不同颜色表示, 箭头指示CorEx基序的反向平行配对, 三角形指示CorEx基序形成的四股螺旋束

Figure 2　The hierarchical assembly of the exocyst complex. Sec3-Sec5, Sec6-Sec8, Sec10-Sec15 and Exo70-Exo84 form binary complexes through anti-parallel pairing of the CorEx motif located at the N-terminus of each subunit. Then, Sec3-Sec5-Sec6-Sec8 and Sec10-Sec15-Exo70-Exo84 form ternary sub-complexes SC1 and SC2 through helical-bundle formation of the CorEx motif. Finally, SC1 interacts with SC2 to form the octameric exocyst complex. The exocyst subunits are shown in different colors. Arrow heads show the anti-parallel pairing of the CorEx motif. Triangles show the helical-bundle formation of the CorEx motif

机制调控了不同物种中exocyst复合体的动态组装? 目前, 对于exocyst复合体的组装机制研究较多, 而对于其解聚的研究则很少, exocyst的解聚以怎样的方式进行? 受到哪些因素调控? 解聚之后的exocyst亚基会被降解还是进入下一轮组装与解聚的循环? 这些问题仍有待研究.

2 Exocyst复合体介导囊泡与质膜拴系过程

目前, exocyst复合体作为拴系因子发挥功能的研究主要来源于酵母和哺乳动物细胞. 早期研究表明, exocyst复合体各亚基广泛存在于胞吐和细胞扩张活跃区域[28], 这提示exocyst可能参与囊泡运输过程. 另外, exocyst任何亚基的缺失或突变都会导致胞吐过程出现缺陷, 使分泌囊泡在出芽区域细胞质内积累[29], 这提示exocyst参与了囊泡运输中的运输、拴系或膜融合环节. 活细胞成像表明, 定位于分泌囊泡上的Sec8随着囊泡转运至质膜上, 并在那里停留直至膜融合发生[30]. 这提示exocyst很可能参与了运输囊泡与质膜的拴系和融合过程. 另外, 酵母细胞异源定位实验发现, 定位到线粒体上的Sec3能够募集exocyst的其他亚基, 并使原本运输至质膜的分泌囊泡转运至线粒体, 堆积在线粒体周围[31], 这表明exocyst复合体在囊泡运输的拴系过程中发挥了关键作用. 最近, Rossi等人[32]建立了分泌囊泡的体外拴系实验, 发现在Sro7存在的情况下, exocyst复合体能够大大促进分泌囊泡聚集成簇. 这是目前关于exocyst复合体介导囊泡拴系的唯一体外重构实验, 表明exocyst复合体在分泌囊泡之间的拴系中起重要作用. 然而, 该体系缺乏细胞质膜成分的参与, 并且Sro7的存在对于分泌囊泡间的拴系也不可或缺, 因此并不能为exocyst复合体介导分泌囊泡与细胞质膜间的拴系提供直接证据.

尽管如此, exocyst复合体作用机制的研究为其拴系功能提供了大量依据, 其不同亚基可以与囊泡和细胞质膜上的磷脂和小G蛋白发生直接相互作用(图3), 从而为整个exocyst复合体在两种膜性结构之间建立直接连接提供了物理基础. 酵母细胞中, 首先被报道与小G蛋白相互作用的exocyst亚基Sec15能够与定位在分泌囊泡上的Sec4-GTP结合[33], 可能在多个exocyst亚基的分泌囊泡定位中发挥作用. Sec3通过其N端PH结构域(pleckstrin homolgy domain)与质膜上的PI(4,5)P2

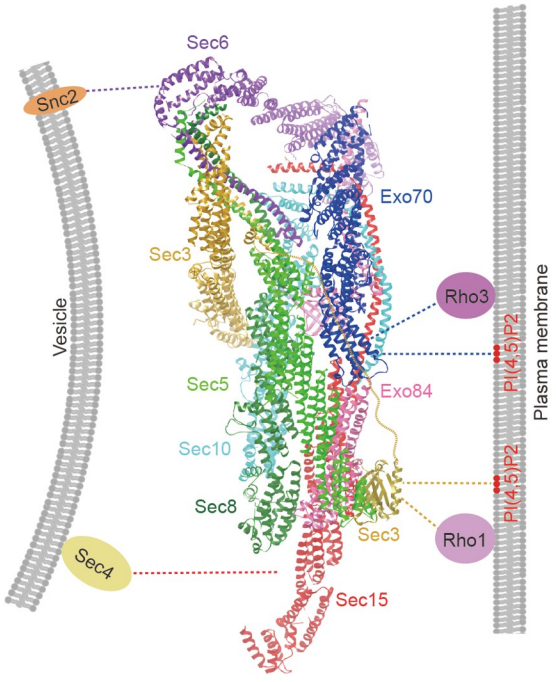

图 3 酵母中exocyst复合体与分泌囊泡和细胞质膜的相互作用. Exocyst复合体通过其Sec3亚基的N端结构域与细胞质膜上的PI(4,5)P2和Rho1相互作用, 通过其Exo70亚基的C端结构域与细胞质膜上的PI(4,5)P2和Rho3相互作用, 通过其Sec15亚基与分泌囊泡上的Sec4相互作用, 通过其亚基Sec6的N端结构域与分泌囊泡上的Snc2相互作用. 图中exocyst各亚基用不同颜色表示, 虚线表示已报道的相互作用

Figure 3 Interactions between exocyst and secretory vesicles or plasma membrane in yeast. The PH domain of Sec3 interacts with PI (4,5)P2 and Rho1, and the C-terminal domain of Exo70 interacts with PI (4,5)P2 and Rho3 on the plasma membrane. Sec15 interacts with Sec4, and the N-terminal domain of Sec6 interacts with Snc2 on the secretory vesicles. The exocyst subunits are shown in different colors. Dotted lines indicate reported interactions

和Rho家族小G蛋白Rho1结合, 从而定位到细胞质膜[15,34,35]. 另外, Exo70通过其C端结构域与PI(4,5)P2和Rho3相互作用, 从而促进exocyst复合体定位到质膜[36,37]. 同时破坏Sec3和Exo70与PI(4,5)P2结合或降低细胞内PI(4,5)P2的含量都会抑制exocyst在质膜上的组装, 导致exocyst与质膜解离并阻碍分泌[36]. 哺乳动物细胞中, exocyst复合体亚基Exo70通过其C端结构域与PI(4,5)P2以及通过其N端结构域与酵母Rho3的同源蛋白TC10相互作用定位到质膜, 这种相互作用是葡萄糖转运体-4(glucose transporter type 4, GLUT4)响应胰岛素刺激而向质膜转运所必需的[38~40]. 另一方面, 哺乳动物和果蝇中Sec15与循环内吞体上的小G蛋白Rab11相互作用, 这种相互作用可能将Sec15定位在循环内吞

体膜上，并在循环内吞体的分泌过程中发挥功能[41,42]. 并且，胰岛素刺激下Sec5与定位在GLUT4储存囊泡上的小G蛋白RalA相互作用，并在GLUT4储存囊泡上共定位, 参与GLUT4囊泡的质膜转运[43,44]. 在植物中, 拟南芥Sec3通过在酵母和人类中部分保守的碱性残基直接与PI(4,5)P2结合[45]. 拟南芥中小分子ES2(endosidin2)结合到Exo70亚基上，破坏Exo70与质膜的结合, 从而抑制了植物的胞吐作用，并增强了植物液泡的运输[22,46]. 此外，exocyst与SNARE蛋白的相互作用也对其定位到囊泡起重要作用. 酵母细胞中, Sec6通过其近N端结构域与分泌囊泡上的Snc蛋白直接相互作用. 在不能结合Sec6的Snc2突变酵母株中，exocyst的定位出现异常且胞吐被抑制，而Sec4的过表达能够部分挽救Snc2突变造成的表型, 提示Sec15与Sec4的相互作用以及Sec6与Snc2的相互作用在建立exocyst与分泌囊泡的连接中协同发挥功能[47].

囊泡拴系是一个时空动态过程，那么exocyst复合体是怎样通过其与分泌囊泡和细胞质膜间的联系以及组装来介导这个过程? 在酵母细胞中，exocyst各亚基和分泌囊泡主要定位在细胞胞吐旺盛的位点，如酵母芽顶端或芽颈处. 荧光漂白恢复实验显示，Sec3恢复质膜定位的速率与其他exocyst亚基不同，并且该过程不依赖于肌动蛋白纤维[48]. 另外, 在Sec3的N端融合质膜跨膜域从而将Sec3锚定到细胞质膜上，并不影响其他exocyst亚基的功能以及胞吐过程[49]. 这些研究提示Sec3在行使功能时定位于细胞质膜. 在荧光漂白恢复实验中，exocyst的其余亚基恢复膜定位的速率与分泌囊泡一致，且依赖于肌动蛋白纤维[48]，提示这些亚基首先定位到分泌囊泡上，然后随着囊泡运输到达细胞质膜. 值得一提的是, 部分Exo70与Sec3的定位和功能相似, 而另一部分则与其他亚基相似[48,49]. 此外, 线粒体异源定位实验也显示，只有Sec3能够招募其他exocyst亚基和分泌囊泡，提示Sec3在拴系过程中发挥至关重要的作用[31]. 基于这些研究和exocyst复合体的组装机制，Mei等人[24,27]提出一个exocyst介导囊泡拴系的模型: Sec3通过其PH结构域与PI(4,5)P2和Rho1相互作用定位于细胞质膜上的分泌位点，其余exocyst亚基通过Sec15-Sec4和Sec6-Snc或Sec15-Rho11的相互作用在囊泡表面发生预组装, 并随着囊泡向细胞外围运输; 当囊泡到达质膜时, Sec3通过其CorEx基序与其他亚基相互作用，从而启动exocyst复合体的组装; 随着exocyst复合体组装完成，Exo70与质膜的相互作用进一步促进了囊泡与质膜间的联系，完成囊泡拴系过程. 然而，对哺乳动物细胞中exocyst动态组装的研究提示, 哺乳动物Sec3初始定位于胞质中，并与Sec5，Sec6和Sec8预组装成亚复合体，提示exocyst介导囊泡拴系的过程与酵母中有所不同[26,27,50]. 目前, 对于exocyst复合体介导囊泡拴系的具体过程还存在争议，其物种特异性的调控机制也有待进一步研究，而基因编辑技术、超高时空分辨率显微镜成像、体外拴系重构、单分子动态分析等新技术和新方法的应用及开发将有助于解答这些问题.

3　Exocyst复合体促进SNARE介导的膜融合过程

Exocyst复合体的完全组装伴随着囊泡与质膜拴系完成, 随后的膜融合过程由囊泡与质膜上的SNARE蛋白介导[2~4,51]. SNARE蛋白按定位可以分为两类，位于囊泡上的v-SNARE与位于靶膜上的t-SNARE. 酵母中介导囊泡与质膜融合的t-SNARE蛋白包括Sso1/2(与哺乳动物中Syntaxin为同源蛋白)和Sec9(与哺乳动物中SNAP-25为同源蛋白), v-SNARE蛋白包括Snc1/2(与哺乳动物中VAMP/Synaptobrevin为同源蛋白). 在介导囊泡与质膜融合的过程中，Sso首先与Sec9形成二元t-SNARE复合物，然后再与Snc结合组装成完整的SNARE复合物[11,52]. 除了通过拴系拉近囊泡与质膜的距离从而使v-SNARE蛋白和t-SNARE蛋白在空间上靠近，exocyst还通过其亚基与SNARE及SNARE调控蛋白相互作用来促进SNARE复合体的组装及其介导的膜融合过程.

Exocyst亚基Sec3通过其PH结构域与Sso2相互作用并诱导Sso2发生构象变化，促进Sso2与Sec9形成二元复合物，从而促进膜融合[53]. Sec6既可以与Sec9直接相互作用，也可以与Sso1-Sec9二元复合物和Sec9-Sso1-Snc2三元复合物结合，另外还与调控SNARE组装的SM(Sec1/Munc-18)蛋白Sec1相互作用，这些相互作用可能促进SNARE复合体的组装[54,55]. 在拟南芥中, Exo70可能通过与v-SNARE蛋白VAMP721的直接相互作用将携带VAMP721的囊泡招募到质膜，并进一步通过与t-SNARE蛋白SYP121的直接相互作用，促进SNARE复合体的组装和膜融合[56]. 在哺乳动物中，有

研究表明, exocyst亚基Sec6和Sec8能够分别与SNAP23和SNAP25相互作用[57]. 另外, 免疫共沉淀与质谱鉴定发现, SNAP23与Exo70也存在相互作用[26]. 但迄今为止, 哺乳动物中尚无确凿的实验数据支持exocyst在SNARE的组装和SNARE介导的膜融合过程中起促进作用.

4 Exocyst复合体的细胞功能

Exocyst复合体除参与胞吐过程外, 还广泛参与多种细胞生物学过程, 如细胞极化、细胞迁移和侵袭、胞质分裂、初级纤毛发生、细胞自噬等.

4.1 Exocyst复合体参与细胞极化过程

细胞极性建立对真核生物生长发育至关重要, 它依赖于分泌囊泡不对称结合到质膜特定区域, 从而将脂质和蛋白质运送到细胞表面的特定位置, exocyst复合体在这一过程中起到精密调控的作用. Exocyst复合体定位于胞吐活跃位点, 参与分泌囊泡靶向和拴系到质膜的过程. 酵母细胞中, Rho GTPases和PI(4,5)P2直接与exocyst复合体亚基Sec3和Exo70相互作用参与细胞极性建立[37,58~61]. 神经元是高度极化的细胞, 具有专门的区域负责突触输入(树突)和突触输出(轴突), 对神经回路形成和功能发挥至关重要[62]. Exocyst定位于神经生长锥、神经突尖端或分支点, 这些区域都需要活跃的胞吐作用来增加膜成分从而扩展表面[63~66]. 值得注意的是, exocyst似乎不参与突触小泡的分泌, 抑制exocyst不影响神经递质的释放[67,68], 但NMDA(*N*-methyl-D-aspartic acid)和AMPA(α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid)受体向突触后膜的递送受exocyst复合体调节[69~71]. 另外, exocyst还可以介导囊泡向顶端-基底外侧运输, 这对于上皮细胞的极性建立非常重要[72,73]. 在果蝇中, Sec6与Rop(脊椎动物Munc18-1的同源蛋白)相互作用, 调节树突的发育[74]. 在线虫中, RAB-10和exocyst是树突化所必需的[75], Sec8的缺失会影响树突的形态发生[76]. 植物细胞中, exocyst复合体亚基Sec3a是尖端生长过程中极性胞吐作用的关键因素, 对花粉管的生长至关重要. 烟草花粉管中Sec3a的N端PH结构域(Sec3a-N)通过与PI(4,5)P2相互作用而与质膜结合, 而拟南芥中极性定位和Sec3a的功能发挥不需要与PI(4,5)P2相互作用[45]. 另外, exocyst的两个亚基Sec6和Exo70A1与拟南芥中调控生长素极性运输的PIN蛋白的回收有关. 研究发现, 拟南芥中Sec6下调导致囊泡聚积在初级根尖细胞中靠近质膜的胞质区域, 致使主根生长受阻、侧根起始受到抑制[77]. Exo70A1突变体也会导致PIN1和PIN2蛋白的回收过程被延迟, 从而导致PIN1和PIN2蛋白的重新定位在这些突变体中受到很大的损害[78].

4.2 Exocyst复合体参与细胞迁移和侵袭过程

细胞迁移和侵袭由肌动蛋白纤维的动态组装、质膜重构和极化分泌协同完成. Exocyst作为复合物主要参与细胞的极化分泌, 而其亚基Exo70还通过多种独立于exocyst复合体的机制参与了肌动蛋白纤维的动态组装和质膜重构过程. Arp2/3复合体是调控肌动蛋白纤维的动态组装和分支的关键蛋白, Exo70在细胞迁移的前沿直接与Arp2/3相互作用, 促进肌动蛋白纤维的组装和分支, 从而促进板状伪足的形成和细胞定向迁移[79,80]. 同时, Exo70还与成核促进因子WAVE复合体和WAVE调控复合体(WAVE regulatory complex, WRC)相互作用, 并将WRC招募到细胞质膜上突起形成的位点, 这两个复合体可以增强和激活Arp2/3复合体的活性, 从而调控细胞迁移[81]. 此外, Exo70还可以通过形成同源多聚体以及其与细胞膜的互作来诱导膜弯曲, 从而形成细胞膜突起, 促进细胞迁移[82].

细胞侵袭通常伴随着入侵伪足的形成和细胞外基质的降解, 入侵伪足的形成基于肌动蛋白纤维的动态组装, 而细胞外基质的降解依赖于基质金属蛋白酶(matrix metalloproteinases, MMPs)的分泌. Exo70通过与Arp2/3互作促进入侵伪足的形成, 并通过exocyst的组装促进MMPs的分泌, 从而促进细胞侵袭[83]. 敲低Exo70会阻断入侵伪足的形成并显著降低细胞外基质的降解. 在Exo70敲低的细胞中, MMP-2和MMP-9的蛋白水平显著降低; 而重新过表达Exo70, 可以恢复这两种蛋白的分泌水平[83]. 在肿瘤细胞中敲低Exo70会导致肌动蛋白纤维聚合减弱和MMPs分泌阻断, 从而显著降低肿瘤细胞的侵袭能力[79,83~85]. 有意思的是, 在不同条件下Exo70受到不同激酶的调控, 促进或抑制肿瘤细胞的迁移和侵袭. 在饥饿条件下, ULK1通过磷酸化Exo70抑制其同源寡聚化以及参与exocyst复合体组装, 从而抑制细胞膜突起形成和MMPs的释放, 抑制细胞侵袭过程; 而在生长因子刺激时, ERK1/2会磷酸

化Exo70, 反过来抑制ULK1对Exo70的磷酸化, 从而促进细胞生长和迁移[86]. 另外, 哺乳动物中Exo70有多种可变剪切形式, Exo70-E(epithelial isoform)和Exo70-M(mesenchymal isoform)这两种亚型在表皮细胞和间叶细胞中分别高表达, 其中Exo70-M可以与Arp2/3互作并促进肿瘤细胞侵袭, 而Exo70-E则不与Arp2/3互作, 增强Exo70-E的表达会抑制小鼠中肿瘤细胞的转移[87].

4.3　Exocyst复合体参与胞质分裂过程

在细胞周期的分裂末期, 染色体分离后会发生胞质分裂, 从而形成两个子细胞. 哺乳动物细胞的胞质分裂过程包括分裂沟收缩、中体形成、囊泡向中体区域运输以及细胞质膜在胞质分裂桥处断裂这几个环节, 该过程在除植物外的真核细胞中大致保守[88]. Exocyst复合体在胞质分裂的多个环节发挥功能. CDK1通过磷酸化Exo84调控exocyst复合体的组装, 使细胞周期在从中期到分裂后期的过程中发生阻滞[89]. 在裂殖酵母胞质分裂早期, 大量分泌囊泡向分裂沟运输, exocyst定位在分裂沟并促进囊泡与质膜的拴系, 从而在分裂沟处产生新的细胞膜[90]. 在果蝇精母细胞中, Exo84和Sec8能够招募Rab11和磷脂酰肌醇转移蛋白Giotto到细胞断裂位点, Exo84和Sec8突变导致Rab11和Giotto定位异常, 胞质分裂在分裂沟收缩阶段被中断[91]. 在囊泡向中体运输的过程中, exocyst被MKLP-1和centriolin招募并定位在中体上, 敲除这些蛋白会阻碍exocyst在中体的定位, 导致细胞膜断裂严重阻滞[92]. Exocyst在Rab11和RalA的调控下介导分泌囊泡向分裂沟和中体的囊泡运输[93~95]. 在胞质分裂后期, exocyst介导囊泡到胞质分裂桥处的运输, 从而促进细胞膜的断裂[96,97]. Exocyst通过其亚基Sec6与Rab5相互作用, 在胞质分裂后期共定位于胞质分裂桥的断裂位点. ESCRT III复合体介导胞质分裂桥的最终断裂, 敲除Rab5或者exocyst的亚基Sec6和Sec8会导致ESCRT III的亚基到胞质分裂桥的定位缺陷, 从而导致胞质分裂桥的断裂延滞[98]. 在线虫中, Rab5和Sec6的同源蛋白在分裂沟上富集, 敲除会导致早期胚胎的胞质分裂缺陷[98]. 植物细胞在胞质分裂前期通过囊泡运输和膜融合在分裂面上生成一个细胞板, 这是植物细胞特有的结构. Sec6, Sec8和Sec15b在细胞板形成起始和成熟过程中主要定位于细胞板, Exo70a1和Exo84b突变导致细胞板组装异常[99]. 在拟南芥中, Sec6与SM蛋白KEULE直接相互作用, 共定位于细胞板调控胞质分裂[100].

4.4　Exocyst复合体参与初级纤毛发生

初级纤毛是突出于细胞表面能够感受外界环境并传导信号的特殊细胞器, 其功能异常会导致多种人类疾病, 如恶性肿瘤、视网膜变性、多囊肾病和朱伯特综合征等. 免疫荧光显微镜或电子显微镜可在初级纤毛基底部或内部检测到exocyst的亚基[101]. 敲低SEC10可导致纤毛变短或消失, 而过表达SEC10会导致纤毛变长[102]. Arl13b是小G蛋白Ras超家族成员, exocyst亚基Sec8, Exo70和Sec5都可以结合Arl13b-GTP. 研究发现, 小鼠和斑马鱼Arl13b或exocyst亚基Sec10缺失会引发多囊肾、水肿和肾发育异常等纤毛异常表型[103]. 斑马鱼、小鼠和人类遗传学研究表明, exocyst对心脏瓣膜上的纤毛生物合成至关重要, exocyst功能障碍可能导致流出道阻塞、钙化性主动脉狭窄和二叶式主动脉瓣等心脏疾病[104].

4.5　Exocyst复合体参与细胞自噬过程

细胞自噬是真核生物中高度保守的蛋白质和细胞器降解途径. 自噬发生时, 自噬小泡包裹“废弃物”后与溶酶体或液泡融合, 从而将其降解成小分子供细胞回收利用, 该过程异常与肿瘤发生、神经病变和免疫失调等多种疾病相关. 在自噬小泡形成初期, RalB结合Exo84, 使其从自噬抑制复合体Sec5-ULK-PI3K上替换掉Sec5, 形成Exo84-ULK-PI3K自噬激活复合体, 从而促进自噬小泡的形成和成熟[105]. 另外, 酵母双杂交实验显示, exocyst与自噬相关蛋白FIP200, ATG14和Rubicon都存在直接相互作用[105]. 进一步研究发现, RalB与Sec5和Exo84的相互作用受泛素化调控, RalB泛素化后与Sec5相互作用, 促进Sec5-TBK1复合物的形成从而抑制自噬过程; 相反, RalB去泛素化后促进RalB-Exo84-Beclin 1复合物的组装, 从而促进自噬体的形成[106]. 另一项研究发现, 在果蝇中RalB调控exocyst参与自噬具有组织特异性. 在唾液腺发育导致的自噬过程中, RalB可以调控exocyst参与自噬, 而在脂肪体中RalB并不调控饥饿诱导的自噬[107]. 有趣的是, EGFR可以通过exocyst而不依赖于其激酶活性来促进自噬. 在血清饥饿的情况下, 失活的EGFR会与LAPTM4B结合从而进入内吞体, EGFR-LAPTM4B复合体会招募含

有Sec5的exocyst亚复合体, 进一步促进了EGFR与自噬抑制蛋白Rubicon的结合和Beclin 1从Rubicon上解离, 从而促进自噬[108]. 在拟南芥中, Exo70B1与自噬相关蛋白ATG8共定位, 并在刀豆素A处理后进入液泡, 且Exo70B1的突变会导致自噬相似的表型, 提示exocyst参与了植物细胞的自噬过程[109].

5 总结与展望

从最初鉴定出exocyst复合体至今已有30年, 对exocyst复合体的结构、组装机制和功能的研究成果丰硕. 这些研究不仅帮助人们更好地理解exocyst复合体在胞吐过程中发挥拴系功能的作用机制, 而且还揭示了exocyst复合体多种多样的功能. 除了上述几种主要功能, exocyst还参与病原菌入侵、细胞黏附、细胞间紧密连接形成和维持等过程. 另外, 关于exocyst在植物细胞生长和免疫中作用的报道也越来越多. Exocyst复合体就像一个巨大的信号中心, 接受众多小G蛋白和激酶等的调控, 并与SNARE蛋白、SNARE调控蛋白、细胞骨架、磷脂等相互作用, 从而在各种细胞过程中发挥功能. Exocyst的这些功能具有高度的时空特异性, 但在不同物种和细胞中的功能机制和调控机制大都还不清楚, 有待进一步的研究. 清楚了解exocyst在不同生理和病理条件下的调控机制, 将为了解exocyst的致病机理和开发相关的医学干预方法提供知识基础.

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Advances in understanding the structure and function of the exocyst complex

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Vesicle trafficking mediates material exchange between organelles in eukaryotic cells. The four major steps of the process are: vesicle budding, transportation, tethering, and membrane fusion. Tethering proteins regulate the initial contact between transport vesicles and target membranes to establish a connection between them, which facilitates SNARE-mediated membrane fusion. The exocyst is a conserved octameric complex that mediates the tethering of post-Golgi vesicles to the plasma membrane during exocytosis. Here we describe current knowledge on the structure and assembly mechanism of the exocyst complex, discuss the molecular mechanism underlying vesicle tethering and regulation of SNARE-mediated membrane fusion, and summarize its major cellular functions, aiming to help understanding of this complex.

vesicle trafficking, vesicle tethering, membrane fusion, tethering protein, exocyst complex, SNARE

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