初级纤毛发生起始的分子机制

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摘要 初级纤毛是一种位于细胞表面基于微管的膜突起, 在真核生物中广泛存在。作为细胞的“天线”, 初级纤毛参与多种重要的细胞内信号转导通路, 在动物组织器官的发育、分化及稳态维持中发挥重要作用。因此, 纤毛结构和功能的缺陷会导致多种器官病变, 引发一系列人类遗传疾病。纤毛由中心粒顶端延伸而出, 但纤毛形成的起始过程目前尚不十分清楚。纤毛的发生起始涉及到中心粒/基体的锚定、中心粒帽蛋白CP110的去除以及纤毛芽的形成。该文将对近年来这三个方面的研究进展进行综述, 为深入理解纤毛发生起始的分子机制提供参考。

关键词 初级纤毛; 纤毛发生起始; 基体锚定; 纤毛囊泡; CP110; 纤毛芽

The Molecular Mechanism underlying the Initiation of Primary Cilia Biogenesis

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Abstract Primary cilia are microtubule-based membrane protrusions, locate on the surface of cells and are widely present in eukaryotic organisms. As the “antennae” of cells, primary cilia participate in the transduction of various important intracellular signaling and play a crucial role in the development, differentiation, and homeostasis maintenance of animal tissues and organs. Therefore, defects in ciliary structure and function lead to various organ pathologies and trigger a series of human genetic diseases. Cilia arise from the top of the mother centriole, but the molecular mechanism of ciliogenesis initiation is still not well understood at present. The initiation of ciliogenesis primarily involves basal body docking, removal of the centriole cap protein CP110, and formation of the ciliary bud. This review aims to summarize recent advancements in these three areas, offering references to enhance the comprehension of the molecular mechanisms underlying ciliogenesis initiation.

Keywords primary cilia; ciliogenesis initiation; basal body docking; ciliary vesicles; CP110; ciliary bud

纤毛(cilia)是基于微管的突出于细胞表面的呈毛发状的细胞器, 长度一般为3~10微米[1]。纤毛在真核生物中广泛存在, 尤其是在哺乳动物中, 几乎所有的细胞类型表面都具有纤毛。根据运动能力, 纤毛可分为运动纤毛和不动纤毛[2]。运动纤毛分布于少数特殊类型的细胞表面, 如精子鞭毛、呼吸道上皮细胞纤毛和输卵管上皮细胞纤毛等, 在精子运动、呼吸道黏液清除及卵子的运输等方面发挥作用[3-4]。不动纤毛, 又称为初级纤毛(primary cilia)。大部分组织器官细胞表面的纤毛为初级纤毛。初级纤毛的膜表面分布着众多受体, 包括G蛋白偶联受体(G protein-coupled receptor, GPCR)和离子通道, 这些受体能够调控多条重要的信号转导通路, 如Hedgehog、Wnt、受体酪氨酸激酶(receptor protein

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tyrosine kinase, RTK)和TGFβ/BMP信号通路等, 在动物组织器官的发育、分化及稳态维持中发挥关键作用[4]。因此, 纤毛结构或功能的异常可引发一系列器官的病变, 例如肾小管上皮细胞的初级纤毛充当着机械感受器的角色, 其功能受损会导致肾囊肿(kidney cyst)的发生[5]; 下丘脑神经元细胞上的初级纤毛调控食物摄取, 其功能紊乱可导致肥胖[6]; 视锥细胞和视杆细胞中的连接纤毛对光感受器的功能至关重要, 其功能异常可导致视网膜色素变性、Leber先天性黑矇(Leber's congenital amaurosis, LCA)等疾病[7]。近年来, 随着测序技术的进步, 越来越多的遗传疾病被发现是由纤毛结构异常或功能障碍引起的。目前, 人们将由纤毛基因突变、纤毛的结构异常或功能缺陷引起的遗传疾病统称为纤毛病(ciliopathies)[2,8]。纤毛病通常以综合症的形式表现, 常见症状包括多囊肾、肥胖、多趾、视网膜变性、呼吸障碍、不育等。了解纤毛形成的分子机制对纤毛病的预防和治疗至关重要。

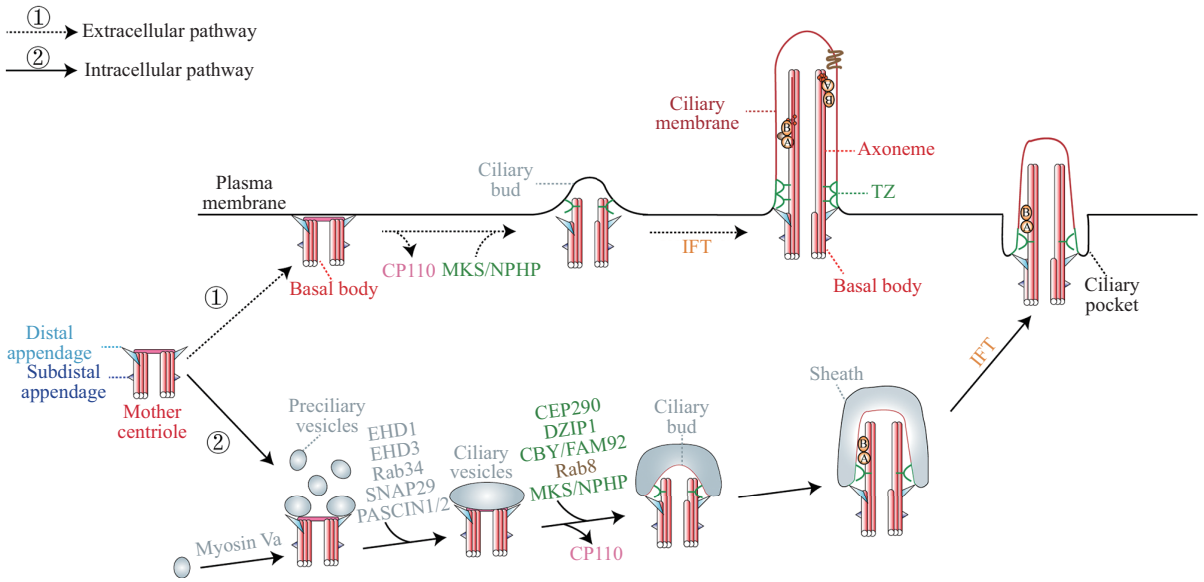
纤毛由中心粒(centriole)顶端延伸而出, 其结构在进化过程中高度保守[9], 主要包括基体(basal body, BB)、过渡区(transition zone, TZ)、轴丝(axoneme)和纤毛膜(ciliary membrane)四部分。中心粒是细胞微管组织中心——中心体(centrosome)的核心组分。一个中心体包含两个中心粒, 其中一个是成熟的(older), 作为复制模板的中心粒, 被称为母中心粒(mother centriole), 而另一个则被称为子中心粒(daughter centriole)。母中心粒的远端具有其特有的亚远端附属结构(sub-distal appendages)和远端附属结构(distal appendages)。纤毛的形成可分为发生起始和轴丝延伸两个主要过程: 首先, 在发生起始阶段, 母中心粒通过其特有的远端附属结构锚定到纤毛囊泡(ciliary vesicles)膜或质膜上, 母中心粒转化为纤毛基体, 其远端附属结构则转化为纤毛基部的过渡纤维(transition fibers)[10-11]; 随后, 中心粒顶端帽子蛋白CP110被去除, 顶端微管得以延伸[10,12]; 接着, 在Rab11-Rabin8-Rab8信号轴的介导下形成早期纤毛膜, 同时过渡区开始组装, 形成纤毛芽(ciliary bud)[10,13]; 最后, 在鞭毛内转运复合体(intraflagellar transport, IFT)的作用下纤毛的轴丝开始延伸, 形成纤毛[10,14]。下面我们将对基体锚定、CP110去除及纤毛芽形成的过程进行综述, 为更好地理解纤毛发生起始的分子机制提供参考。

1 基体锚定(basal body docking)

早在20世纪60年代, 美国科学家索罗金(Sorokin)通过电子显微镜发现纤毛的形成有两种不同的途径[15](图1)。一种为"细胞外"生成途径(extracellular pathway): 纤毛在细胞质膜表面形成, 极化的上皮细胞纤毛采用此种方式[15-17]; 在该途径中, 母中心粒锚定到质膜上, 并直接从质膜表面突出形成纤毛。另一种是"细胞内"生成途径(intracellular pathway): 纤毛在细胞质内组装, 存在于由细胞质膜内陷形成的纤毛口袋(ciliary pocket)中, 成纤维细胞和间充质细胞多采用此种方式形成纤毛[15-17]; 在该途径中, 纤毛形成起始于母中心粒与纤毛囊泡(ciliary vesicle, CV)的膜结合[18]。在形成初期, 首先, 纤毛囊泡前体小泡(preciliary vesicle, PCV)停靠在母中心粒的顶端; 接着, PCV在EHD1/EHD3蛋白的介导下融合成CV; 随后, 微管从母中心粒顶端延伸以起始纤毛轴丝的形成; 与此同时, 伴随着CV的生长, 延伸的轴丝被CV形成的双层膜包裹; 最后, 基体向质膜迁移, 外侧的CV膜(ciliary vesicle sheath)与质膜融合, 完成纤毛与质膜的对接, 并将纤毛释放到细胞外空间[19-21]。可以看出, 两种纤毛形成途径的最大不同在于基体锚定的位置和方式不同。

1.1 细胞内纤毛生成途径中的基体锚定

1.1.1 纤毛囊泡前体小泡　在细胞内纤毛生成途径中, 母中心粒顶端纤毛囊泡的形成是纤毛发生起始的关键。纤毛囊泡由小囊泡融合而成, 这些小囊泡被称为PCV或远端附属结构囊泡(distal appendage vesicle, DAV)[16-17,21]。PCV可能来源于高尔基体或再循环内体。在纤毛发生起始时, PCV开始在母中心粒的顶端聚集, 这应该是中心粒向基体转化的第一个可见标志。随后, 这些前体小泡在母中心粒的顶端融合成一个大的CV。研究表明, 肌球蛋白Va(Myosin Va, MyoVa), 一种微丝依赖性的分子马达, 在PCV向中心粒顶端的运输中发挥关键作用[22]。结合MyoVa的PCV在细胞质动力蛋白的驱动下沿着微管运输到中心粒周围, 然后在MyoVa的作用下通过中心体微丝骨架网络被运输到母中心粒远端附属结构上[23]。最近有研究表明, $P27^{kip1}$/CDKN1B[细胞周期蛋白依赖性激酶(cyclin-dependent kinase, CDK)抑制蛋白Cip/Kip家族成员]也参与了PCV向中心粒顶端的运输。在*$P27^{kip1}$*敲除的人视网膜色素上皮细胞(hTERT-RPE1)中, 透射电镜结果显示中心粒顶端PCV的数量



①“细胞外途径(extracellular pathway)”: 母中心粒(mother centriole)锚定到质膜(plasma membrane)上并转化为基体(basal body), 随后其顶端帽子蛋白CP110被去除, 微管得以延伸, 伴随着过渡区(transition zone, TZ)的组装, 纤毛芽从质膜表面突出, 然后在鞭毛内转运复合体(intraflagellar transport, IFT)的作用下, 纤毛轴丝(axoneme)进一步延伸。②“细胞内途径(intracellular pathway)”: 在Myosin Va的介导下, 纤毛囊泡前体小泡(preciliary vesicle)被招募到母中心粒周围并停靠在母中心粒的远端附属结构上, 随后, 在EHD1/EHD3、SNAP29、PACSIN1/2等蛋白的作用下融合形成纤毛囊泡(ciliary vesicle), 接着中心粒顶端帽子蛋白CP110被去除, 微管得以向上延伸, 从而起始纤毛芽的形成。CEP290、DZIP1、CBY-FAM92等蛋白在纤毛芽的形成中发挥重要作用。纤毛芽的形成需要早期纤毛膜的形成和过渡区的组装协调进行。Rab8信号轴在纤毛膜形成中发挥作用。随后, 在鞭毛内转运复合体的作用下, 纤毛轴丝进一步延伸。然后, 基体向质膜迁移, 外侧的纤毛囊泡膜(ciliary sheath)与质膜融合, 完成纤毛与质膜的对接, 并将纤毛释放到细胞外空间。

① Extracellular pathway: the mother centriole docks to the plasma membrane and transforms into the basal body, and then the distal end-capping protein CP110 is removed, allowing the extension of microtubules. Subsequently, the TZ (transition zone) assembles, and the ciliary bud emerges from the surface of plasma membrane. Finally, the ciliary axoneme is assembled via IFT (intraflagellar transport). ② Intracellular pathway: preciliary vesicles are recruited to the mother centriole by Myosin Va, where they subsequently attach to the distal appendages of the mother centriole. These vesicles then merge into a larger ciliary vesicle, facilitated by proteins such as EHD1/EHD3, SNAP29, PACSIN1/2 and Rab34. Following this fusion, the distal end-capping protein CP110 is removed, permitting microtubule extension and initiating the formation of ciliary buds. CEP290, DZIP1, and CBY-FAM92 is critical for ciliary bud formation. The formation of ciliary bud requires the coordination between the formation of early ciliary membrane and the assembly of transition zone. The Rab8 signal axis is involved in ciliary membrane formation. Subsequently, the axoneme extends further via IFT. After that, the basal body migrates towards the plasma membrane, and the ciliary sheath membrane fuses with the plasma membrane, ultimately releasing the cilium into the extracellular space.

图1 纤毛形成的两种途径

Fig.1 Two pathways of ciliogenesis

严重减少, 但具体机制仍有待探索[24]。

1.1.2 PCV与母中心粒远端附属结构的结合 目前普遍认为, 纤毛囊泡停靠(docking)在母中心粒的远端附属结构上[25]。远端附属结构是附着在母中心粒远端微管上的9重对称的、径向突出的、风车状结构, 是母中心粒特有的结构, 这可能也是只有母中心粒具有纤毛形成能力的原因。在纤毛形成过程中, 远端附属结构转化为纤毛的过渡纤维(transition fibers)[26-27]。在电镜下, 过渡纤维呈现九叶螺旋桨样结构, 连接着基体微管和细胞膜[28]。最近已鉴定了超过10种定位在中心粒远端附属结构/纤毛过渡纤维上的蛋白质, 包括CEP164、CEP83(CCDC41)、CEP89(CCDC123)、SCLT1、LRRC45、ANKRD26、FBF1、TTBK2、KIZ和NCS1等[29-31]。研究已表明, 细胞中缺失CEP83、CEP164或CEP89蛋白, 都会造成基体锚定的异常[32-35]。CEP164可能通过CBY影响基体的锚定[36]。而CEP89则通过NCS1介导PCV与远端附属结构的结合。CEP89招募NCS1到远端附属结构上, 随后NCS1的N-端被豆蔻酰化(myristoylation), 从而使其具备了与囊泡膜连接的能力。豆蔻酰化是NCS1捕获PCV所必需的。然而, NCS1并不是唯一介导PCV锚定的分子, 还存在其他未被鉴定的蛋白[37]。CBY是否与NCS1在介导PCV锚定中存在功能冗余, 值得进一步探索。另外, 也有报道指出, MyoVa的球形尾部结构域(globular tail domain, GTD)与纤毛过渡区重要蛋白MKS5/RPGRIP1L的C2

结构域能够直接相互作用，使其靶向中心体。它们之间的相互作用也有可能在PCV与中心粒顶端的结合中发挥一定作用[22]。

1.1.3 纤毛囊泡的形成 聚集在中心粒顶端的PCV，招募调控膜形成的蛋白EHD1/EHD3、SNAP29和PACSIN1/2促使PCV融合形成一个大的纤毛囊泡。EHD1和EHD3蛋白之间有87%的相似性，可能存在一定的功能冗余性[38]。SNAP29是一个SNARE膜融合调控因子，与EHD1和EHD3直接相互作用，介导PCV之间的膜融合[38]。而PACSIN1/2能够帮助EHD1/EHD3形成和重塑脂质双层[39]。PASCIN具有F-BAR结构域，也被称为突触连接蛋白(syndapins)，能够形成同源或异源二聚体，具有感知膜弯曲和管状脂质双分子层的能力[40]。PASCIN不仅在PCV融合中发挥功能，而且它和EHD1/EHD3一起促进了膜通道(extracellular membrane channel, EMC)的形成[39]。膜通道与质膜的融合，使得纤毛得以释放到细胞外空间。

最近有研究发现，小分子GTP酶Rab34是细胞内纤毛形成途径特异性需要的，在细胞外途径的纤毛形成中不发挥作用[41-43]。在*Rab34*突变的细胞里，PCV的招募不受影响，但PCV向纤毛囊泡的转变受阻，表明它作用于EHD1/EHD3介导的膜融合过程[42]。

1.2 细胞外纤毛生成途径中的基体锚定

与细胞内途径不同，在细胞外纤毛生成途径中，母中心粒通过其远端附属结构直接在质膜上锚定并启动纤毛的形成。目前有关细胞外途径中基体锚定的研究甚少[44]。已有研究表明，MyoVa在该过程中仍发挥作用[23]，提示小的囊泡在该途径中仍然有一定的作用。透射电镜结果显示，在敲除CEP164或CBY的具有极性的呼吸道上皮细胞中，许多基体未能正确锚定到质膜上，而是滞留在细胞质中[36,45]。此外，这些细胞质中的基体结合囊泡的能力明显受损。这些结果表明，CEP164和CBY在极性细胞纤毛形成过程中的基体锚定中发挥着一定的作用。但CEP89-NCS1模块是否在极性细胞的纤毛形成起始中发挥作用尚不清楚。在细胞外途径中，有一个极其重要的问题亟需解答：中心粒在质膜上锚定的位置是随机的，还是特异性选择的？如果是后者，相关的分子机制是什么？

另外，最近有研究表明，在具有极性的马丁达比犬肾上皮细胞(Madin-Darby canine kidney, MDCK)中，胞质分裂中间体残余物(midbody remnant, MBR)在纤毛形成中发挥重要作用[46]。中间体残余物含有纤毛蛋白。在细胞分裂时的剪切过程中，中间体残余物位于细胞间紧密连接处的顶端边缘，被其中一个细胞继承，残余物随后在该细胞的顶端表面向停靠在质膜的中心体移动，与中心体相遇时，促进初级纤毛的形成。但中间体残余物与中心体对接的分子机制仍有待进一步探索。

1.3 远端附属结构/纤毛过渡纤维在基体锚定中的作用和进化

目前的观点认为，在哺乳动物细胞中，无论是细胞内纤毛发生途径还是细胞外纤毛发生途径，远端附属结构在基体与膜的结合中都发挥关键作用。但两种方式中远端附属结构与膜结合的分子机制是否相同仍不清楚。

一个值得探索和回答的问题是，在早期的进化中，纤毛形成过程中的基体锚定是否依赖于远端附属结构/纤毛过渡纤维？在无脊椎动物或单细胞纤毛生物中的纤毛形成过程中基体如何锚定以及是否也存在两种不同的方式，目前还了解甚少。在无脊椎模式生物秀丽隐杆线虫和果蝇中存在着保守的过渡纤维相关蛋白，虽然它们的中心粒缺少远端附属结构，但在纤毛形成时，仍然能够形成过渡纤维相关结构[47-48]。然而，我们的研究表明，这些过渡纤维相关蛋白的缺失并不影响线虫和果蝇的基体锚定和纤毛发生起始。特别是在果蝇中，我们发现，UNC/OFD1、CEP164和CEP89这三个关键的过渡纤维蛋白都不会影响纤毛形成过程中基体的锚定以及过渡区的装配[48]。除了介导基体锚定外，过渡纤维还在纤毛门控(ciliary gating)中发挥重要作用，调控纤毛蛋白的输入。FBF1作为过渡纤维调控纤毛蛋白输入的核心组分，它不参与基体的锚定，而是特异性地调节纤毛蛋白的输入。在线虫和果蝇中，FBF1都是高度保守的，我们发现它在纤毛物质运输方面的功能也是保守的[47-49]，这表明过渡纤维调控纤毛蛋白输入的功能在进化早期就已存在，而调控基体锚定的功能很可能是后期演化发展而来的。这一结果也提示我们，在进化早期，很可能存在其他机制来进行基体的锚定，相关机制值得进一步探索。

在果蝇的精母细胞中，电镜实验观察到在纤毛发生早期，纤维状物质从中心粒的远端延伸而出与细胞质膜直接连接，并且，膜的细胞质侧在这些纤维前面积累了一薄层电子致密物质[50]。随着纤毛轴丝

开始伸长, 细胞表面凸出, 质膜延伸形成纤毛样。目前并不清楚这些纤维状物质的分子基础, 但它们并不位于过渡纤维的位置, 且过渡纤维相关蛋白的缺失并不影响果蝇基体的锚定, 暗示其可能不是过渡纤维。

2 CP110去除的分子机制

CP110定位于中心粒的顶端, 被认为是一种帽子蛋白, 其主要作用是抑制中心粒的延伸。在纤毛形成过程的起始阶段, CP110需要被去除才能使中心粒微管得以延伸形成纤毛轴丝[51-52]。CP110在中心粒顶端的定位受到KIF24-MPP9-CEP97复合体的调控。KIF24(kinesin family member 24)是一种定位于中心粒顶端的微管解聚驱动蛋白, 它招募MPP9(M-phase phosphoprotein 9)到中心粒顶端, 随后MPP9通过与CEP97的直接结合将CP110-CEP97复合物募集到中心粒的远端[53]。CEP97是CP110在中心粒顶端作为微管帽子的重要辅助因子, 它与CP110直接相互作用形成复合体, 并且它们在中心粒顶端的定位相互依赖[54-55]。

有关CP110去除的分子机制, 受到了广泛的关注(图2)。多项研究表明, 泛素化介导的蛋白酶体降解途径在CEP97-CP110帽子复合体的去除中发挥作用。XIE等[56]的研究表明, 定位于中心体微卫星上的HERC2和MIB1是CP110泛素化的E3连接酶。EHD1能够介导微管依赖性的运输中心体微卫星物质和HERC2到母中心粒区域, 促使HERC2对CP110的泛素化, 进而被蛋白酶体降解。SHEN等[51]报道了线性泛素链组装复合物LUBAC(linear ubiquitin chain assembly complex)直接靶向CP110并催化其线性泛素化。随后, 位于母中心粒远端的前mRNA剪接因子PRPF8与CP110的线性泛素链相互作用, 并促进CP110从母中心粒上移除, 从而启动纤毛形成。此外, NAGAI等[57]报道了cullin-3-RBX1-KCTD10复合体是介导CEP97降解的E3连接酶, 在CEP97-CP110复合体的去除中发挥作用。

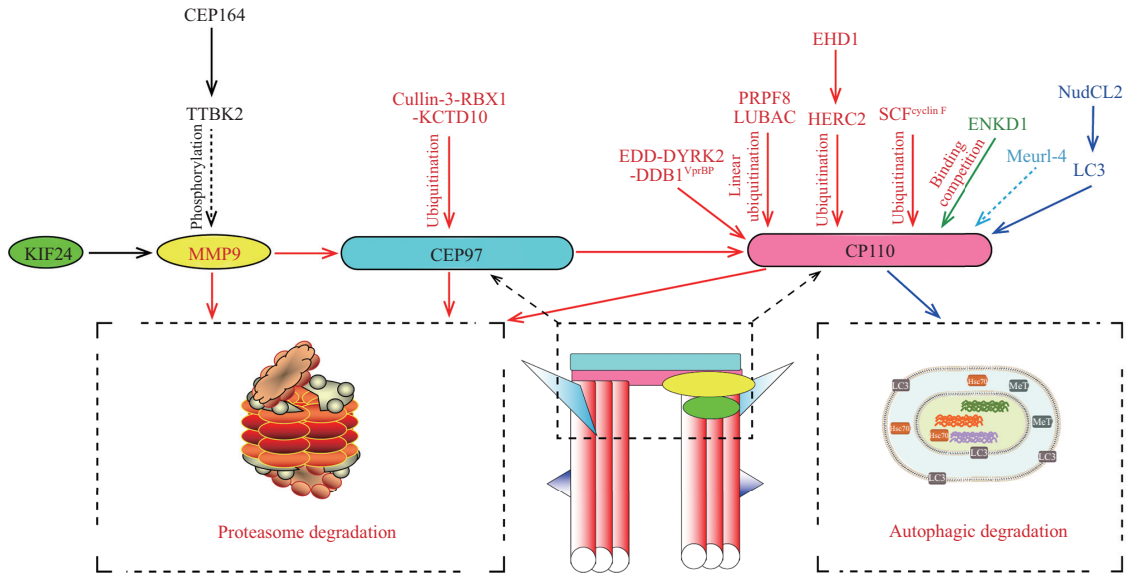
CP110在中心粒中具有多种功能, 除了在G_0/G_1期作为微管帽子蛋白外, 在S期和G_2/M期中还可以调节中心粒的复制、长度和稳态等。因此, 在整个细胞周期中其蛋白水平需要受到严格控制。已报道$SCF^{cyclin\ F}$泛素连接酶复合物和EDD-DYRK2-$DDB1^{VprBP}$复合物能够泛素化CP110, 而USP33是CP110的去泛素化酶, 它们调控中心体复制和稳态过程中的CP110水平[58-59]。但目前尚不清楚它们是否在G_0/G_1期CP110的去除中发挥作用。因此, 全面研究HERC2、MIB1、LUBAC和其他泛素连接酶在CP110泛素化和去除中的作用及相互关系将非常有意义。

此外, LIU等[60]发现CP110的去除也可以由自噬介导的降解来调节。他们发现, NUDCD2/NudC样蛋白2(NudC-like protein 2, NudCL2)作为一种新型选择性自噬受体, 通过自噬介导CP110从母中心粒去除。NudCL2倾向于定位在母中心粒上, 在血清饥饿后, 自噬体标记物LC3开始在母中心粒处与NudCL2相互作用, 诱导CP110的自噬降解, 最终促进纤毛形成。

另外, CP110的去除还受到多种蛋白的调节。已报道远端附属结构CEP164招募TTBK2, 促进CP110的去除以及纤毛形成[61]。HUANG等[53]的研究表明, MPP9能够被TTBK2磷酸化, 磷酸化后的MPP9通过泛素–蛋白酶体系统UPS被降解, 促进了CEP97-CP110的去除。但MPP9的E3连接酶仍未被鉴定。也有研究表明, Enkurin结构域蛋白1(Enkurin domain-containing protein 1, ENKD1)能够通过与CEP97竞争结合CP110, 从而调控该复合物从母中心粒的移除。一旦ENKD1缺失, CP110-CEP97的相互作用则显著增强, 从而使CP110滞留在母中心粒而无法启动纤毛的组装, 而敲除*CP110*可以显著逆转ENKD1缺失引起的纤毛形成缺陷[62]。还有报道称, Neurl-4是一种定位于子中心粒的CP110相互作用蛋白, 在纤毛形成时, 它易位至母中心粒促进CP110的去除和纤毛发生起始, 但具体机制不清楚[63]。除以上蛋白外, MARK4和Centrin2也被报道能够调控CP110的水平, 但作用机理尚不清楚[64-65]。

3 纤毛芽(ciliary bud)的形成

纤毛囊泡在中心粒/基体的顶端形成后, 随着CP110的去除, 轴丝从中心粒/基体的顶端延伸而出, 同时, 过渡区也开始组装, 纤毛膜逐渐扩张, 最终形成纤毛芽。因此, 纤毛芽的形成是过渡区组装和纤毛膜形成的协同过程。过渡区在电镜下呈现连接着纤毛基部微管和纤毛膜的Y字形连接结构(Y-linker)作为纤毛基部的扩散屏障(diffusion barrier)控制着纤毛蛋白质的进出。来自秀丽隐杆线虫和哺乳动物的研究表明, 过渡区主要由MKS复合体、NPHP复合体和CEP290组成[66]。其中, MKS复合体包含MKS1、MKSR1/B9D1、MKSR2/B9D2、MKS3/TMEM67、MKS6/CC2D2A、TMEM107、TMEM237、TMEM218等, NPHP复合物主要包含



在中心粒的远端, KIF24招募MPP9, 随后MPP9通过与CEP97的直接结合将CP110-CEP97复合物招募到中心粒的顶端。CP110的去除有多条途径。①通过降解MPP9: 定位于远端附属结构的CEP164招募TTBK2, TTBK2磷酸化MPP9, 促进泛素–蛋白酶体系统降解MPP9, 进而使CEP97-CP110复合体被移除; ②通过降解CEP97: cullin-3-RBX1-KCTD10复合体作为E3连接酶介导CEP97的降解, 在CEP97-CP110复合体的去除中发挥作用; ③通过竞争结合: ENKD1通过与CEP97竞争结合CP110, 从而促进CP110的去除; ④泛素–蛋白酶体系统降解CP110: EHD1促进中心粒招募HERC2, 随后HERC2对CP110进行泛素化, 最终使其被蛋白酶体降解; LUBAC直接靶向CP110并催化其线性泛素化, PRPF8与CP110的线性泛素链相互作用, 并促进CP110从母中心粒上移除; $SCF^{cyclin\ F}$泛素连接酶复合物和EDD-DYRK2-DDB1VprBP复合物能够泛素化CP110, 从而调控中心体复制和稳态过程中的CP110水平; Neurl-4也能促进CP110的去除, 推测通过蛋白酶体系统, 但具体机制不清楚。⑤自噬降解CP110: 在血清饥饿后, LC3开始在母中心粒处与NudCL2相互作用, 诱导CP110的自噬降解。

At the distal centriole, KIF24 recruits MPP9, and then MPP9 recruits CP110-CEP97 complex to the top of centriole through direct interaction with CEP97. There are several ways to remove CP110. ① Degradation of MPP9: TTBK2 is recruited to the distal appendages by CEP164, and then phosphorylates MPP9, promoting its degradation through the ubiquitin-proteasome system, resulting in the removal of CEP97-CP110 complex. ② Degradation of CEP97: the cullin-3-RBX1-KCTD10 complex acts as an E3 ligase, mediating the degradation of CEP97, leading to the removal of CEP97-CP110 complex. ③ Binding competition: ENKD1 competes with CEP97 to bind CP110, facilitating the removal of CP110. ④ Degradation of CP110 by the ubiquitin-proteasome system: EHD1 promotes the recruitment of HERC2 to centriole, leading to the ubiquitination of CP110, which is finally degraded by proteasome. LUBAC directly targets CP110 for linear ubiquitination. PRPF8 interacts with the linear ubiquitination chain of CP110 to aid its removal from the mother centriole. $SCF^{cyclin\ F}$ ubiquitin ligase complex and EDD-DYRK2-DDB1VprBP complex can ubiquitinate CP110, regulating its level during cell division. Neurl-4 can also promote the removal of CP110, likely through proteasome system, although the exact mechanism remains unclear. ⑤ Autophagic degradation of CP110: after serum starvation, LC3 interacts with NudCL2 at the mother centriole, initiating autophagic degradation of CP110.

图2 CP110去除的分子机制

Fig.2 The molecular mechanism of CP110 removal

NPHP8/MKS5、NPHP1和NPHP4等[67]。CEP290位于过渡区核心, 不仅可以结合MKS复合物, 也可以结合NPHP复合物。虽然过渡区组成成分已知, 但是关于它如何建立及Y字形连接结构的具体分子基础仍有待进一步研究。至于早期纤毛膜的形成过程目前仍了解甚少, 但多种证据表明, Rab11-Rabin8-Rab8复合物在早期纤毛膜的形成中发挥作用, 它们可能通过作用于囊泡的形成、运输、拴系和融合促进纤毛囊泡及早期纤毛膜的生长[13]。

最近, 以果蝇为模式生物, 我们课题组发现CEP290-DZIP1-CBY/FMA92复合体在纤毛芽的形成中发挥关键作用[68]。我们发现CEP290是果蝇纤毛发生起始所必需的, DZIP1是CEP290调控纤毛发生起始的下游关键因子, 其缺失突变体与*Cep290*缺失突变体表型一致[68]。我们进一步的研究发现, CEP290与DZIP1直接相互作用, 通过其N-端招募DZIP1, 随后DZIP1通过CBY/FAM92模块和Rab8通路促进早期纤毛膜的形成[68]。鉴于CEP290在过渡区组装中的重要作用, 我们认为, CEP290协调早期纤毛膜形成与过渡区的组装, 将过渡区的组装和纤毛膜的形成偶联。值得注意的是, 许多证据提示该机制在哺乳动物中可能也是保守的。首先, 有报道表明, 在DZIP1L缺失的小鼠中, 停靠有PCV/CV的中心粒数量明显减少, 即使可观察到具有CV的基体, 但也未观察到纤毛芽的形成[69-70], 而且结果发现DZIP1L缺失会影响CP110的去除和过渡区蛋白

MKS5/RPGRIP1L的招募, 表明DZIP1L在哺乳动物的过渡区建立和纤毛芽的形成中发挥作用[69]。其次, 在哺乳动物中, DZIP1/DZIP1L与CBY/FAM92模块的相互作用也是保守的, 而且DZIP1与Rab8相互作用在纤毛形成中也发挥作用[69]。最后, CBY、FAM92在哺乳动物基体锚定中的作用也有报道[71]。因此, CEP290-DZIP1-CBY/FAM92复合体在纤毛芽形成中的作用很可能是进化保守的。

4 纤毛发生起始相关纤毛病

由于纤毛广泛分布于人体的全身并具有重要功能, 其缺陷往往会导致人体多种组织器官的病变。目前已报道了大约35种由于纤毛结构异常或功能缺陷引起的人类遗传疾病, 包括常染色体显性遗传性多囊性肾病(autosomal dominant polycystic kidney disease, ADPKD)、肾单位肾痨(nephronophthisis, NPHP)、Bardet-Biedl综合征(Bardet-Biedl syndrome, BBS)、Meckel综合征(Meckel-Gruber syndrome, MKS)、Joubert综合征(Joubert syndrome, JBTS)、口–面–指综合征(oral-facial-digital syndrome, OFDS)等, 这些遗传疾病被统称为纤毛病[72]。许多与纤毛发生起始相关的蛋白已经被鉴定为纤毛病的致病基因。例如: 基体锚定相关蛋白CEP164(NPHP15)和CEP83(NPHP18)的突变会导致肾单位肾痨[73-74]; SCLT1、OFD1和C2CD3蛋白的突变会导致口–面–指综合征[75]; CP110去除相关蛋白TTBK2的突变会导致神经退行性疾病–脊髓小脑萎缩症(spinocerebellar ataxia, SCA)。纤毛芽形成相关蛋白DZIP1L的突变会导致常染色体隐性遗传性多囊肾病(autosomal recessive polycystic kidney disease, ARPKD)。表1中列举了与纤毛发生起始相关的主要蛋白的功能研究及相关纤毛病, 为更好地理解纤毛发生起始机制及相关遗传疾病提供了参考。

5 总结和展望

虽然初级纤毛被发现已有100多年的历史, 但长期以来被认为是退化且无功能的细胞器。然而, 在过去的20~30年里, 随着科研水平的进步和研究的深入, 我们对微小纤毛在哺乳动物发育和疾病中的重要功能有了更深入的了解, 同时对纤毛形成的分子机制也有了越来越多的认识。尽管如此, 纤毛形成起始的机制中仍存在许多未解之谜。因此, 在本综述中, 我们总结了已经了解的基体锚定、CP110的去除和纤毛芽形成机制, 希望能够为深入理解纤

表1 纤毛发生起始相关蛋白功能研究及其关联的纤毛病

Table 1 Functions of proteins related to ciliogenesis initiation and their associated ciliopathies

类型 Style	蛋白 Protein	纤毛相关的模式动物和哺乳动物细胞研究 Cilia-related studies in model organisms and mammalian cells					相关纤毛病 Ciliopathies
		线虫 *C. elegans*	果蝇 *Drosophila*	斑马鱼 *Zebrafish*	小鼠 Mouse	哺乳动物细胞 Mammalian cells	
Ciliary vesicle formation	MyoVa				*MyoVa*$^{-/-}$ (Dilute-Lethal) mice have severe seizures and die before postnatal day 21[76]; the cilia-related phenotype has not been reported yet	A plus-end filamentous-actin motor protein[76]; trafficking preciliary vesicle to mother centriole[23]; KO inhibits ciliogenesis in RPE cells and IMCD3 cells[77]	
	EHD1			Morphants show defects in ciliogenesis[38]	KO mice exhibit partial embryonic lethality, gross ocular defects, and developmental defects[38]; increased levels of microglobulin in the urine and high-frequency hearing impairment; male infertility; cilia appear normal in kidney[78]	Membrane shaping protein; preciliary vesicle fusion and ciliary vesicle formation[38]; knockdown impairs ciliation in RPE cells[56]	Patient with *Ehd1* (*R398W*) mutation had tubular proteinuria and high-frequency hearing loss[78]

续表1

类型 Style	蛋白 Protein	纤毛相关的模式动物和哺乳动物细胞研究 Cilia-related studies in model organisms and mammalian cells					相关纤毛病 Ciliopathies
		线虫 *C. elegans*	果蝇 *Drosophila*	斑马鱼 *Zebrafish*	小鼠 Mouse	哺乳动物细胞 Mammalian cells	
	EHD3			Morphants show defects in ciliogenesis[38]	Healthy and fertile[38]	Membrane shaping protein[38]	
	SNAP29				KO mice exhibit neonatal lethality[79-80]; the cilia-related phenotype has not been reported yet	Knockdown impairs ciliation in RPE cells[38]	
	PACSIN1/2			Disruption results in ciliary defects[39]	KO mice are viable and no ciliopathy defects[39]	Knockdown impairs ciliation in RPE cells[39]	
	Rab34				KO mice show ciliogenesis defects; exhibiting polydactyly, cleft lip and palate preweaning lethality[81]	Mediating ciliary membrane formation specifically in intracellular pathway; KO blocks ciliogenesis in fibroblast cells (MEFs, NIH/3T3, MCF10A, RPE-1)[41,82]	OFDS[83], skeletal ciliopathy[84]
Basal body docking	CEP164 (NPHP15)		Dispensable for ciliogenesis[48]	Morphants show ciliopathy phenotypes: tubule cysts, hydrocephalus, and retinal dysplasia[74,85]	KO mice show defects in both primary cilia and motile cilia, and exhibit male infertility, hydrocephalus, and kidney cysts[45,85-86]	Distal appendage protein; KO results in early defects in cilium assembly in RPE cells[87-88]	NPHP[29,74,89], SLS[90]
	CEP83 (NPHP18)					Distal appendage protein; blocking ciliogenesis initiation in RPE cells[33]	NPHP[29,73]
	CEP89		Essential for ciliogenesis, but dispensable for ciliogenesis initiation[48]			Distal appendage protein; KO affects ciliogenesis initiation in RPE-1 cells[33,37]	ADPKD[91]
	SCLT1				KO mice show defects in ciliogenesis, and exhibit ciliopathy phenotypes: abnormal craniofacial, cystic kidney, and polydactyly[92]	Distal appendage protein; KO affects ciliogenesis initiation in RPE cells[33]	BBS[93], OFDS[75], SLS[94], cone dysfunction[95]
	OFD1		Essential for ciliogenesis, but dispensable for ciliogenesis initiation[96]		Required for cilia formation and left-right axis specification; KO mice die early during gestation, displaying neural tube closure defects and polydactyly[97-98]	Required for distal appendage formation; regulating ciliogenesis initiation and inhibiting centriole elongation[99]	OFDS[100-102], JBTS[102-104], Retinitis pigmentosa[105], SGBS[102]

续表1

类型 Style	蛋白 Protein	纤毛相关的模式动物和哺乳动物细胞研究 Cilia-related studies in model organisms and mammalian cells					相关纤毛病 Ciliopathies
		线虫 *C. elegans*	果蝇 *Drosophila*	斑马鱼 *Zebrafish*	小鼠 Mouse	哺乳动物细胞 Mammalian cells	
	C2CD3 (OFD14)				Required for cilia formation; KO mice show embryonic lethality and exhibit ciliopathy related phenotypes[106-107]	Required for distal appendage formation; regulating ciliogenesis initiation and promoting centriole elongation[108]	OFDS[106,109-110], JBTS[110-111]
CP110 removal	TTBK2				A null mutation blocks Shh signaling and ciliogenesis, leading to death at midgestation[112-113]	Recruited by Cep164, required for CP110 removal[113]	SCA[114-115]
	KIF24					Aberrant cilia assembly in RPE-1 cells[116]	
	MPP9				KO mice show a higher percentage of ciliation; exhibiting abnormal development and a twisted body axis at midgestation[53]	Knockdown promotes cilia formation in RPE cells[53]	
	CEP97		Required for centriole integrity and ciliogenesis[117]			Ciliogenesis defects in 3T3 fibroblasts and RPE cells[54]	
	CP110		Dispensable for cilia formation[118]		Required for SDA assembly and ciliary vesicle docking; KO mice die shortly after birth because of organogenesis defects, and exhibiting polydactyly, cardiac abnormalities, severe skeletal defects[12] Shh signaling is impaired in null embryos, and primary cilia are reduced in multiple tissues	Aberrant centriole elongation[54]	
Ciliary bud formation	Rab8				No abnormalities in cilia of *Rab8a* and *Rab8b* double KO mice[119]	Abnormal cilia morphology in MEFs[120]	
	Rab11				Embryonic lethal[121]	Inhibition of Rab11 compromises ciliogenesis in RPE cells[122]	
	Rabin8						

续表1

类型 Style	蛋白 Protein	纤毛相关的模式动物和哺乳动物细胞研究 Cilia-related studies in model organisms and mammalian cells					相关纤毛病 Ciliopathies
		线虫 *C. elegans*	果蝇 *Drosophila*	斑马鱼 *Zebrafish*	小鼠 Mouse	哺乳动物细胞 Mammalian cells	
	CEP290	Involved in ciliogenesis; essential for MKS module localization at transition zone[123]	Essential for ciliogenesis initiation and transition zone assembly; recruiting DZIP1 to initiate ciliogenesis[68]	Acute *cep290* morpholino knockdown caused severe cilia-related phenotypes, whereas defects in chronic deletion mutant were restricted to photoreceptor cilia[124]	Exhibiting ciliopathy phenotypes: early-onset retinal degeneration, anosmic phenotype, hydrocephalus, and severely cystic kidneys[125-127]	Aberrant cilia biogenesis; early defects in ciliary membrane formation[128]	LCA[129], NPHP[130], SLS[131], JBTS[131-132], MKS[133], BBS[134]
	DZIP1		Blocking ciliogenesis at the initiation stage[68,135]	Defects in cilia formation[136-137]	Required for ciliogenesis and Hedgehog signaling; death during embryogeneisis[69,138]	Required for ciliogenesis[138]	MVP[139]
	DZIP1L				DZIP1L binds Cby, promotes ciliary bud formation; KO mice exhibit embryonic lethal, hedgehog signaling defects, enlarged brain and polydactyly[70]	KO has no primary cilia in MEF cells[138]	ARPKD[140]
	CBY		Required for cilia formation[36,141]		KO mice exhibiting cilia related phenotypes: chronic airway infection and rhinitis and sinusitis[36,142]	Required for cilia formation; interacting with DZIP1 and FAM92[69]	
	FAM92A/B		Involved in cilia formation[69,135]		*Fam92a* KO mice show abnormal digit morphology, including metatarsal osteomas and polysyndactyly[143]	Required for cilia formation in RPE cells; interacting with Cby[71]	PAP[143]

OFDS: 口–面–指综合征; NPHP: 肾单位肾痨; ADPKD: 常染色体显性遗传性多囊肾病; BBS: Bardet-Biedl综合征; SLS: Senior-Loken综合征; JBTS: 朱伯特综合征; RP: 视网膜色素变性; SGBS: 辛普森变异综合征; SCA: 脊髓小脑性共济失调; ARPKD: 常染色体隐性遗传性多囊肾病; MVP: 二尖瓣脱垂; MKS: Meckel-Gruber综合征; LCA: Leber先天性黑蒙; PAP: 轴后多指畸形。

OFDS: oro-facio-digital syndrome; NPHP: nephronophthisis; ADPKD: autosomal dominant polycystic kidney disease; BBS: Bardet-Biedl syndrome; SLS: Senior-Loken syndrome; JBTS: Joubert syndrome; RP: retinitis pigmentosa; SGBS: Simpson-Golabi-Behmel syndrome; SCA: spinocerebellar ataxia; ARPKD: autosomal recessive polycystic kidney disease; MVP: mitral valve prolapse; MKS: Meckel-Gruber syndrome; LCA: Leber congenital amaurosis; PAP: postaxial polydactyly.

毛形成起始的分子机制提供参考。目前已经报道了许多纤毛形成起始过程中的基因突变与纤毛病有关，但是这些纤毛病发生的具体机制仍有待探索。未来，通过解析更多的母中心粒顶端/纤毛基部蛋白在纤毛发生起始中的分子功能，并使用多种模式生物来研究纤毛形成起始的机制，将为纤毛形成起始机制的解析以及纤毛病致病机理的理解作出重要贡献。

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