

胚胎结纤毛运动的生物力学研究进展

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摘要 纤毛是细胞的重要功能性细胞器, 可感知细胞外的机械和化学信号, 并协助信号传导引发细胞应答. 在脊椎动物胚胎发育早期, 胚胎结中存在若干旋转运动的单细胞纤毛, 具有9+0的超微结构, 即其轴丝主要组成表现为9个环绕排列的双联体微管. 相邻双联体微管间的动力蛋白通过ATP水解为纤毛运动提供动力和控制, 从而引起纤周流体的左向流动, 进而引起胚胎对左右轴方向的感知. 胚胎结纤毛的结构和功能异常会引起严重的发育障碍, 其相关探索对理解脊椎动物的发育过程有着重要作用. 自20世纪70年代起, 越来越多的研究者对纤毛进行了多方面研究, 本文从分子、细胞和细胞外流体3个层级, 对这些研究进展进行了总结.

关键词 胚胎结纤毛, 动力蛋白, 胚胎结流体, 数值模拟

纤毛最初是在1675年被荷兰光学显微镜专家Antoni van Leeuwenhoek发现的, 但直到电子显微镜的出现, 才有越来越多的科学家关注纤毛领域. 纤毛是一种从细胞表面伸出的细胞器, 它体形微小, 基本结构包括轴丝和纤毛膜, 可以感知细胞外的机械和化学信号变化, 并协助信号转导引发相应的细胞应答. 纤毛功能缺陷是多种人类发育或退化性疾病发生的基础, 这些疾病被统称为纤毛病, 如鼻窦炎、多囊肾病、内脏发育随机分布等^[1].

纤毛一般被分为两类: 9+2型纤毛和9+0型纤毛, 其中9是指存在于纤毛轴突中外围环绕排列的9个双联体微管, 2或0是指中央单微管对. 在哺乳动物中, 9+2型纤毛是运动纤毛, 多存在于气道、脑和输卵管中, 径向辐条在这种纤毛中桥接外周双联体微管和中央微管对. 不动的9+0型纤毛被称为初级纤毛, 多起到感受器的作用. 而运动的9+0型纤毛, 如胚胎结纤毛, 能够通过自身的旋转运动驱动纤周流体单向流动从而激活胚胎左

右轴信息, 如果这个过程被打破会导致内脏不对称组织发生偏侧性缺陷. 在新生儿中, 胃肠道、脾、心脏出现偏侧性缺陷的发生率约为1/10000^[2].

纤毛和左右不对称之间的连接起源于20世纪70年代中期, Afzelius^[3], Pedersen和Mygind^[4]分别研究Kartagener综合征时发现这种遗传病是由不动纤毛引起的. 胚胎结纤毛引导胚胎打破左右对称的作用引起了广泛关注^[5-10]. 科学家们对纤毛的研究大致可以分为3类: 分子层级、细胞层级和细胞外层级, 随着相关研究的开展, 人们对胚胎结纤毛的结构和功能地了解不断加深, 但纤毛发生运动的内部机制以及胚胎结流体流动传播的机制仍存疑问, 单一尺度量级的研究已经无法解决这些问题, 耦合相关尺度进行跨尺度的研究是一个迅速发展的热点.

1 分子层级的研究

在脊椎动物发育初期, 胚胎表面会形成暂时性

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的凹陷结构(胚胎结). 在小鼠(*Mus musculus*)胚胎中, 当从胚胎结上方(腹侧)观察时大致呈梨形, 约50 μm 宽、10 μm 深, 覆盖着Reichert膜, 并充满胚胎外液. 在胚胎结腔基底存在200~300个1~4 μm 长的运动纤毛. 从上方观察, 这些纤毛顺时针旋转, 且周期约为10 Hz^[11], 纤毛的旋转运动能驱动纤周流体流动. 纤毛的运动和变形由内部分子结构决定. 由于近来的分子结构分析^[12~14], 纤毛轴丝的成分逐渐被澄清. 图1是胚胎结纤毛轴丝结构示意图. 每个外周双联体微管都是由一个A微管和一个B微管组成, 为纤毛运动提供灵活的支架, 微管是 α -和 β -微管蛋白二聚体聚合的长的中空圆柱体, A微管是由13根原丝组成的完整圆柱体且在横截面上呈椭圆形, B微管是由11根原丝构成的不完整圆柱体^[15]. 纤毛运动是由外周相邻双联体微管对之间滑动产生的, 而将化学能转化为微管滑动机械力的马达是轴丝动力蛋白^[16], 一般运动纤毛具有内部和外部动力蛋白臂. 通过纤毛的透射电子显微照片发现, 小鼠胚胎结纤毛的外部动力蛋白臂具有两个头部, 但没有识别到内部动力蛋白臂^[17,18]. 目前还无法确定胚胎节纤毛缺少内动力蛋白臂对其运动的影响, 但有研究发现缺少辐条是进行旋转运动的必要条件之一^[17].

轴丝动力蛋白和细胞质动力蛋白具有相同的基本结构. X射线晶体学、核磁共振(nuclear magnetic resonance, NMR)、电子显微镜单颗粒分析和电子断层扫描已被用于研究动力蛋白的结构生物学. 动力臂由重链、中间链、动力蛋白轻链和辅助蛋白组成^[19], 重链可以分为N-端尾部、连接子、6个成一个环的AAA+域、一个卷曲螺旋茎、微管结合区域(microtubule binding domain, MTBD)和C端结构域. 外部动力蛋白是负端定向电动机, 其通过与三磷酸腺苷(adenosine triphosphate, ATP)水解相关的构象依赖性变化附着并沿微管行进^[12]. 动力蛋白的尾巴铆钉在双联体微

管的A小管上, 且头部伸向相邻双联体微管的B小管上, 当ATP结合到AAA+头部时, MTBD迅速从微管分离, 而ADP-Pi和ADP结合状态分别对应弱结合和强结合^[12,20,21]. 当动力蛋白的头部与目标小管接触时, 会在相邻的双联体微管之间产生一对作用方向相反的剪切力从而驱动这对微管进行滑动^[22]. 虽然动力蛋白分子力的产生机制已经被阐明, 但这仍不足以解释纤毛的运动机制.

纤毛或鞭毛轴丝的运动机制一直是纤毛领域活跃的研究方向之一, 科学家们已经进行了一系列的实验、理论和计算研究^[23~33]. 例如, Brokaw^[23]开发了一种滑动丝模型, 该模型由弯曲关节连接的直线段组成且弯曲激活由鞭毛的曲率局部控制. Hines和Blum^[24]在1984年改进了模型, 考虑了三维滑动丝改进延迟曲率反馈的鞭毛连续模型. Sugino和Naitoh^[25]认为相邻双联体微管之间的距离会影响动力蛋白桥的形成. Stair和Matsuoka^[32]在1989年基于开关点假说(Switch Point)提出了一个异步动力蛋白活动模型, 表明摆动的多样性可能通过动力蛋白臂激活与非激活状态切换时间的变化来解释. Lindemann^[33]针对纤毛超微结构中动力蛋白与双联体微管的活动提出了几何离合器理论(Geometric Clutch). Hilfinger和Julicher^[26]也开发了一个圆柱模型, 使用二次弹性能确定圆柱体的弯曲和扭转阻力, 并且以傅里叶模式表示相邻双联体微管之间的内部滑动位移. Yang等人^[27]利用浸入式边界法, 通过切换两个微管之间的动力连通性, 他们成功地模拟了纤毛运动的动力和恢复冲程. Eloy和Lauga^[28]用细长体理论研究了壁弹性细丝的时间周期变形, 研究结果表明能量最优的运动学取决于纤毛的弯曲刚度. 在这些先前的研究中, 纤毛轴丝被表示为一根或两根细丝, 并且不考虑九微管结构的全部动力学. 纤毛轴丝有着复杂的性质, 应该明确轴丝的完整三维力学, 以更好地理解纤毛运动. 此外, 轴丝动力蛋白的特征是这些动力蛋白需要在精确的时间和空间控制下被激活. 通过模拟试验发现, 只有当相邻双联体微管上动力蛋白的激活传递沿顺时针方向进行时, 胚胎结纤毛才能进行顺时针的旋转运动^[29]. 动力蛋白激活的传递方向猜测是有两种可能的原因, 一种是双联体微管的极性, 也就是说动力蛋白的位置及其头部的朝向可能决定了激活传递方向^[34]; 另一种是距离控制假说, 也就意味着相邻双联体微管之间的距离变化会影响它们

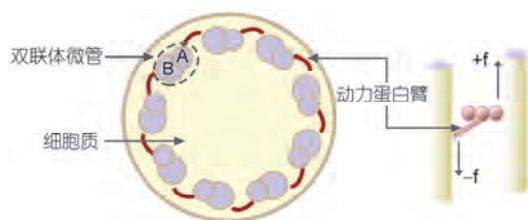


图1 (网络版彩色) 胚胎结纤毛轴丝结构示意图

Figure 1 (Color online) The diagrammatic representation of axonemal structure

之间动力蛋白桥的建立和解离,距离变小有利于动力蛋白桥的建立,间距增大有助于动力蛋白与目标微管的解离^[35].本研究组^[29-31]已基于上述理论及研究基础,构建了胚胎结纤毛三维理想化模型和真实模型(图2(a)),计算结果在一定程度上证明了距离控制假说的合理性,并提出了沿着每对双联体微管之间动力蛋白的激活速度在纤毛底部较快而尖端部分较慢的规律.

2 细胞层级的研究

在1998年,Nonaka等人^[36]使用荧光珠的体外实验发现,小鼠正常的左右(left-right, LR)不对称发育是源于胚胎结内流体的向左流动(nodal flow).这种流动如何转变为不对称发育呢?对于胚胎结流体流动赋予侧向性的机制已经提出了3种主要的假说,形态发生素假说^[36]、胚胎结囊泡包裹模型^[37]和双纤毛假说^[38].

胚胎结囊泡包裹模型是基于形态发生素假说提出的一种模型,它的基本原理是认为形态发生素在胚胎结左右两侧浓度差异是决定左右不对称的因素(化学传感)^[39],如图3(a)所示.双纤毛假说是指在胚胎结中向后倾斜的运动纤毛产生向左的流体流动,而不能动的纤毛作为感受器,感知流体流动并放大机械负荷产生左侧信号^[38,40],如图3(b)所示.虽然目前的实验观察技术并不能完全验证这些机制的正确性,但已有相关数值模拟研究证明了其在一定程度上的合理性.Cartwright等人^[11]通过数值模拟的方法

进行了进一步的实验,模拟研究发现,胚胎结囊泡包裹(nodal vesicular parcels, NVPs)的小颗粒确实会因为流体的流动聚集在胚胎结的左侧,但由于这些囊泡的生物物理特性,它们所包含的形态发生素可能不会通过纤毛或流动的机械破裂传递到周围的细胞,这需要特殊的生化机制引起破裂,这种想法可以将胚胎结囊泡包裹模型与双纤毛假说结合起来.Chen等人^[41]提出的一个主动和一个被动纤毛的双纤毛计算模型,研究结果表明,主动纤毛的顺时针旋转能为周围的被动纤毛产生逆时针方向的椭圆旋转和整体的横向位移,支持了双纤毛假说.非运动初级纤毛的机械感觉假说最常见的解释是通过纤毛内钙可渗透离子通道响应机械力^[42].但Delling等人^[43]的研究证明,初级纤毛不能通过 Ca^{2+} 信号传导感知机械力,并在体内观察到,当左右方向建立时,胚胎结腔里只有5%的不动纤毛,初级纤毛数量过低会无法感知流体的流动方向,这就对双纤毛假说提出了挑战.考虑到运动纤毛可能是化学传感器^[44],LR对称性破坏可以依靠机械感受和化学感应机制的组合,这些机制可以并行工作.不论是胚胎结囊泡包裹模型还是双纤毛假说都需要进一步的验证或更直接的证据证明.

纤周流体的有效流动离不开胚胎结纤毛位置和状态.Cartwright等人^[45]通过斯诺克斯方程的“旋转”点转矩奇异解来建立流体动力学模型,将纤毛假设为一个圆柱体.研究发现,当纤毛垂直于细胞表面时,纤周流体呈现涡流状态,当纤毛倾斜且离表面越近时,就会在纤毛上方产生更强的单向流动,研究表

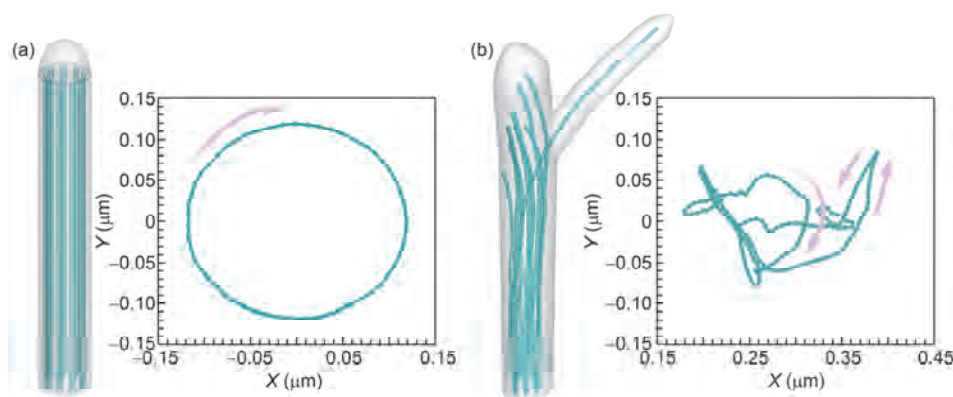


图2 (网络版彩色)数值计算的模型及结果. (a) 左侧是正常胚胎结纤毛计算模型, 右侧是在正弦动力蛋白激活模式下纤毛顶点运动轨迹; (b) 左侧是 inv 变异胚胎结纤毛计算模型, 右侧是距离控制方案正弦动力蛋白激活模式下纤毛顶点运动轨迹

Figure 2 (Color online) The models and results numerical simulation. (a) The left side is the calculation model of wildtype nodal cilia, the right side is the ciliary tip trajectory in the sine dynein activation mode; (b) the left side is the calculation model of inv nodal cilia, and the right side is the ciliary tip trajectory in sine dynein activation mode of the distance-control scenario

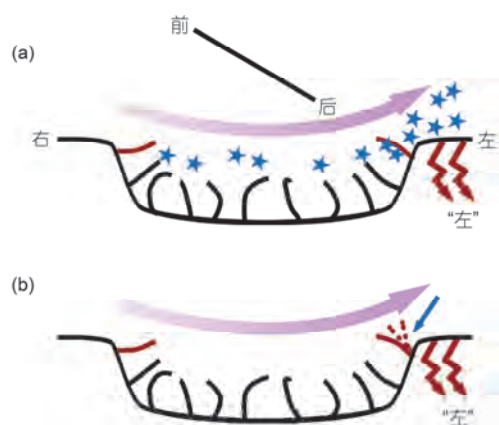


图3 (网络版彩色)胚胎结流体作用机制的模型。(a)形态发生素假说,运动纤毛顺时针旋转将形态发生素或胚胎结囊泡包裹送到左侧^[36,37]; (b)双纤毛假说,运动纤毛产生的流体流动可以被不动纤毛感知^[38]

Figure 3 (Color online) Models to explain the function of nodal flow in left-right asymmetry. (a) The “morphogen” hypothesis, clockwise rotating of motile cilia transports the morphogen or NVPs towards the left side of the node^[36,37]; (b) the “two-cilia” hypothesis, nodal fluid flow generated by the motile cilia is sensed by immotile cilia^[38]

明,排成阵列的后倾纤毛是产生纤周流体单向流动的条件,随后Okada等人^[46]的实验研究证明了这一点。Omori等人^[47]基于三维胚胎结纤毛轴丝模型通过使用有限元-边界耦合方法研究了人造胚胎结纤毛运动所产生的平流。研究发现,胚胎结纤毛的旋转轨道依赖于动力蛋白的激活频率,而且纤周流体强度在远离纤毛的地方衰减为 r^{-3} ,与此同时也发现纤毛 $2\pi/9$ 的倾斜角度(纤毛与垂直于表面方向的夹角)就可以产生向左的流体流动。Shinohara等人^[17]和本团队的合作实验表明,缺少径向辐条是胚胎结纤毛旋转运动的关键,而且外周双联体微管的规律的环形排列也是纤毛进行稳定的旋转运动必不可少的条件。本研究组在此基础上针对小鼠*inv*变异胚胎结纤毛进行了计算模拟研究,证实了距离控制假说的合理性,也说明变异胚胎结纤毛9个双联体微管形态的改变会影响胚胎结纤毛进行有效稳定的旋转运动,从而对纤周流体的流动造成一定的影响(图2(b))。

3 细胞外流体的研究

目前的研究中发现,除了小鸡(*Gallus gallus domesticus*)和猪(*Sus scrofa domestica*)以外,在非洲爪蟾(*Xenopus laevis*)的原肠腔顶板(gastrocoel roof plate, GRP)和斑马鱼(*Danio rerio*)库氏囊泡(Kupffer's vesicle, KV)都存在类似的旋转纤毛驱动流体流动促

进左右不对称形态的发生。与小鼠胚胎结相比,这两种左右发育的组织者有一定的不同。非洲爪蟾只有左侧的GRP需要流体流动^[48],斑马鱼KV的腹侧和背侧表面存在运动纤毛并形成左侧流体流动^[49,50]。这说明定向的纤周流体流动对左右不对称发育具有重要意义。Watanabe等人^[51]研究发现,小鼠*inv*突变体中的侧向缺陷通过人为的快速向左流动来纠正,这说明快速的左向流体流动是胚胎正确进行左右判断的一个必要条件。从平流的角度看,体内胚胎结是封闭的结构,被Reichert膜覆盖,所以向左流动的流体必须向右返回^[36,45]。但在小鼠胚胎结中向右的流动没有被观察到^[52],这是由于纤毛后倾使得靠近细胞表面的向右流动的流体在黏滞阻力的作用下无法形成连续的流体层^[53,54]。

纤周流体流动既能传输小分子信号,也能通过产生的机械力影响胚胎发育^[55]。虽然左右信号传递和感知机制还不明确,但左右不对称建立的分子层级机理已在众多科学家的努力下被逐渐阐明。最初,在小鼠胚胎中,Nodal, Wnt以及它们的拮抗物Cerberus like-2(*cerl2*)在结旁冠状细胞中均匀地表达,通过胚胎结的左向流体开始流动导致左侧结旁冠状细胞胞内 Ca^{2+} 增加并触发*Cerl2*的mRNA的降解^[56]。*Cerl2*的减少,减轻了对Wnt和Nodal的抑制作用,随后通过Wnt-*Cerl2*的互联反馈环进一步减少左侧*Cerl2*的mRNA的量,Nodal正调控自身的表达从而放大初始不对称^[57]。然后不对称的结旁Nodal信号被转移到中胚层(lateral plate mesoderm, LPM)。在左侧中胚层(L-LPM)中,Nodal正调节自身的表达,并诱导其拮抗剂Lefty1和Lefty2的表达抑制Nodal的活性^[58]。被诱导的Lefty1的表达在中线会建立一个屏障阻碍Nodal向右侧中胚层(R-LPM)的转移^[59]。在之后的发育过程中,这些初始不对称被稳定并传播,从而指导内脏器官的不对称生长。机械力在控制哺乳动物胚胎早期不对称中的作用仍存在争议。但由透明带(卵细胞外包裹的非细胞膜)所施加的机械力被认为会影响细胞排列和定位胚胎轴^[60]。纤周流体流动会造成胚胎结内的不动纤毛变形从而激活不对称的钙内流^[40,61]。

现已有几种方法来可视化 and 量化纤毛驱动的流体流速:流动微珠的粒子图像测速(particle image velocimetry, PIV)、人造流体灌注测速、数值模拟计算测速等。Okada等人^[62]使用被动示踪剂测得正常小

鼠胚胎结的单个纤毛产生的流速约为20~50 $\mu\text{m/s}$; 后来, 通过将人造流体灌注到小鼠胚胎的实验发现, 胚胎结流体以110 $\mu\text{m/s}$ 的流速流动可以诱导正常的L-R发育^[46], 这说明通过示踪剂测量的流速可能低估了胚胎结流体的流动. 此外, Chen等人^[29]用数值模拟方法计算得到的单个纤毛产生的平均流速可达139 $\mu\text{m/s}$, 这种方法更大地估算了胚胎结流量的最大速度, 也有助于进一步研究胚胎结内的信号传输.

4 结论与展望

综上所述, 关于胚胎结纤毛多方面的研究揭露了胚胎结纤毛的结构和功能, 但对于其运动的内部机制和纤周流体流动传递机制还不明确. 目前的研究还存在着一些局限: 关于单个纤毛的实验, 样本获取较为困难, 实验周期也较长, 而且通过现在的提取技术获得的单个纤毛已经失去活性, 这给直接观察纤毛运动内部机制带来了不便; 另外, 观察胚胎结流体流动需要破坏胚胎结上方的覆膜, 这破坏了原有的流动环境, 也使得原有的流体流失无法进行直接

观察. 跨尺度模拟分析能够打破单一尺度量级固有局限性, 抓住不同时空尺度下结构响应特征、阐明关键性结果并且能模拟无法在实验条件下观察的结构变化过程. 当然, 数值模拟还存在着一些缺陷, 如在模拟分析过程中往往对边界条件和材料属性进行了简化, 而且结构的离散化程度不同, 得到的结果和精度也有一定的差异, 这些都会或多或少地降低分析结果的可信度. 但是数值模拟能够模拟较复杂的过程, 可以快速得到结果, 也可以施加实验方法达不到的条件, 能够提供很好的平台去研究纤毛运动机制和纤周流体的流动传播机制. 针对已有的纤毛数值模拟研究, 现存的问题有: 纤毛各部分的属性测定还不精确; 一些数值模拟得出的结论还无法通过实验进行验证. 在未来的研究工作中, 一方面实验研究能检验数值模拟结果的可行性, 对于现有技术的改进势在必行; 另一方面需要更先进的胚胎结纤毛属性测定技术的支持; 此外, 优化仿真模型, 将正常胚胎结纤毛和变异胚胎结纤毛的研究进行相互验证也是得到更准确纤毛运动机理分析的方法之一.

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Summary for “胚胎结纤毛运动的生物力学研究进展”

Advances in biomechanical studies of nodal ciliary movement

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In recent years, cilia have become a research hotspot in the biomedicine field. As the important functional organelles of cells, cilia can not only perceive extracellular mechanical and chemical signals, but also transduce signals to initiate cellular responses. Based on ultrastructural and functional differences, cilia can be classified as immotile cilia or motile cilia. The immotile cilia are also called primary cilia, and its principal functions are sensing cellular antennae and transducing various morphogenetic and sensory signals. By contrast, motile cilia are usually present on a cell's surface, and the presence of dynein arms confers them with the ability to beat. Even though exteriorly the vertebrate body is bilaterally symmetric, the internal organs and associated vasculature show a clear left-right (L-R) asymmetry in the placement and patterning. L-R asymmetries in embryos occur earlier than visceral organs. Thus, for bilaterally symmetric embryo, it is a challenging problem to distinguish left from right. In the early stage of vertebrate embryonic development, there are several rotating monocilia in the embryonic node, with a 9+0 ultrastructure. Namely, the main composition of the axoneme is represented by nine surrounding doublet microtubules. The dynein in the axoneme forms bridges among neighbouring microtubule doublets, and it provides the power and control for cilia movement through ATP hydrolysis. Then, the nodal cilia rotate to drive the surrounding fluid to flow leftward, which causes the embryo to sense the left and right axis directions. Beyond that, abnormalities in the structure and function of nodal cilia can lead to serious developmental disorders. Thus, the study of nodal cilia contributes to our understanding in the developmental processes of vertebrates. The connection between cilia and L-R asymmetry originated in the mid-1970s. Through the studies of Kartagener syndrome, Afzelius and Pedersen found that the Autosomal recessive genetic disorder was caused by immotile cilia. From then on, more and more researchers have studied cilia in many aspects. Although the understanding of the structure and function of nodal cilia continues to deepen, the internal mechanism of cilia movement and the mechanism of nodal flow propagation remain questionable. This paper will summarize the progress of these studies from the molecular, cellular, and extracellular fluid levels. Above all, the related researches on cilia structure, dynein structure, and numerical simulation of dynein activity model are introduced. Furthermore, based on morphogen hypothesis, nodal vesicular parcel (NVP) hypothesis and two-cilia hypothesis, the role of nodal cilia in embryonic development and the effect of cilia position and state on pericapillary fluids are described. After that, the necessity of unidirectional nodal flow for embryo development is introduced from the aspects of transmitting small molecule signals and generating the mechanical force. At the same time, several methods for measuring flow velocity are introduced. Finally, the problems to be solved in the biomechanical research of ciliary movement are summarized and prospected. Cross-scale simulation analysis can break the inherent limitations of single-scale magnitude. In the future, research work can be carried out in the following aspects: improving experimental observation techniques, measuring the precise properties of the nodal cilia, and optimizing simulation models.

nodal cilia, dynein, nodal flow, numerical simulation

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