《中国科学》杂志社
SCIENCE CHINA PRESS

How is asymmetry determined in the embryo?

哺乳动物早期胚胎第一次细胞谱系形成

聂晓庆, 卢绪坤, 李磊*

中国科学院动物研究所, 干细胞与生殖生物学国家重点实验室, 北京 100101
* 联系人, E-mail: lil@ioz.ac.cn

摘要   哺乳动物早期胚胎发育过程中, 全能的受精卵和早期卵裂球如何打破对称分裂并建立第一次细胞谱系, 是发育生物学乃至生命科学研究最具挑战的科学问题之一. 为研究哺乳动物第一次细胞谱系建立, 早期研究提出过多种理论模型, 但都不能很好地解释早期胚胎的可塑性. 近年来, 随着显微观察、生物数学、以及单细胞测序等技术的发展, 人们认识到哺乳动物早期胚胎中, 细胞内大分子的表达和分布、细胞的位置和极性, 以及细胞间相互作用等多种因素, 造成了同一个胚胎中的不同细胞差异显著, 即早期胚胎的异质性. 哺乳动物早期胚胎的异质性可能是其可塑的主要原因. 哺乳动物早期胚胎中不对称分裂的打破和第一次细胞命运决定是一个高度动态和复杂的过程, 需要新的技术和理论来阐述这一重要生命现象.

关键词   哺乳动物, 小鼠, 早期胚胎发育, 第一次细胞谱系建立, 不对称分裂, 细胞命运决定

哺乳动物的生命起始于高度特化的精子和卵子通过受精作用形成的具有全能的受精卵(即合子, zygote), 受精卵经过卵裂形成不同卵裂期胚胎, 然后发育形成包括分子特性、位置及命运均不同的两种细胞组成的囊胚(blastocyst)[1~3], 经过进一步增殖和分化, 最终形成包括各种类型细胞的个体. 着床前胚胎发育(pre-implantation development)是哺乳动物特有的发育过程, 包括从受精卵到囊胚等多个发育时期(图1). 着床前各期胚胎中的卵裂球具有很强的可塑性和异质性[4~6]. 这些生物学特征加上研究材料的稀缺制约了相关领域的发展. 目前, 人们对着床前胚胎发育认知非常有限, 早期胚胎对称分裂的打破和第一次细胞谱系的形成是生命研究领域最具挑战的科学问题之一. 本文以哺乳动物中应用最为广泛的小鼠(*Mus musculus*)为例, 简单描述着床前胚胎发育过程和第一次细胞命运决定的经典理论, 主要介绍和讨论相关领域的最新发展, 以期为人类生命起始和

干细胞命运调控等提供理论参考.

1 小鼠着床前胚胎发育和早期细胞谱系形成

小鼠着床前胚胎发育过程中, 伴随着细胞的不对称分裂, 经历了两次细胞命运决定. 第一次细胞命运决定中, 发生两次主要的不对称细胞分裂(8-到16-细胞和16-到32-细胞两个阶段), 进而产生囊胚, 它包含分子特性和位置均不相同的两种细胞, 即滋养外胚层(trophectoderm, TE)和内细胞团(inner cell mass, ICM)细胞[3]. 此后, 滋养外胚层继续发育成为胚外外胚层(extraembryonic ectoderm, ExE)和外胎盘锥(ectoplacental cone); 而内细胞团经过增殖和第二次细胞命运决定形成原始内胚层(primitive endoderm, PE)和上胚层(epiblast, EPI), 它们为三胚层形成、器官原基发生和胚胎继续发育打下基础[7,8].
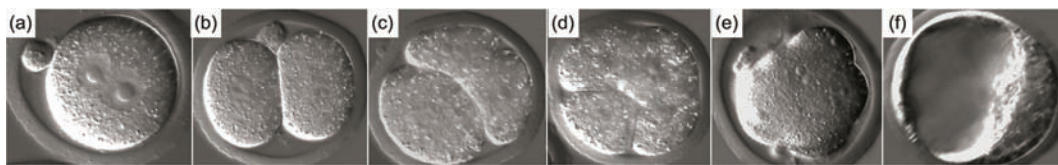
图 1 小鼠植入前胚胎发育(E0.5~E3.5). (a) 精子和卵子受精卵后形成具有雌雄原核的受精卵(zygote); (b) 受精卵分裂形成的二细胞阶段胚胎; (c) 二细胞不同步分裂形成的三细胞阶段胚胎; (d) 发育过程中的四细胞阶段胚胎; (e) 致密化的桑椹胚(morula); (f) 包括滋养外胚层和内细胞团的囊胚

Figure 1 Mouse pre-implantation development (E0.5–E3.5). (a) The zygote at E0.5; (b) two-cell stage embryo at E1.5; (c) three-cell embryo resulted from the asynchronic division of blastomeres; (d) the four-cell stage embryo; (e) the compacted morula at E2.5; (f) the blastocyst with trophectoderm and ICM at E3.5

小鼠早期胚胎具有很强的可塑性(plasticity), 能够适应和补偿细胞位置和数量的改变继续发育. 例如, 当胚胎外层的细胞移植到内部时, 它们能够适应环境的改变分化成ICM细胞; 而去除少量细胞的早期胚胎也能够继续发育成正常的个体[5,9]. 此外, 同一时间分离到的胚胎可以处于不同的发育时期, 而且同一个胚胎不同位置细胞的基因表达和极性等也可能不同, 即早期胚胎的异质性(heterogeneity)[5,8]. 小鼠早期胚胎的可塑性和异质性等特征使得有关早期胚胎细胞命运决定的研究异常困难.

2 哺乳动物第一次细胞命运决定的经典模型

从卵母细胞成熟到合子基因组启动(zygotic genome activation, ZGA)以前, 基因转录处于沉默状态, 早期胚胎发育主要受到母源效应基因调控[10,11]. 在很多非哺乳动物中, 母源效应基因决定了第一次细胞谱系的建立[12~16]. 根据从低等模式生物中观察到的这种现象, Dalcq[17]最早提出了哺乳动物早期胚胎第一次细胞命运决定的预塑模型或镶嵌模型(prepattern model或mosaic model)(图2(a)). 该模型认为卵母细胞受精后, 母源决定因子在细胞分裂时分别进入不同的子细胞, 进而导致细胞异质性产生, 子细胞根据继承的母源决定因子的不同, 分化形成不同的细胞[17]. 小鼠中, 母源效应基因调节母源RNA降解、DNA复制和损伤修复, 以及合子基因组启动等多个过程[18~21], 而且很多母源效应基因在桑椹胚甚至囊胚的外层和内层细胞分布明显不同[22~24], 但是母源效应基因是否与第一次细胞谱系形成有关仍不清楚. 由于缺少实验证据支持相关模型, 随着实验胚胎学的发展和需要, 该模型很快被搁置.

1959年, Tarkowski[9]用实验证明了小鼠2-细胞胚胎中的单个卵裂球在体内能够发育成独立个体; 此外, 从4-或8-细胞期胚胎中分离的单个卵裂球也能发育成小型囊胚[25]; 这些结果表明, 8-细胞以前的单个卵裂球都可能具有全能性, 明显与上述预塑模型相矛盾. 于是, Tarkowski和Wróblewska[25]在1967年提出了哺乳动物第一次细胞命运决定的内外模型或位置决定模型(inside-outside model或positional model)(图2(b)). 该模型认为早期胚胎细胞的空间位置决定其将来发育为TE还是ICM细胞[25]. 由于内外模型相对简单且容易验证, 该模型提出以后, 很多实验室都对它进行了验证, 发现位置确实能够改变着床前胚胎细胞的命运, 因此该模型在20世纪60~70年代的哺乳动物早期发育研究中得到广泛认可[26]. 尽管内外模型能够较好地解释了当时实验中观察到的现象, 但该模型没有考虑早期胚胎内部和外部位置产生的原因[26].

实际上, 小鼠早期胚胎中, 在8-细胞胚胎致密化(compaction)后, 微绒毛、细胞器及极性蛋白等在卵裂球细胞的极膜区(apical region)特异分布[27,28]. 这些极化细胞由于分裂面方向的不同, 极膜区可以被不同的细胞继承. 因此, 考虑到16-细胞期胚胎不同位置的细胞产生的来源, Johnson等人[2,26]20世纪80年代初提出了更为灵活的极性模型(polarization model), 并尝试用实验证实该假说(图2(c)). 他们认为桑椹胚细胞不对称分裂导致继承了极膜区(apical domain)的子细胞发育为TE, 而未继承极膜区的细胞则分化成ICM[2]. 例如, 在8-细胞期胚胎中, 由于分裂面的不同, 极膜区可以在对称分裂后同时被两个子细胞继承, 也可以通过不对称分裂由一个子细胞继承(图2(c)). 极性模型实际上是位置模型的改进, 该模型引入了细胞分裂面和极膜区的物质继承等因素与细胞命运的关系[26]. 从8-到16-细胞过程中, 分裂轴的方
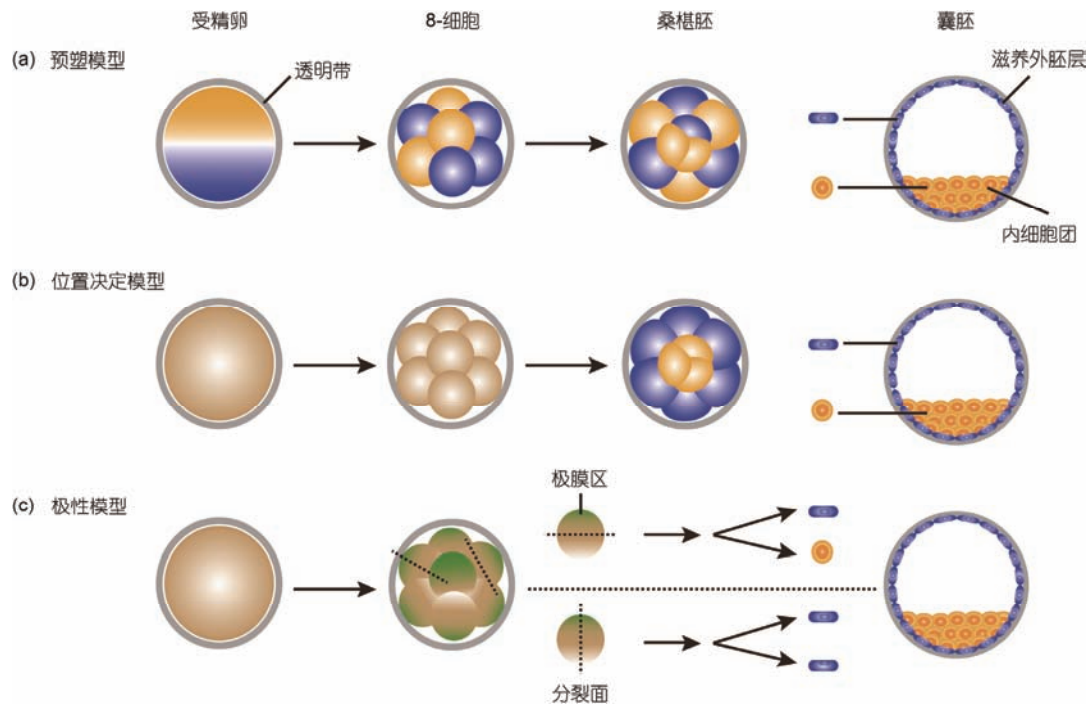
图 2 (网络版彩色)小鼠胚胎第一细胞谱系形成的经典模型. (a) 预塑模型. 预塑模型认为第一细胞谱系的分化取决于母源决定因子, 这些母源决定因子在卵母细胞或受精卵中分布不均等, 细胞分裂时, 不同的母源决定因子分别被分配到不同的子细胞中, 子细胞命运将由其继承的母源因子决定. (b) 位置决定模型. 该模型认为细胞在桑椹胚时的位置决定其后来的分化命运, 桑椹胚时, 位于外层的细胞发育为TE, 位于内层的细胞形成ICM. (c) 极性模型. 极性模型假设细胞的命运与细胞是否继承其祖细胞的极膜区(apical membrane domain)有关, 极膜区的分子能诱导细胞的滋养外胚层命运; 极性细胞不对称分裂形成一个滋养外胚层细胞和一个内细胞团细胞(上侧分裂方式); 极性细胞对称分裂形成两个滋养外胚层细胞(下侧分裂方式)

Figure 2 (Color online) Classic models for the first cell lineage specification in the early mouse embryo. (a) The prepattern model: the segregation of trophectoderm and ICM cells is determined by the maternal factors that are asymmetrically distributed in the oocyte. After fertilization, these factors are inherited from zygote division by the daughter cells, thus determining their fate of the cells; (b) the positional model: the cell fate is determined by the cell position in the morula stage embryo, the cells inside of the morula form the ICM, while cells outside form the trophectoderm; (c) the polarization model: the cell will differentiate into trophectoderm when it inherits the apical membrane domain, otherwise, the cell will develop into ICM at blastocyst stage embryos

向受细胞形状、黏附、极性和分裂时分裂球极性面的大小与纺锤体位置及细胞变形等多种因素影响[29,30], 因此, 细胞极性模型的测试, 除了需要鉴定细胞谱系标志分子外, 还需要评估细胞形态和相互作用等多种因素对细胞命运的影响.

3 早期胚胎第一次细胞谱系形成的分子调节

着床前胚胎发育的关键转录因子和重要信号通路的研究明显促进了对第一次细胞谱系形成的理解[5,8,31]. 小鼠第一次细胞谱系在16-到32-细胞阶段形成, 该时期的胚胎包括外层的TE细胞和内部的ICM细胞[32,33]. 其中, 小鼠滋养外胚层重要转录因子*Cdx2*特异表达在TE中; 它缺失的胚胎能够基本正常发育到囊胚阶段, 但TE的标记分子*Eomes*等不能表达, 而ICM特异分子*Oct4*等表达基本不受影响[34]; Cdx2能够抑制*Oct4*的表达; 此外, 过表达*Cdx2*能直接将胚胎干细胞转变成滋养外胚层干细胞[35]; 因此, Cdx2被认为是TE命运的关键转录因子. 实际上, *Cdx2*可能仅是TE形成以后的标记分子, 它对TE细胞命运的维持非常重要, 但它在第一次细胞命运决定中的作用仍不清楚. 尽管在TE形成中, *Cdx2*的表达水平与极膜区的存在呈现高度相关性, 而且*Cdx2*的mRNA在极膜区的定位等被认为与TE形成有关[32]; 但是目前有关*Cdx2*在早期胚胎发育过程中的精确作用时间还不清楚, 因此Cdx2在TE形成过程中的功能仍有争议[36~38]. 此外, 转录因子*Arid3a*也可能是小鼠TE形成必需的[39].

近期大量研究表明, *Cdx2*表达受到Hippo/Yap/Tead4通路调控. Hippo/Yap信号最早在果蝇(*Drosophila melanogaster*)中发现[40], 后来研究表明, 它在哺乳动物中保守存在, 能够响应细胞的密度和位置等外界环境变化[41]. 小鼠早期胚胎中, Hippo/Yap信号只有在与其他细胞存在完全相互接触的ICM细胞中才被激活, 而在TE细胞中没有活性[8,31,42,43]. 研究表明, 早期胚胎外层细胞中, Hippo通路的Amot(Angiomotin)和Nf2被分别锚定在极膜区或基膜区[44,45], 从而抑制了Hippo信号通路对Yap的磷酸化[42], 非磷酸化的 Yap 进入细胞核以后与 Tead4 结合形成Yap/Tead复合体, 激活TE的关键转录因子*Cdx2*和*Gata3*等的表达, 促使外层细胞向TE分化[42,44~47]; 而在内层细胞中, Amot被磷酸化后从极膜区解离并可能与Nf2和Lats等形成复合体并磷酸化Yap, 将其滞留在胞质中使其不能进入细胞核, 导致*Tead4*不能启动其下游靶基因表达, 内层细胞继续维持全能性, 并最终形成ICM细胞(图3)[31,42,44,45]; 而且TE关键转录因子Cdx2还可能抑制*Oct4*的表达并维持外层细胞的命运(图3)[7,31,48]. 此外, Notch信号与Hippo通路可能共同调控Cdx2表达参与TE的形成[48].

除了调控TE分化, Hippo/Yap信号通路还可能调控ICM特异性基因*Sox2*的表达[47]. 如果在TE细胞中抑制Yap入核, 则可以导致*Sox2*在TE细胞中异位表达[47]. 这些细胞中, Cdx2能够抑制*Oct4*表达, 但不抑制*Sox2*表达, 提示Yap可能通过其他机制调控*Sox2*表达[35,47]. 尽管Hippo/Yap/Tead4信号在TE形成过程中至关重要, 但*Tead4*缺失的胚胎在低氧条件下能够形成TE[49], 表明Yap在不同条件下可能结合不同的DNA结合蛋白调控下游基因[50]. 当然, 阐明TE中调控*Yap*上游调控因素(可能是极性、化学或机械张力等), 以及这些因素产生的原因对理解哺乳动物第一次细胞谱系形成机制至关重要. 实际上, 最近研究表明, 早期胚胎卵裂球的收缩性(contractility)自主调控细胞的内化, 并可能利用其产生的皮质下张力通过Yap参与早期细胞谱系的形成[30,51~54]. 以上结果说明, 小鼠胚胎第一次细胞谱系形成的分子调节机制非常复杂.

4 早期胚胎中卵裂球的异质性与分化潜能

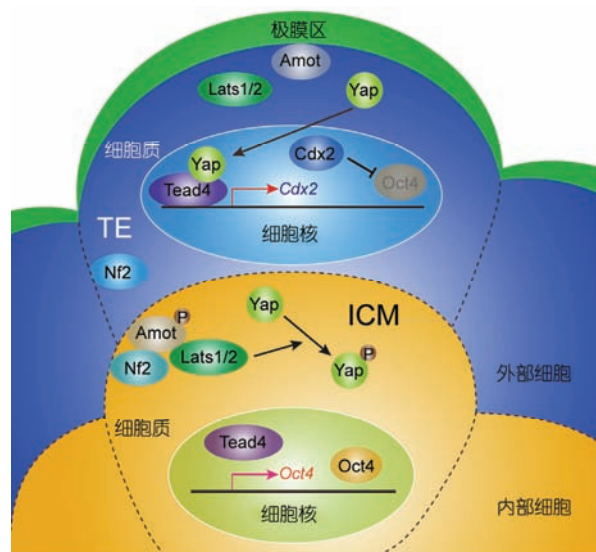实际上, 滋养外胚层形成以前的谱系调控更为复杂. 近年, 越来越多的实验证据表明, 异质性可能是小鼠早期卵裂球具有可塑性的关键因素[4~6]. 除了

图 3 (网络版彩色)Hippo信号通路调控小鼠滋养外胚层与内细胞团分化的模式图. 在小鼠胚胎中, Hippo(小鼠中的同源蛋白为Stk3/Mst)只有在与其他细胞存在完全相互作用的内层细胞内才能被激活, 而在外层细胞中没有活性. 在内层细胞中, 活化Hippo的激活通过Lats等因子磷酸化Yap, 磷酸化的Yap不能被转运入细胞核, 导致其下游基因*Tead*不能激活其靶基因, 从而使内层细胞继续保持全能性, 并最终形成ICM. 在外层细胞中, 由于没有完全的相互作用, 导致Hippo信号的下调, Yap保持非磷酸化状态. 未磷酸化的Yap进入细胞核, 与Tead结合, Yap-Tead结合TE特化的基因, 如*Cdx2*等, 从而激活并促进它们的表达, 促使外层细胞向TE分化

Figure 3 (Color online) Specification of the TE and ICM mediated by Hippo signaling. In mouse blastocyst embryos, Hippo (homologous protein Stk3/Mst in mice) can only be activated in the inner cells that interact with other cells completely, while it is not active in the outer cells. In the inner cells, the Hippo pathway is activated, Lats and other factors phosphorylates Yap, and then the phosphorylated Yap cannot be transported into the nucleus to activate this pathway, therefore blocking Tead activity and maintaining the totipotency of the ICM. In the outer cells, however, Hippo signaling is down-regulated, and the unphosphorylated Yap translocates to the nucleus and interacts with Tead, thus promoting the expression of TE specific genes, such as *Cdx2*, and inducing the TE differentiation

上面提到的极性外, 早期胚胎异质性还包括细胞周期、分裂面和基因表达等多方面因素[6,26]. 在分离2-细胞或4-细胞小鼠胚胎时, 经常可以发现3-细胞期胚胎(图1), 提示小鼠早期胚胎中卵裂球细胞周期的异质性最早出现在2-细胞阶段, 细胞周期可能与卵裂球分化潜能相关, 但2-细胞卵裂球分化潜能长期存在争议[55,56]. 4-细胞期卵裂面或卵裂轴方向不同可以导致其子代细胞继承的动植物极或极性面不同, 也能使子代细胞产生异质性, 这种异质性可能影响细胞谱系形成[57]; 但也有报道称, 分裂球的分裂角度在早期胚胎分化中的影响有限[58].

最近, 大量的研究表明, 哺乳动物早期胚胎中细胞的基因表达明显不同. 单细胞测序分析发现小鼠2–8细胞期卵裂球的基因表达存在异质性[59~64]. 第一次卵裂后的差异可能源于分裂时细胞内母源mRNA分配的随机性; 其中, 表达量高的基因趋于均匀分配; 而表达量低的因子往往在两个卵裂球中的含量差异明显[59,60]. 伴随着ZGA, 这种卵裂球之间的随机差异出现两种趋势: 一些分子趋向于消除在各个卵裂球之间的差异; 而另一些分子在卵裂球之间的差异越来越显著. 表达差异显著的分子往往与分化相关, 提示它们可能与早期卵裂球命运相关[60]. 从8-或16-细胞期以后, 很多重要细胞谱系转录因子, 如Nanog, Oct4和Cdx2等基因开始呈现明显异质性表达[5,6,31]. 随着胚胎进一步发育, Cdx2表达逐渐稳定在滋养外胚层细胞, 而同期的内部细胞依然具有可塑性[65,66].

尽管小鼠2-细胞胚胎的异质性是否与其后期命运的关系有很大争议, 但最近研究表明, 4-细胞期卵裂球的异质性可能与它们的分化潜能有关. 最早报道的小鼠4-细胞期卵裂球中分子异质性表达的是组蛋白H3的修饰(H3R26)和它的调控酶Carm1[67]. 后来利用OCT4-GFP融合蛋白标记实验发现Oct4出入细胞核的动力学特性在4-细胞期卵裂球中存在差异[68]. 有意思的是, 最近两个实验室独立报道了Carm1/Sox2/Sox21通路可能与4-细胞期卵裂球分化潜能相关[69,70]. 上述研究结果支持偏向的异质性模型(biased heterogeneity model), 该模型实际上是早期预塑模型的改进[71].

当然, 上面的结果还是不能排除第一次细胞谱系形成过程的其他可能性. 例如, Wennekamp等人[4]将控制论中的自组织概念应用到哺乳动物早期胚胎图式形成中, 更新了他们早期主张的随机模型, 并提出自组织模型(self-organization model). 他们认为第一次细胞谱系建立和体内首次对称性的打破不取决于单个因子(相对于命运决定子模型), 而是多个关键

因素(包括基因表达、极性和表面张力等细胞内部特性和细胞外信号等因素)一起触发, 经过纠正和优化产生的最终结果[4]. 小鼠早期胚胎的8-细胞致密化过程中, 不同卵裂球的表面张力存在异质性, 胚胎通过产生不同收缩性的卵裂球, 使相应细胞倾向TE或ICM分化, 并能够通过收缩自主调控细胞内化, 这些证据在一定程度上支持哺乳动物早期胚胎自组织性的存在[30,51~54]. 自组织概念近期也被用于小鼠和人的囊胚体外培养系统中[72~74]. 但是, 自组织模型中的多因素相互作用的结局很难利用目前的单因素实验来证实.

5 结语与展望

哺乳动物着床前胚胎发育在相对独立的输卵管中进行, 不对称分裂在第一次细胞谱系命运决定中发挥了重要作用, 但是阐明不对称分裂建立的关键因素是生物学研究领域的巨大挑战. 基于低等生物发育的特点早期研究提出了预塑模型, 母源效应基因在囊胚中不同细胞类型的分布支持该模型的成立, 但这些因子在囊胚中的功能还有待于阐明; 随着研究的进展和需要, 出现了内外模型和极性模型, 它们能够解释早期胚胎实验过程中观察到的一些现象, 但早期胚胎的可塑性依然得不到合理解释; 其间, 也有人提出了随机模型; 最近研究者又提出了自组织模型和偏向的异质性模型, 且异质性模型也得到了较强的分子实验证据; 其实, 最近的模型在一定程度上只是极性模型或预塑模型的改进, 使得它们更加包容. 当然, 这些模型并不完全互相排斥, 反而是越来越互相兼容. 随着计算生物技术的发展、新的观察手段和模型的建立, 以及对小鼠早期胚胎发育分子机制认识的深入[65,66,75], 哺乳动物第一次细胞谱系的决定可能被证明异质性和随机性共存, 同时受到内外因素的互相影响, 并最终融合于生命起始阶段的可塑性之中.

参考文献

1　Fleming T P. A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. Dev Biol, 1987, 119: 520–531

2　Johnson M H, Ziomek C A. The foundation of two distinct cell lineages within the mouse morula. Cell, 1981, 24: 71–80

3　Morris S A, Teo R T Y, Li H L, et al. Origin and formation of the first two distinct cell types of the inner cell mass in the mouse embryo. Proc Natl Acad Sci USA, 2010, 107: 6364–6369

4　Wennekamp S, Mesecke S, Nedelec F, et al. A self-organization framework for symmetry breaking in the mammalian embryo. Nat Rev

Mol Cell Biol, 2013, 14: 452–459

5 Martinez Arias A, Nichols J, Schroter C. A molecular basis for developmental plasticity in early mammalian embryos. Development, 2013, 140: 3499–3510

6 Bedzhov I, Graham S J, Leung C Y, et al. Developmental plasticity, cell fate specification and morphogenesis in the early mouse embryo. Philos Trans R Soc London B Biol Sci, 2014, pii: 20130538

7 Rossant J, Tam P P. Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. Development, 2009, 136: 701–713

8 Chazaud C, Yamanaka Y. Lineage specification in the mouse preimplantation embryo. Development, 2016, 143: 1063–1074

9 Tarkowski A K. Experiments on the development of isolated blastomers of mouse eggs. Nature, 1959, 184: 1286–1287

10 Li L, Lu X, Dean J. The maternal to zygotic transition in mammals. Mol Aspects Med, 2013, 34: 919–938

11 Li L, Zheng P, Dean J. Maternal control of early mouse development. Development, 2010, 137: 859–870

12 Driever W, Nusslein-Volhard C. The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell, 1988, 54: 95–104

13 Driever W, Nusslein-Volhard C. A gradient of bicoid protein in drosophila embryos. Cell, 1988, 54: 83–93

14 Goldstein B, Hird S N. Specification of the anteroposterior axis in *Caenorhabditis elegans*. Development, 1996, 122: 1467–1474

15 Huynh J R, St Johnston D. The origin of asymmetry: Early polarisation of the *Drosophila* germline cyst and oocyte. Curr Biol, 2004, 14: R438–R449

16 Vincent J P, Oster G F, Gerhart J C. Kinematics of gray crescent formation in *Xenopus eggs*: The displacement of subcortical cytoplasm relative to the egg surface. Dev Biol, 1986, 113: 484–500

17 Dalcq A. Introduction to General Embryology. Oxford: Oxford University Press, 1957

18 Liu Y, Lu X, Shi J, et al. BTG4 is a key regulator for maternal mRNA clearance during mouse early embryogenesis. J Mol Cell Biol, 2016, 8: 366–368

19 Yu C, Ji S Y, Sha Q Q, et al. BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. Nat Struct Mol Biol, 2016, 23: 387–394

20 Xu Q, Wang F, Xiang Y, et al. Maternal BCAS2 protects genomic integrity in mouse early embryonic development. Development, 2015, 142: 3943–3953

21 Yu C, Ji S Y, Dang Y J, et al. Oocyte-expressed yes-associated protein is a key activator of the early zygotic genome in mouse. Cell Res, 2016, 26: 275–287

22 Li L, Baibakov B, Dean J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. Dev Cell, 2008, 15: 416–425

23 Ohsugi M, Zheng P, Baibakov B, et al. Maternally derived FILIA-MATER complex localizes asymmetrically in cleavage-stage mouse embryos. Development, 2008, 135: 259–269

24 Yu X J, Yi Z, Gao Z, et al. The subcortical maternal complex controls symmetric division of mouse zygotes by regulating F-actin dynamics. Nat Commun, 2014, 5: 4887

25 Tarkowski A K, Wróblewska J. Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. J Embryol Exp Morphol, 1967, 18: 155

26 Johnson M H. From mouse egg to mouse embryo: Polarities, axes, and tissues. Annu Rev Cell Dev Biol, 2009, 25: 483–512

27 Ducibella T, Anderson E. Cell shape and membrane changes in the eight-cell mouse embryo: Prerequisites for morphogenesis of the blastocyst. Dev Biol, 1975, 47: 45–58

28 Handyside A H. Distribution of antibody- and lectin-binding sites on dissociated blastomeres from mouse morulae: Evidence for polarization at compaction. J Embryol Exp Morphol, 1980, 60: 99

29 Dard N, Louvet-Vallee S, Maro B. Orientation of mitotic spindles during the 8- to 16-cell stage transition in mouse embryos. PLoS One, 2009, 4: e8171

30 Korotkevich E, Niwayama R, Courtois A, et al. The apical domain is required and sufficient for the first lineage segregation in the mouse embryo. Dev Cell, 2017, 40: 235–247

31 Frum T, Ralston A. Cell signaling and transcription factors regulating cell fate during formation of the mouse blastocyst. Trends Genet, 2015, 31: 402–410

32 Jedrusik A, Parfitt D E, Guo G, et al. Role of Cdx2 and cell polarity in cell allocation and specification of trophectoderm and inner cell mass in the mouse embryo. Genes Dev, 2008, 22: 2692–2706

33 Skamagki M, Wicher K B, Jedrusik A, et al. Asymmetric localization of *Cdx2* mRNA during the first cell-fate decision in early mouse development. Cell Rep, 2013, 3: 442–457

34 Strumpf D, Mao C A, Yamanaka Y, et al. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the

mouse blastocyst. Development, 2005, 132: 2093–2102

35 Niwa H, Toyooka Y, Shimosato D, et al. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell, 2005, 123: 917–929

36 Jedrusik A, Bruce A W, Tan M H, et al. Maternally and zygotically provided Cdx2 have novel and critical roles for early development of the mouse embryo. Dev Biol, 2010, 344: 66–78

37 Jedrusik A, Cox A, Wicher K, et al. Maternal-zygotic knockout reveals a critical role of Cdx2 in the morula to blastocyst transition. Dev Biol, 2015, 398: 147–152

38 Blij S, Frum T, Akyol A, et al. Maternal Cdx2 is dispensable for mouse development. Development, 2012, 139: 3969–3972

39 Rhee C, Lee B K, Beck S, et al. Arid3a is essential to execution of the first cell fate decision via direct embryonic and extraembryonic transcriptional regulation. Genes Dev, 2014, 28: 2219–2232

40 Pan D J. The Hippo signaling pathway in development and cancer. Dev Cell, 2010, 19: 491–505

41 Plouffe S W, Hong A W, Guan K L. Disease implications of the Hippo/Yap pathway. Trends Mol Med, 2015, 21: 212–222

42 Nishioka N, Inoue K, Adachi K, et al. The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. Dev Cell, 2009, 16: 398–410

43 Sasaki H. Roles and regulations of Hippo signaling during preimplantation mouse development. Dev Growth Differ, 2017, 59: 12–20

44 Cockburn K, Biechele S, Garner J, et al. The Hippo pathway member Nf2 is required for inner cell mass specification. Curr Biol, 2013, 23: 1195–1201

45 Hirate Y, Hirahara S, Inoue K, et al. Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. Curr Biol, 2013, 23: 1181–1194

46 Ralston A, Cox B J, Nishioka N, et al. Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. Development, 2010, 137: 395–403

47 Wicklow E, Blij S, Frum T, et al. HIPPO pathway members restrict SOX2 to the inner cell mass where it promotes ICM fates in the mouse blastocyst. PLoS Genet, 2014, 10: e1004618

48 Rayon T, Menchero S, Nieto A, et al. Notch and hippo converge on Cdx2 to specify the trophectoderm lineage in the mouse blastocyst. Dev Cell, 2014, 30: 410–422

49 Kaneko K J, DePamphilis M L. Tead4 establishes the energy homeostasis essential for blastocoel formation. Development, 2013, 140: 3680–3690

50 Zanconato F, Forcato M, Battilana G, et al. Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol, 2015, 17: 1218–1227

51 Fierro-Gonzalez J C, White M D, Silva J C, et al. Cadherin-dependent filopodia control preimplantation embryo compaction. Nat Cell Biol, 2013, 15: 1424–1433

52 Maitre J L, Niwayama R, Turlier H, et al. Pulsatile cell-autonomous contractility drives compaction in the mouse embryo. Nat Cell Biol, 2015, 17: 849–855

53 Maitre J L, Turlier H, Illukkumbura R, et al. Asymmetric division of contractile domains couples cell positioning and fate specification. Nature, 2016, 536: 344–348

54 Samarage C R, White M D, Alvarez Y D, et al. Cortical tension allocates the first inner cells of the mammalian embryo. Dev Cell, 2015, 34: 435–447

55 Zernicka-Goetz M, Huang S. Stochasticity versus determinism in development: A false dichotomy? Nat Rev Genet, 2010, 11: 743–744

56 Kurotaki Y, Hatta K, Nakao K, et al. Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape. Science, 2007, 316: 719–723

57 Piotrowska-Nitsche K, Perea-Gomez A, Haraguchi S, et al. Four-cell stage mouse blastomeres have different developmental properties. Development, 2005, 132: 479–490

58 Watanabe T, Biggins J S, Tannan N B, et al. Limited predictive value of blastomere angle of division in trophectoderm and inner cell mass specification. Development, 2014, 141: 2279–2288

59 Biase F H, Cao X, Zhong S. Cell fate inclination within 2-cell and 4-cell mouse embryos revealed by single-cell RNA sequencing. Genome Res, 2014, 24: 1787–1796

60 Shi J, Chen Q, Li X, et al. Dynamic transcriptional symmetry-breaking in pre-implantation mammalian embryo development revealed by single-cell RNA-seq. Development, 2015, 142: 3468–3477

61 Deng Q, Ramskold D, Reinius B, et al. Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. Science, 2014, 343: 193–196

62 Piras V, Tomita M, Selvarajoo K. Transcriptome-wide variability in single embryonic development cells. Sci Rep, 2014, 4: 7137

63　Zhu C, Gao Y, Guo H, et al. Single-cell 5-formylcytosine landscapes of mammalian early embryos and ESCs at single-base resolution. Cell Stem Cell, 2017, doi: 10.1016/j.stem.2017.02.013

64　Yang L, Ma Z, Cao C, et al. Mr-seq: Measuring a single cell's transcriptome repeatedly by RNA-seq. Sci Bull, 2017, 62: 391–398

65　Posfai E, Petropoulos S, de Barros F R, et al. Position- and hippo signaling-dependent plasticity during lineage segregation in the early mouse embryo. eLife, 2017, pii: e22906

66　Dietrich J E, Panavaite L, Gunther S, et al. Venus trap in the mouse embryo reveals distinct molecular dynamics underlying specification of first embryonic lineages. EMBO Rep, 2015, 16: 1005–1021

67　Torres-Padilla M E, Parfitt D E, Kouzarides T, et al. Histone arginine methylation regulates pluripotency in the early mouse embryo. Nature, 2007, 445: 214–218

68　Plachta N, Bollenbach T, Pease S, et al. Oct4 kinetics predict cell lineage patterning in the early mammalian embryo. Nat Cell Biol, 2011, 13: 117–123

69　Goolam M, Scialdone A, Graham S J L, et al. Heterogeneity in Oct4 and Sox2 targets biases cell fate in 4-cell mouse embryos. Cell, 2016, 165: 61–74

70　White M D, Angiolini J F, Alvarez Y D, et al. Long-lived binding of Sox2 to DNA predicts cell fate in the four-cell mouse embryo. Cell, 2016, 165: 75–87

71　Wu J, Izpisua Belmonte J C. The molecular harbingers of early mammalian embryo patterning. Cell, 2016, 165: 13–15

72　Bedzhov I, Zernicka-Goetz M. Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation. Cell, 2014, 156: 1032–1044

73　Deglincerti A, Croft G F, Pietila L N, et al. Self-organization of the *in vitro* attached human embryo. Nature, 2016, 533: 251–254

74　Shahbazi M N, Jedrusik A, Vuoristo S, et al. Self-organization of the human embryo in the absence of maternal tissues. Nat Cell Biol, 2016, 18: 700–708

75　Strnad P, Gunther S, Reichmann J, et al. Inverted light-sheet microscope for imaging mouse pre-implantation development. Nat Methods, 2016, 13: 139–142

李磊
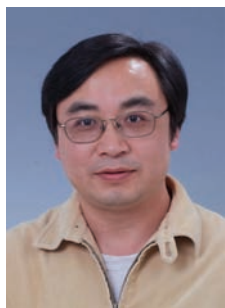
中国科学院动物研究所研究员，分子胚胎发育生物学研究组组长，博士生导师. 2009 年入选中国科学院"百人计划". 长期从事哺乳动物早期胚胎发育分子调控机制研究，相关成果已发表在 *Dev Cell*, *Nat Commun*, *Development* 和 *J Biol Chem* 等学术期刊上，应邀为 *Development* 和 *Mol Aspects Med* 等撰写哺乳动物早期胚胎发育综述论文. 目前实验室主要研究哺乳动物胚胎发育母源调控、早期细胞谱系和生殖细胞形成等的分子调控机制.

First cell lineage specification in mammalian early development

NIE XiaoQing, LU XuKun & LI Lei[*]

State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
* Corresponding author, E-mail: lil@ioz.ac.cn

Mouse pre-implantation embryogenesis is featured by several rounds of sequential cleavages, followed by the first cell lineage formation, specification of the inner cell mass (ICM) and trophectoderm (TE) at blastocyst stage. Establishment of the first cell lineage through asymmetric cell division is a longstanding question in developmental biology. Based on analogy to other lower model organisms or early achievements in mammalian development, three classic models of the first cell lineage specification are proposed to address this question: The prepattern or mosaic model, the inside-outside or positional model, and the polarization model. However, none of these models could well explain the highly regulatory nature of early mammalian embryos. During the past decades, great efforts have been made to elucidate when and how the pre-implantation blastomeres become different and finally segregate from each other. Mounting evidences show that the fate of trophectoderm is regulated by the Hippo/Yap/Tead signaling cascade in mouse early embryogenesis. In addition, single cell profiling and living imaging illustrate the great heterogeneity between blastomeres, providing some explanations for the regulatory nature of mammalian early embryos. Accompanying these progresses, a self-organization model was recently proposed to explain the blastocyst patterning in mammalian early embryos. This new model reconciles the experimental findings that seem to be contradictory to the three classic models and thus is regarded as a reformulation but improvement of classic models. Even so, however, the exact molecular mechanisms underlying this highly dynamic, complex and self-regulative process remain enigmatic. In this review, using mouse as the model system, we firstly summarize the preimplantation development and the classic models of the first cell lineage specification. Then, we highlight recent progresses, especially, the contributions of molecular regulators and heterogeneity of blastomeres in orchestrating the segregation of ICM and TE in mouse early embryos. At last, we summarize the current view on the first cell lineage specification in mammalian early embryogenesis and provide some clues for the future investigations. Altogether, the molecular basis of first cell lineage specification in mammals not only promotes the understanding for the beginning of life, but also contributes to stem cell biology and reproductive medicine.

mammals, mouse, early embryonic development, first cell lineage specification, asymmetric division, cell fate determination