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Review

Position- and polarity-dependent Hippo signaling regulates cell fates in preimplantation mouse embryos

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ABSTRACT

During the preimplantation stage, mouse embryos establish two cell lineages by the time of early blastocyst formation: the trophectoderm (TE) and the inner cell mass (ICM). Historical models have proposed that the establishment of these two lineages depends on the cell position within the embryo (*e.g.*, the positional model) or cell polarization along the apicobasal axis (*e.g.*, the polarity model). Recent findings have revealed that the Hippo signaling pathway plays a central role in the cell fate-specification process: active and inactive Hippo signaling in the inner and outer cells promote ICM and TE fates, respectively. Intercellular adhesion activates, while apicobasal polarization suppresses Hippo signaling, and a combination of these processes determines the spatially regulated activation of the Hippo pathway in 32-cell-stage embryos. Therefore, there is experimental evidence in favor of both positional and polarity models. At the molecular level, phosphorylation of the Hippo-pathway component angiomotin at adherens junctions (AJs) in the inner (apolar) cells activates the Lats protein kinase and triggers Hippo signaling. In the outer cells, however, cell polarization sequesters Amot from basolateral AJs and suppresses activation of the Hippo pathway. Other mechanisms, including asymmetric cell division and Notch signaling, also play important roles in the regulation of embryonic development. In this review, I discuss how these mechanisms cooperate with the Hippo signaling pathway during cell fate-specification processes.

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1. Introduction

Mouse embryogenesis occurs under powerful regulatory control. Embryos of other oviparous vertebrates such as zebrafish and *Xenopus* also employ regulatory mechanisms during embryogenesis, but axis formation depends on maternal determinants localized in unfertilized eggs and zygotes (see reviews [1–5]). In contrast, the development of mouse embryos does not critically depend on such factors. For example, manipulations such as removal of portions of the zygote or destruction of a single blastomere in embryos at the 8-cell stage do not affect mouse embryonic development. Furthermore, isolated blastomeres at the 4- or 8-cell stage embryos show totipotency when aggregated with host embryos [6–11].

The ability of mouse embryos to develop properly without using localized information has been a hotly debated topic in developmental biology. Preimplantation mouse development has been under intense scientific scrutiny for many years and several models have been proposed. Recent molecular biology-based insights have revealed Hippo signaling as one of the earliest mechanisms influencing cell fate specification. In this review, I will summarize the role and regulation of the Hippo signaling pathway during the first cell-fate specification of mouse embryo development and discuss their relationships with the historical models.

2. First cell-fate specification in preimplantation embryos

2.1. Preimplantation mouse development

During preimplantation development, mouse embryos form a cyst-like structure called a blastocyst by 3.5 days post-coitus (dpc) (Fig. 1A). The early blastocysts consist of two cell types. The outer epithelial cells constitute the trophectoderm (TE) that is required for implantation into the uterus. At later developmental stages, the TE forms extraembryonic tissues, including the embryonic part of the placenta. The inner cells attached to one end of the TE form the inner cell mass (ICM). The ICM further differentiates into the epiblast and primitive endoderm by 4.5 dpc; the former tissue gives rise to the embryo proper, while the latter forms another group of extraembryonic tissues.

At the cellular level, up to the 8-cell stage, all blastomeres are loosely connected, and individual blastomeres are morphologically identifiable. At the 8-cell stage, intercellular adhesion mediated by the hemophilic cell-adhesion molecule E-cadherin strengthens [12–15], and cell boundaries become less evident. This process is known as compaction. Upon compaction, each blastomere becomes polarized along the apicobasal axis (Fig. 1B). During development after the 16-cell stage, some blastomeres occupy the inner position. While the outer cells remain polarized, the inner cells become apolar. Most inner cells are formed during the two rounds of cell divisions occurring between the 8- and 32-cell stages. The number of inner cells generated during each round of cell division varies [16–23].

Cell fates are controlled by the expression of cell type-specific transcription factors (Fig. 1B). The inner cells transform into the ICM following a gradual increase in the expression of the transcription factor Sox2 of the Sox family, a homeodomain protein Nanog, and the transcription factor Pou5f1 (also known as Oct3/4) of the POU family [24–27]. Sox2 is the first factor that is selectively upregulated in the inner cells as early as the 16-cell stage [28] and is confined to the ICM progenitors prior to blastocyst formation [29]. Clear and uniform expression of Oct3/4 and Nanog in all cells is observed as early as the 8-cell stage [19,30]. Strong and uniform expression of Oct3/4 continues in all cells up to the middle-to-late blastocyst stage (approximately the 96-cell stage), after which Oct3/4 expression is confined to ICM-derived epiblast and primitive endoderm cells at the late blastocyst stage (approximately the 104-cell stage) [19,30]. Nanog is also expressed widely at variable levels up to the middle blastocyst stage (approximately the 64-cell stage), and then its expression is confined to epiblast cells at the late blastocyst stage [19,29,31]. Differentiation of outer cells into the TE is associated with expression of the homeodomain transcription factor Cdx2 and the zinc-finger transcription factor Gata3 [31,32] (Fig. 1B). Expression of both proteins becomes noticeable in some cells by the 8-cell stage. Their initial expression patterns appear to be stochastic and they gradually become restricted to the outer cells, by approximately the 32-cell stage [19,32,33]. The helix-loop-helix protein Id2 is the earliest transcription factor that demonstrates specificity for the outer cells, which is observed by the 16-cell stage [28]. However, *Id2*-null mutants exhibit postnatal abnormalities [34–36], and the precise role of Id2 in TE development remains unknown.

2.2. Historical models

Because mouse embryo development is subject to plastic regulatory control, understanding the mechanisms of TE and ICM fate specification has been a long-standing conundrum. Historically, two main models have been proposed (Fig. 1C). The first model is described by the positional (inside–outside) model proposed by Tarkowski [10] (Fig. 1C). After analyzing the development of dissociated 4- and 8-cell-stage embryos, Tarkowski proposed that an intercellular environment (*i.e.*, internal positioning of cells) is required for ICM formation [10]. Later, this model was directly tested by manipulating the cell positions [6,37,38]. In support of the positional model, it was found that when the blastomere positions of 16- and 32-cell stage embryos were altered, their cell fates were determined by their new cell positions within the embryos [38].

A variation of the "inside–outside" model has been proposed by the polarity (polarization) model that was originally formulated by Johnson et al. [39] (Fig. 1C). According to the original polarity model, the polarization of each blastomere along the apicobasal axis at the 8-cell stage is of crucial importance. During the subsequent cell divisions, depending on the division plane, each blastomere undergoes one of the following modifications: (1) both daughter cells inherit the apical domain and form two polar cells (symmetric division), or (2) only one daughter cell inherits the apical domain, so that one polar and one apolar cell are formed (asymmetric division). Thus, cell fates are controlled by the differential inheritance of determinants caused by cell polarization. In support of this model, it has been demonstrated that, although polarization of the cytoplasm and cytoskeleton [40] and most junctional contacts between cells [41] are lost during the division of dissociated blastomeres of 8-cell embryos (1/8 cells), the polarized organization of the apical cortical domain is maintained [42] and the daughter cells differentially inheriting apical domains produce 2/16 couplets of polar TE and apolar ICM cells [16,42].

A new version of the polarity model was proposed by Rossant's group (Fig. 1C). In this model, the presence or absence of cell polarity or an apical domain controls cell fates [33,43]. The authors suggested that the molecular mechanism underpinning this control involves controlled expression of the TE-regulator gene *Cdx2* by the Par–aPKC system, a key modulator of apicobasal cell polarity [43]. Although these three models appear to be distinct, they focus on different aspects of the same or overlapping processes of cell fate specification. For example, manipulation of cell position controls both cellular fates and polarization [44–46]. Therefore, it is important to note that these models are not entirely incompatible with each other (Fig. 1C).

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