Apical polarization and lumenogenesis: The apicosome sheds new light

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Establishment of apico–basal polarity is critical for the lumenal epiblast-like morphogenesis of human pluripotent stem cells (hPSCs). In this issue, Taniguchi et al. (2017. *J Cell Biol.* https://doi.org/10.1083.jcb201704085) describe a structure called the apicosome, generated in single hPSCs, that allows them to self-organize and form the lumenal epiblast-like stage.

The formation of an epiblast cavity after fertilization is essential for morphogenesis and survival of the embryo as implantation takes place on the uterine wall. This cavity appears at the beginning of the second week after fertilization within the inner cell mass. It enlarges to become the amniotic cavity, which ultimately contains the developing fetus. The molecular changes that take place in the human embryo at this stage remain unknown. Monkey and mouse embryos as well as cocultures of human blastocysts and endometrial cells have been used as models for human embryo development (Weimar et al., 2013). However, to what extent these systems can recapitulate the development of a human embryo is uncertain. For example, gene expression patterns of mouse and human embryonic stem cells have shown that significant differences exist in the expression of transcription factors (e.g., FoxD3 and ARNT), of cytoskeleton proteins (e.g., vimentin and β-III tubulin), in cell cycle regulation, control of apoptosis, and cytokine expression (Gabdoulline et al., 2015). Previous studies have shown that even in the absence of maternal cues, embryos generated by in vitro fertilization (IVF) are able to self-organize and polarize to form lumenal proamniotic cavities (Deglincerti et al., 2016; Shahbazi et al., 2016). Yet, peri-implantation studies are still restricted because of the limited availability of IVF samples, ethical/legal constraints, and technical challenges of these mechanistic analyses (Rossant, 2016). The self-organization ability of human pluripotent stem cells (hPSCS; including both human embryonic stem cells and induced pluripotent stem cells) and their ability to recapitulate the events of polarization and lumenogenesis were previously documented by Shahbazi et al. (2016) and Taniguchi et al. (2015). This process not only resembles the in vivo expansion of the proamniotic cavity, but it suggests that the underlying molecular pathways may also be involved in epiblast cavity formation (Simunovic and Brivanlou, 2017). How apical polarization emerges in this model remains unclear. In this issue, Taniguchi et al. report an intriguing phenomenon by which a structure generated in single hPSCs allows them to self-organize and form what resembles a lumenal epiblast stage (Fig. 1). This study highlights the remarkable ability of hPSCs to organize into complex structures through self-assembly.

The authors describe a highly organized membrane-bound lumenal compartment that they name the "apicosome." They find that the apicosome is an intracellular perinuclear entity rich in apical proteins, positively expressing F-actin, EZRIN, and PODOCALYXIN (PODXL), among others. High accumulation of the marker PODXL has been identified in apicosomal-like structures of mouse blastocysts (Bedzhov et al., 2014). Taniguchi et al. (2017) also show that the apicosome is not present in mouse embryonic stem cells, which makes it a unique property of single-plated hPSCs and mouse epiblast cells. Its novelty is also highlighted by the fact that it does not costain with other organelle markers, making it an independent structure.

By the combination of ultrastructural analyses and live-cell imaging of isolated hPSCs expressing an EZRIN-GFP fusion protein, Taniguchi et al. (2017) characterize the apicosome as a membrane-bound lumen with a diameter of 3–5 µm. It is demarcated by a membrane containing highly dynamic microvillus-like protrusions as well as primary cilia, resembling the physical characteristics of the exterior surface of the cell. Interestingly, Ca^{2+} seems to accumulate in this compartment in a more robust fashion than in the endoplasmic reticulum, and at concentrations similar to those in the extracellular environment. These observations suggest that this newly characterized organelle-like structure provides a fully polarized, membrane-bound lumenal compartment with extracellular properties inside of a single hPSC.

Based on their previous study that two-cell lumen formation depends on actin assembly (Taniguchi et al., 2015), Taniguchi et al. (2017) probed the apicosome for actin assembly pathways. Targeting Arp2/3 and diaphanous-related formin-1 (mDIA)/formin with chemical inhibitors resulted in a reduced number of apicosomes. In contrast, expression of high levels of mDIA generated an increased amount of apicosomes compared with controls. This finding underlines the importance of actin assembly programs for apicosome formation as it was seen in two-cell lumen generation.

Using time-lapse imaging in single cells, the researchers demonstrate that the apicosome forms de novo during interphase as early as 30 min after plating, increasing in size until mitosis, demonstrating dynamic growth of the organelle. By labeling the outer membrane before plating, Taniguchi et al. (2017) were

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The Rockefeller University Press
J. Cell Biol. Vol. 216 No. 12 3891–3893
https://doi.org/10.1083/jcb.201710028

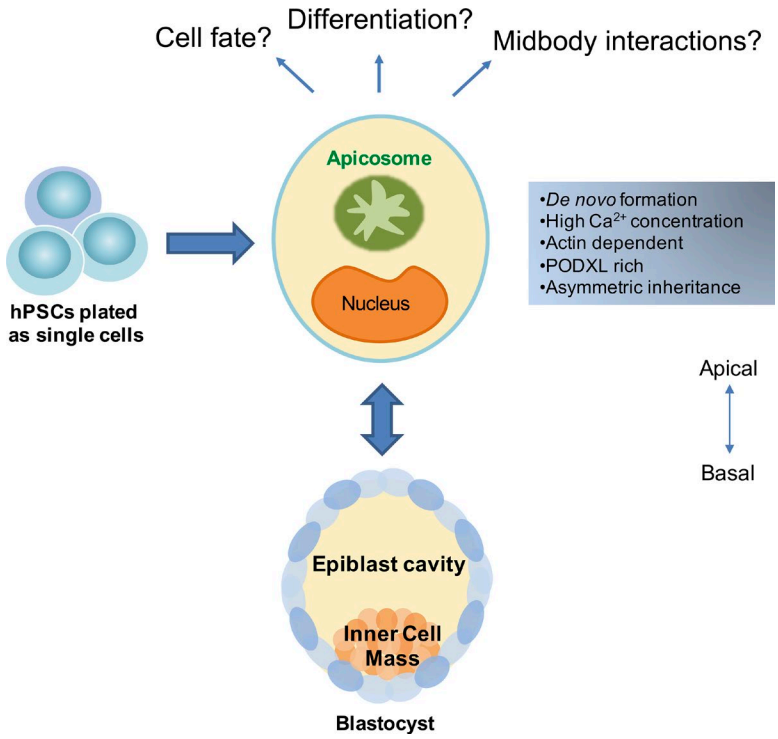

Figure 1. **The apicosome in hPSCs initiates lumenogenesis in vitro resembling epiblast cavity formation.** When plated as single cells, hPSCs self-organize and form a structure that resembles the lumenal epiblast stage. This membrane-bound structure, termed the apicosome, depends on actin assembly pathways for its formation. The inside resembles the extracellular environment. Illustration courtesy of Megan Rasmussen and Alejandra I. Romero-Morales.

able to examine the membrane dynamics using fluorescent recovery after photobleaching. Apicosomes labeled with EZR IN or PODXL-GFP showed rapid fluorescence recovery after laser photobleaching, indicating active membrane trafficking. Moreover, using live-cell imaging, they observed that the apicosome has an asymmetrical inheritance pattern upon cytokinesis. This finding is particularly fascinating from a cell fate perspective. Although Taniguchi et al. (2017) note that both cells maintain the expression of the stemness marker POUF51, it would be interesting to follow the fate of the cells under differentiating conditions. Does inheritance of the apicosome or lack thereof prime the cells for differentiation? Could this inheritance affect other cell fate decisions?

Initially in the blastocyst, cells from the inner cell mass exist as an unpolarized aggregate of cells. Their first morphogenic change is the apico–basal polarization that results in the radial organization of the epiblast cells and the formation of the proamniotic cavity. When aggregated in vitro, Taniguchi et al. (2017) observe that apicosomes form inside individual hPSCs after 24 h in culture. Moreover, after 48 h, self-organized cell rearrangement takes place to form cysts with a central lumen as a result of apicosome fusion. The molecular machinery governing the positioning of the apicosome and how it could potentially transmit cues to differentiating cells remains unknown. Taniguchi et al. (2017) suggest that the apicosome may play a role in lumenogenesis because it requires the actin cytoskeleton and other proteins shown to be vital for mouse blastocyst formation. In addition, it could become a valuable model to understanding epiblast cavity formation in vivo. It is intriguing that the apicosome and a structure known as the midbody (Schiel et al., 2013) share similar features. It would be interesting to examine the interplay and any potential signaling crosstalk between these cellular structures. Future studies centered on revealing the dynamics and function of the apicosome in progenitor cells and fully differentiated cell types will provide insight into its role in later stages of development.

We are still a long way from having a full understanding of all the complex cell–cell and cell–ECM interactions as well as of the signaling networks that take place during development. However, the studies by Taniguchi et al. (2017) and collaborators are instrumental to lead the way. The integration of developmental biology, physics, and bioengineering would be crucial to generate a more robust and reproducible understanding of the peri-implantation events of early human development.

Acknowledgments

We apologize to those colleagues whose work could not be cited because of length restrictions.

Research in the Gama laboratory is funded by the National Institutes of Health/National Cancer Institute (5R00CA178190-05).

The authors declare no competing financial interests.

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