STEM CELLS

It takes two to make an embryo

***In vitro* culture of mouse embryonic and extra-embryonic stem cells recapitulates embryogenesis.**

As far as systems to study embryogenesis go, embryonic stem cell (ESC)-based *in vitro* culture systems have been a vital tool. But they do not mimic the changes that occur as the embryo implants in the uterus, making it difficult to study this process of dramatic changes in blastocyst architecture *in vitro*. Now, a report from Magdalena Zernicka-Goetz at the University of Cambridge and colleagues shows that culturing mouse ESCs with extra-embryonic cells leads to formation of embryos that recapitulate this complex process with remarkable veracity (Harrison *et al.*, 2017). The approach is poised to enable unprecedented dissection of the molecular pathways that mediate critical steps in embryogenesis.

The beginnings of tissue identity are established during the first days of embryonic development as the epiblast, from which all tissues in the body originate, is defined in parallel to trophectoderm and primitive endoderm, both extra-embryonic tissues that give rise to the placenta and yolk sac. It is the communication among these three tissues that, during implantation, promotes formation of a lumen in the epiblast and another one in the trophectoderm, which eventually unite to form the 'egg cylinder'. Symmetry then breaks at the tissues' boundary as development continues.

Studying these critical events in embryogenesis has not been easy. As the embryo implants, engulfment by uterine tissues obscures the changes that occur in the embryo's architecture, preventing *in vivo* analysis. *In vitro* culture of mouse embryos (Bedzhov *et al.*, 2014), previously reported by the Zernicka-Goetz group, has enabled important discoveries by allowing imaging of morphogenetic events but doesn't enable the degree of molecular manipulation possible with cell-culture-based methods. While culture of ESCs into organoids, embryonic bodies, or patterned colonies has been useful, ESCs alone do not support development of the postimplantation egg cylinder.

To better mimic natural events with cell culture, Zernicka-Goetz and her team mixed two out of the three cell types involved: single ESCs and clusters of trophoblasts, with

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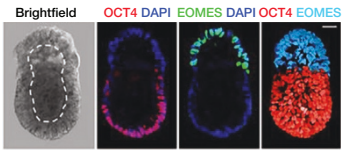
A LIGHT SWITCH FOR KINASES

A photodissociable dimer of the Dronpa fluorescent protein can cage kinases, making these important signal transducers controllable by light.

Kinases are central to many signaling events in our cells, but they are fickle; their activity needs to be tightly controlled in time and space or they can wreak havoc. Having a way of controlling kinases with high spatiotemporal precision would thus facilitate their study. Michael Lin and his team at Stanford University have succeeded in placing a variety of kinases under light control, which allows them to precisely switch these kinases on and off.

"If you could fuse two domains that bind to each other in the dark and you attach them to a protein of interest so that their binding would occlude the active site, then you would inhibit your protein in the dark," Lin explains. If these two domains could be dissociated by light, illumination would then activate the protein of interest. Lin decided that the fluorescent protein Dronpa was a good candidate for this light-dependent switch, as it undergoes a conformational switch in response to light. The team engineered Dronpa into a dimeric protein and the engineered Dronpa dimer dissociated upon illumination with violet light.

The researchers then inserted copies of the photodissociable Dronpa on each side of the active site of their target kinase. "We need to be close enough to the active site, so that the dimer forms and cages the protein [in the dark]," says Lin. For the examples the researchers tried—Raf1, MEK1, MEK2 and CDK5—they did not observe any deleterious effects on kinase function, and the caged kinases retained similar activity when illuminated as did the endogenous kinases. However, the insertion of two Dronpa moieties adds some bulk to the targeted kinases, which could be cause



An embryo derived from mouse embryonic and trophoblast stem cells, stained for various markers as indicated. Reprinted with permission from Fig. 1b of Harrison *et al.* (2017).

extracellular matrix in Matrigel providing a 3D scaffold. Reproducibly, 22% of structures contained both cell types, and ~92% of those had the typical elongated cylindrical structure of a postimplantation embryo. By 96 h post-plating, the cavities of the ESC and trophoblast compartments merged into a single large cavity.

That the stem cells could self organize was no surprise, recalls Zernicka-Goetz, but that they could replicate the embryo's natural architecture at this developmental stage was. "And they can do this without the third stem cell component for another extra-embryonic tissue that is normally involved in embryo development at that stage," she says.

Demonstrating the utility and versatility of their approach, the researchers studied molecular and cellular events in embryonic development using reporter ESC lines and synthetic inhibitors. For example, they treated cultured embryos with an inhibitor of the Nodal/Activin pathway and tested embryos from Nodal-deficient ESCs. Seeking insights into the generation of mesoderm and the specification of primordial germ cells, they used reporter ESC lines, which enabled them to demonstrate expression of primordial germ cell markers after 120 h of culture.

The approach does not require exogenous treatment, and "it has a built-in reference point—the boundary between the two cell types," says Zernicka-Goetz. Whether it would work with human cells is not clear; and if it did, the use of such a system would almost certainly face ethical questions. But the ability to image and manipulate molecular events in mouse embryogenesis in a culture dish provides a tool that will enable important discoveries with implications for both murine and human biology.

Irene Jarchum

RESEARCH PAPERS

Harrison, S.E. *et al.* Assembly of embryonic and extra-embryonic stem cells to mimic embryogenesis in vitro. *Science* http://dx.doi.org/10.1126/science.aal1810 (2017).

Bedzhov, I. *et al. In vitro* culture of mouse blastocysts beyond the implantation stages. *Nat.Protoc.* **9**, 2732–2739 (2014).

for concern. "It's similar to the concern one would have for fusing a GFP to a protein of interest, meaning most of the time it's fine but there are a few cases where it's not okay, so it's always good to test it with the assay that you care about," reasons Lin.

Lin and his team tested their photoswitchable kinases in a variety of contexts, including in cell culture and *Caenorhabditis elegans*. In particular, the team mimicked the physiological effects typically induced by bacterial infection in the worm rectum simply by activating photoswitchable MEK1 or Raf1 in the animals. Also, they rescued a defect in synaptic vesicle trafficking by illuminating mutant worms expressing photoswitchable CDK5. Since the original Dronpa can be excited by two-photon illumination, it may even be possible to use the photoswitchable kinase tools in animals where light scattering makes it more challenging to use optogenetic tools.

The strategy of caging proteins with Dronpa may be generalizable beyond kinases, and the team has already successfully caged other classes of proteins. "There are other proteins with active sites that you can cage in a similar way, meaning you can put one copy of Dronpa on each side of the active site," says Lin.

It is an exciting time for the optogenetics field, and a number of tools have recently been developed that allow light-dependent control of protein activity or localization. "We are kind of in a fast growth phase in terms of protein engineering technologies," says Lin. "It is good to see that we now have enough knowledge of how to engineer proteins, how to evolve specific kinds of domains, so that we can do what we want with light."

Nina Vogt

RESEARCH PAPERS

Zhou, X.X. *et al.* Optical control of cell signaling by single-chain photoswitchable kinases. *Science* **355**, 836–842 (2017).