STEM CELLS

A question of culture

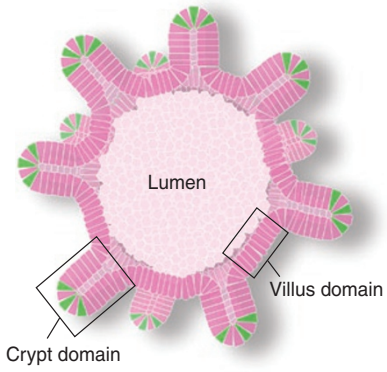
Two groups report culture conditions for long-term *in vitro* growth of intestinal tissue from the mouse.

The surface of the mammalian intestine renews itself very rapidly, needing only about five days for complete regeneration in the mouse. This massive capacity for self-renewal depends on intestinal stem cells, which generate all the lineages that make up a functional intestine.

The study of mouse intestinal stem cells has been largely carried out *in vivo*, because of the lack of a long-term culture system for this tissue. Although *in vivo* studies have several undeniable advantages, they also present complications: imaging and experimental manipulation, for instance, are difficult. But now, in recent papers, two independent groups present methods for long-term *in vitro* culture of intestinal tissue (Sato *et al.*, 2009; Ootani *et al.*, 2009).

Although they share some features, such as three-dimensional culture conditions, the approaches taken by both groups are quite different. Hans Clevers and colleagues at the Hubrecht Institute in the Netherlands, begin with the intestinal crypts—structures known to harbor stem cells—isolated from the mouse small intestine, using standard methods to purify them away from the intestinal villi (Sato *et al.*, 2009). These crypts are embedded in Matrigel, a basement membrane–like extract secreted by mouse cells, and then treated with a Wnt agonist, with the bone morphogenetic protein inhibitor Noggin and with epidermal growth factor. "The insights into what was needed to make these cultures work essentially came from genetic studies in the mouse and from studies on colon cancer," says Clevers.

Calvin Kuo, Akifumi Ootani and colleagues at Stanford University, in contrast, do not specifically isolate the crypts. Rather, they embed minced tissue from the mouse small or large intestine directly in a three-dimensional collagen gel (Ootani *et al.*, 2009). Critically, the preparation includes stromal cells, and the culture must be exposed to an air-liquid interface for robust growth. "It is difficult to know why the air-liquid interface is so important in our system, but it is possible that increased oxygenation is needed," speculates Ootani. In keeping with the observations of Clevers and colleagues, long-term growth of these cultures is also improved by the inclusion of a Wnt agonist.



Schematic showing the structure of a crypt organoid. The green cells in the crypt domain represent the intestinal stem cells. Image reproduced from *Nature*, courtesy of Toshiro Sato and Hans Clevers.

Methodological differences notwithstanding, both groups achieved sustained *in vitro* growth of intestinal tissue over many months, with multilineage differentiation and the appropriate expression of intestinal markers. The tissue grows in the form of spheres or organoids; a single polarized epithelial layer surrounds a lumen, with the apical surface of the cells facing the lumen and the basal surface facing the culture matrix or, in the work of Kuo and colleagues, the accompanying stromal cells.

Long-term growth of the cultures implies that they contain intestinal stem cells, and indeed, both groups confirmed this by identifying cells expressing the appropriate stem cell markers. Clevers and colleagues report that, as expected, the stem cells reside at the base of the crypt-like domains in the *in vitro* organoids.

The preparation of Clevers and colleagues lacks stromal cells, which are thought to make up the intestinal stem cell niche. Remarkably, their culture conditions can also be used to promote long-term growth of intestinal epithelium beginning from just a single sorted intestinal stem cell, although the efficiency of this outgrowth is low (6%). These somewhat unexpected findings indicate that, with the appropriate exogenously supplied signals, intestinal stem cells can be cultured and maintained without an explicitly supplied niche. The inclusion of stroma, however, may be a strength of the culture system developed by Kuo and colleagues. "It will allow the study of the intestinal stem cell in the context of the myofibroblast niche," says Ootani, "and may also allow the interactions of stem cells with other cell types to be studied, such as smooth muscle, endothelium or neurons, which may be relevant *in vivo*."

What is certain is that neither of the two reported culture systems were easy to develop. Both Clevers and Ootani comment, independently, that they and their colleagues tested hundreds of culture conditions and spent many years until they each converged onto the reported conditions.

These systems are likely to be useful for research on intestinal biology and potentially, assuming they work on human tissue, as the basis of therapeutic autologous transplants of intestinal epithelium. What is more, in an exciting possible extension of the work, the conditions described for the intestine may provide useful guidelines for the primary culture of other mammalian tissues as well.

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RESEARCH PAPERS

Sato, T. *et al.* Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* **459**, 262–265 (2009).

Ootani, A. *et al.* Sustained *in vitro* intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat. Med.* **15**, 701–706 (2009).