

## Helping stem cells make a connection

Protein network strengthens cell-cell contacts, promoting pluripotency.

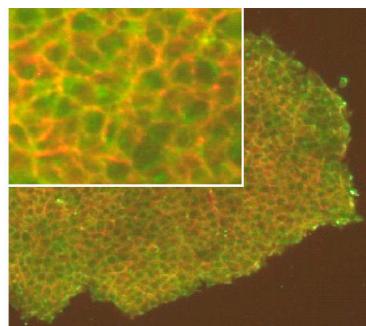
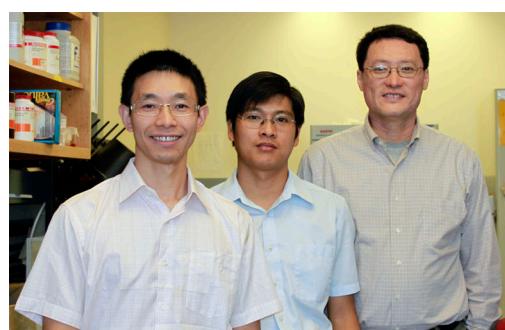
For embryonic stem cells, keeping in touch with the neighbors is crucial. Li et al. (1) have teased out a regulatory network that helps the cells stay close, thus guaranteeing that they remain pluripotent.

As long as they have congenial culture conditions, human embryonic stem cells (hESCs) can divide indefinitely without differentiating. Researchers have identified some of the cells' requirements. The recipe for rearing hESCs typically includes ingredients such as basic fibroblast growth factor and TGF- $\beta$  (2, 3). These compounds stabilize transcription factors—among them SOX2, OCT-4, and NANOG—that work together to curb differentiation (4). hESCs also need company. They huddle together, forming compact colonies with tight connections between cells. These colonies are distinctive—researchers have used their presence to verify the reprogramming of differentiated cells to restore hESC characteristics (5)—and isolated hESCs often die. Li et al. set out to investigate how close connections enable the cells to remain pluripotent.

First, the team dosed hESCs with compounds that might thwart kinases or signaling molecules necessary for pluripotency. They found that blebbistatin spurred the cells to separate and the colonies to become ragged. Blebbistatin also curtailed the activity of the pluripotency-maintaining transcription factors, and treated hESCs boosted their output of lamin A/C, an early differentiation marker.

Blebbistatin inhibits nonmuscle myosin II (NMMII), which helps control cell shape and movement. The researchers showed that only one version of the molecule, NMMIIA, influences pluripotency. The team tracked NMMIIA in hESCs and found that it gathered at cell-cell junctions with E-cadherin, a membrane-spanning protein that fastens adjacent cells together.

As their proximity suggests, the two molecules have an intimate relationship. NMMIIA helps E-cadherin remain in position. When the team cut NMMIIA levels



**Embryonic stem cells start to differentiate unless they remain in contact with surrounding cells.** (L-R) Dong Li, Jiaxi Zhou, Fei Wang, and colleagues (not shown) discovered that a regulatory network containing E-cadherin, NMMIIA, and p120-catenin makes the cells gregarious. The image above shows NMMIIA (green) and E-cadherin (red) in a colony of human embryonic stem cells.

with RNAi, E-cadherin vacated the cell-cell junctions and moved into the cytosol. Prolonged NMMIIA inhibition also prompted cells to manufacture less E-cadherin.

Blebbistatin trimmed the number of cells attached to a surface carpeted with E-cadherin by more than 20%, indicating that NMMIIA makes the cells stickier. Less sticky cells edge toward differentiating, the researchers found. Slashing E-cadherin levels with RNAi dampened the circuit that prevents differentiation, reducing the amounts of SOX2, OCT-4, and NANOG. Levels of several differentiation indicators also increased in these cells.

NMMIIA and E-cadherin may be close, but as Li et al. learned, there's a third party involved in the relationship. NMMIIA inhibition reduced E-cadherin levels by lowering expression of an E-cadherin binding protein called p120-catenin. The scientists also found that E-cadherin and p120-catenin are locked in a mutual enhancement loop—E-cadherin increases p120-catenin production, and p120-catenin boosts E-cadherin levels. The researchers speculate that this feedback loop within a signaling network

ensures that sufficient E-cadherin accumulates at cell-cell junctions.

"This work begins to reveal the molecular tricks that allow these cells to form these really tight adhesions," says senior author Fei Wang. In this case, he says, biochemical and mechanical signals combine to determine the stickiness of hESCs and the closeness of their colonies—and therefore the cells' differentiation status. One question researchers now need to answer, Wang adds, is how the bonds between hESCs affect the pluripotency-controlling circuit that includes SOX2, OCT-4, and NANOG.

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The findings might also provide stem cell researchers with clues about how to tweak the recipe for nurturing hESCs. Small molecules that activate the NMMIIA-E-cadherin regulatory loop might help ensure that hESCs keep dividing without differentiating prematurely.

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