

The two stages of cell fusion

Cells switch molecular lineups for initial and later phases of fusion.

FOCAL POINT

Cells have their own versions of the bankers and lawyers who arrange corporate mergers—proteins and other molecules that orchestrate cell fusion. Leikina et al. reveal that the process goes through two distinct stages that are under the control of different groups of molecules (1).

Fertilization is the first of many cellular mergers during an organism's lifetime (2). Immune cells fuse during inflammation, and multiple myoblasts combine to form muscle fibers during development. The same process also occurs in adults to repair muscle injuries. But studying the mechanics of cell fusion is difficult because researchers have to distinguish the fusion events themselves from the steps that ready cells to meld. For instance, before myoblasts merge, they differentiate, gain the ability to fuse, and stick to one another. Leikina et al. devised a new way to isolate cells undergoing fusion. They added the lipid lysophosphatidylcholine, which stalls cells just as they are about to fuse (3). Removing the compound triggered multiple cells to combine simultaneously, allowing the researchers to track the molecular changes as the cells join.

The researchers labeled myoblasts so they could determine when the cells' membranes and contents began to mix. The first step in the process, the scientists determined, was the fusion of the outer leaflets of each cell's plasma membrane. As fusion proceeds, an opening forms in the membranes of the two cells, and their contents begin to mingle.

The researchers next investigated what controls cell fusion. As myoblasts differentiate, they decorate their surface with two kinds of annexins, proteins that have been implicated in functions as diverse as apoptosis and blood coagulation. Some studies suggest that annexins help bend and fuse membranes (4). Leikina et al. discovered that antibodies that block two annexins, A1 and A5, prevented myoblasts from merging. Knocking down A1 or A5

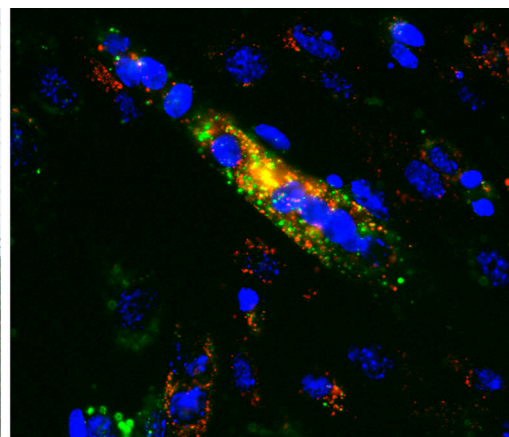


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Evgenia Leikina (left) and colleagues (not pictured) uncovered a control mechanism for muscle cell fusion. They followed the fusion of myoblasts labeled with either red or green dyes, which combine to form doubly labeled myotubes (right) with multiple nuclei (blue). The team discovered that this process occurs in two steps. Annexins 1 and 5 spur the first phase, in which the outer leaflets of the cells' membranes combine. Dynamin and PtdIns(4,5)P2 then prompt the second step, in which the barrier between the cells breaks down and they begin to share their cytoplasm.

with siRNA also stymied cell fusion. The functions of the two annexins appear to overlap. Adding A1 to cells lacking A5 restored their ability to fuse, for example. That result also explains why knockout mice lacking either A1 or A5 are healthy—one annexin can take over for the other.

Further experiments revealed that annexins help orchestrate the merger of the outer leaflets of the cells' membranes. The researchers then identified three molecules that were essential for the next stage, which

concludes with the intermingling of the two cells' cytoplasm. This fusion stage required ATP and dynamin, a GTPase that's also crucial for endocytosis. The team showed that dynamin inhibitors prevented cells from moving past the initial fusion of their plasma membrane outer leaflets. The third essential molecule was the phospholipid phosphatidylinositol(4,5) biphosphate (PtdIns(4,5)P2),

a vital cellular regulator that's involved in everything from lysosome formation to potassium channel activity.

The work suggests that cellular fusion occurs in two steps. Annexin 1 and

annexin 5 spur the first step, which involves the intermingling of the outer layers of the two cells' membranes. "To complete cell-to-cell fusion, there is a shift from extracellular machinery to intracellular machinery," says senior author Leonid Chernomordik. This intracellular machinery includes dynamin and PtdIns(4,5)P2, which help seal the two cells together, a feat that requires ATP. Whether annexins also take part in this second stage isn't clear, but dynamin and PtdIns(4,5)P2 aren't necessary for cells' outer leaflets to fuse.

The discovery that myoblast fusion requires dynamin could have medical implications, Chernomordik points out. Some rare muscle disorders involve dynamin mutations, and the new findings suggest their symptoms could result from faulty cell fusion during muscle development. The big question that needs to be answered, he says, is what functions the two annexins, dynamin, and PtdIns(4,5)P2 perform that enable cells to unite.

1. Leikina, E., et al. 2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201207012>.
2. Chen, E.H., et al. 2007. *FEBS Lett.* 581:2181–2193.
3. Chernomordik, L.V., and M.M. Kozlov. 2005. *Cell.* 123:375–382.
4. Gerke, V., and S.E. Moss. 2002. *Physiol. Rev.* 82:331–371.

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