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Myoblasts Fuse When Loner Meets ARF6

Studies of myoblast fusion in *Drosophila* have revealed numerous signaling and structural proteins that regulate this critical event in muscle development. A paper by Chen et al. published in the September 19 issue of *Cell* adds ARF6, a GTPase, and one of its guanine nucleotide exchange factors to this set of players.

Cell-cell fusion occurs among select groups of cells throughout development in a variety of contexts including the fusion of gametes to form a zygote, macrophages to form the osteoclast, and myoblasts to form myotubes that develop into muscle fibers. Myoblast fusion is the most widely studied of these intercellular fusion processes, and genetic approaches in *Drosophila melanogaster* have been most informative in defining the molecular components involved. Myoblast fusion is a multistep process whereby myoblast cells undergo successive steps of differentiation, cell-cell attraction, adhesion, and finally fusion. There is compelling evidence in *Drosophila* for an asymmetric expression of proteins between the two populations of myoblast cells, founder cells and fusion-competent myoblasts (see Taylor, 2002). Founder cells express the transmembrane protein Dumbfounded (DUF), which allows them to attract and mediate fusion with fusion-competent myoblasts. A cytosolic adaptor protein, Antisocial (ANTS), binds to DUF and is postulated to link fusion receptor signaling to the cytoskeleton through the recruitment of Myoblast City (MBC), leading to Rac activation. Fusion-competent myoblasts express MBC and Rac but do not express either DUF or ANTS, suggesting that DUF and ANTS might have special signaling roles in founder cells.

During a search for new genes involved in muscle development in *Drosophila*, Chen et al. (2003) discovered that *loner*, a gene encoding an ARF6 guanine nucleotide exchange factor (GEF), is required for myoblast fusion. This remarkable finding places ARF6, and its activation by Loner, in myoblast fusion and is the first report identifying an ARF regulatory protein in *Drosophila* development. It suggests that two GTPases, ARF6 and Rac, are necessary to coordinate the membrane and cytoskeletal rearrangements necessary for myoblast fusion. Loner contains domains typical of the ARNO/cytohesin subgroup of the ARF family GEFs including the catalytic Sec7, pleckstrin homology (PH), and coiled-coil domains (Jackson and Casanova, 2000), but it also contains an IQ motif at the amino terminus as does ARF-GEP$_{100}$ (Someya et al., 2001), the human homolog of Loner.

Chen et al. (2003) found that the expression of Loner is temporally and spatially regulated. Expression increases in the mesoderm at the onset of myoblast fusion and then declines when fusion is complete. Loner is expressed exclusively in the founder cells and its recruitment to sites of cell-cell contact is dependent upon DUF. Rescue of the defect in myoblast fusion in *loner* mutants is observed with full-length *loner*, but not with *loner* containing a catalytically inactive point mutation in the Sec7 domain or with a mutant lacking the PH domain required for membrane targeting. The *loner* phenotype is recreated in founder cells that express a dominant-negative mutant of *Drosophila*, ARF6^{T27N}, predicted to act by sequestering the GEF. This, along with biochemical data presented, indicates that Loner is acting as an ARF6 GEF. Significantly, the requirement for ARF6 activation in myoblast fusion is well conserved, as expression of ARF6^{T27N} in mammalian myoblast cultures also inhibits myoblast fusion. Taken together, these findings of Chen et al. (2003) provide new insight into regulation of myoblast fusion. In parallel signaling pathways, DUF can signal through Loner to ARF6 as well as through ANTS and MBC to Rac. It will be important to learn how these two pathways are coordinated and differentially regulated by DUF.

What function could ARF6 serve in cell-cell fusion? ARF6 is a member of the ARF family of GTPases and functions at the plasma membrane (PM) to regulate membrane traffic and the actin cytoskeleton (for review, see Donaldson, 2003). It is expressed in most eukaryotes from yeast to man, and the *Drosophila* homolog (ARF51F) is 97% identical to the human protein. In mammalian cells, ARF6 activity is required for many cortical actin-driven processes including cell spreading, PM ruffling, cell migration, wound healing, and Fc-mediated phagocytosis. ARF6 is required for and enhances the ability of Rac to remodel actin at the PM; indeed, activation of ARF6 leads to increased activation of Rac. ARF6 activation may be necessary to target Rac to sites of actin rearrangement. Consistent with this, Chen et al. (2003) found that Rac localization to discrete sites along founder cell PM was lost and became diffuse in *loner* mutants. Other activities of ARF6, including activation of phosphatidylinositol 4-phosphate 5-kinase and phospholipase D, that influence PM lipid composition and membrane trafficking could also be involved in mediating the specific changes in the PM that occur during myoblast fusion.

ARF6 facilitates endosomal-plasma membrane recycling and regulated secretion of dense core granules (see Donaldson, 2003), which may be of significance for myoblast fusion. A detailed morphologic study of

myoblast fusion in *Drosophila* revealed striking, paired vesicular structures containing electron-dense material on either side of tightly apposed PM of founder and fusion-competent myoblasts (Doberstein et al., 1997). These vesicles appear to be depositing electron-dense material between the two cells. As fusion proceeds, fusion pores are observed along PM areas devoid of these electron-dense plaques, and cytoplasm between the two cells is exchanged (Doberstein et al., 1997). Similar morphologic structures are observed during myoblast fusion in mammalian cells. It will be interesting to determine the contents of these vesicles and whether ARF6 and Rac regulate their localization and fusion with the PM.

Although fusion of intracellular, vesicular membranes during membrane traffic is a frequent event, cell-cell fusion is an unusual event that must be tightly regulated. The work of Chen et al. (2003) has uncovered an elaborate signaling network that coordinates this event during myoblast fusion. The conservation of proteins among multicellular organisms suggests these networks are also functioning in mammalian cells and provides new insight into this fascinating cellular process.

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Ac'septin' a Signal: Kinase Regulation by Septins

Budding yeast monitor shape and the assembly of cytoskeletal structures and convey this information to regulators of cell division, but the molecular mechanisms responsible for monitoring and interpreting spatial information about the cytoskeleton remain poorly understood. A paper in the September issue of *Molecular Cell* shows that direct binding of components of the septin cytoskeleton may relieve autoinhibition of a conserved checkpoint kinase, creating a simple molecular device for sensing septin cytoskeleton organization.

Septins are a family of evolutionarily conserved GTP binding proteins that were discovered over three decades ago in budding yeast in screens for mutants that showed a characteristic cell cycle arrest phenotype. Subsequent genetic and cell biological scrutiny revealed that septins form a unique cytoskeletal substructure that localizes with the cleavage apparatus in yeast and animal cells and is required for cytokinesis (for reviews, see Field and Kellogg, 1999; Gladfelter et al., 2001). Septins are not only found at cell division sites but are often localized to sites on the cell cortex that are destined for spatial reorganization, consistent with a broad role in regulating polarized growth and cell morphogenesis; for example, the septins form a ring-like structure at the incipient bud site in yeast and can be found in actin-rich regions of the cortex in fibroblasts. From these strategic positions on the yeast cell cortex, the septins appear to serve as molecular scaffolds for assembling key signaling modules that orchestrate coordination of bud morphogenesis, cytokinesis, and bud site selection with cell cycle progression.

As in all eukaryotic cells, the yeast cell cycle is monitored by checkpoints that convey information about cell status to cyclin-dependent protein kinases (CDKs), master regulators of cell division. The morphogenesis/size checkpoint in budding yeast induces a mitotic delay in the presence of cell shape perturbations. When bud site assembly is disrupted, cell cycle progression is arrested at the G2/M transition by inhibitory phosphorylation on Tyr19 of the CDK Cdc28 by Swe1, the budding yeast version of the conserved Wee1 kinase in other eukaryotes (Lew and Reed, 1995). This mitotic delay involves sensing by cytoskeletal elements and other factors that affect cell size and shape, transduction by intermediary signaling molecules, and activation of the effector kinase Swe1. Spatial information for downstream transducers appears to emerge from the septin cytoskeleton but exactly how the sensory information is conveyed has remained obscure. In fact, how cytoskeletal assembly and organization are monitored and coupled to other cellular events remains an important frontier in cell biology.

Recent advances using the genetically accessible budding yeast system have implicated members of another family of conserved kinases as direct monitors of septin assembly. The budding yeast has three Nim1-related kinases called Hsl1, Kcc4, and Gin4 that appear to act in a conserved signaling module to impart spatial information to Swe1. A recent paper from the Lew group has proposed that the Nim1-related kinase Hsl1 may act as a monitor for bud emergence in the yeast morphogenesis checkpoint (Theesfeld et al., 2003). They showed that activation of Hsl1 is dependent upon its recruitment to a cortical domain organized by the septins and that Hsl1 hyperphosphorylation and recruitment of the Hsl1 binding protein Hsl7 to the septin cortex only occurs after bud emergence. This is consistent with previous observations that Hsl1 kinase is inactive in mutants that do not form the septin cytoskeleton, indicating that septins are required for Hsl1 function in vivo