Involvement of Claudins in Tight Junctions

Two papers in this issue illuminate the role of claudins in the formation and maintenance of tight junctions, structures critical for the control of epithelial and endothelial tissue permeability. Previous work demonstrated that claudins are a major component of tight junctions, and to date, 16 members of the claudin family have been cloned.

Morita et al. (page 185) developed two polyclonal antibodies, one recognizing claudin-6 and the other recognizing both claudin-5 and claudin-6. Immunofluorescence microscopy and immunoprecipitation electron microscopy with these antibodies demonstrates that claudin-5 is a component of the tight junctions of endothelial cells, but not epithelial cells. When claudin-5 cDNA is introduced into mouse L cells, the cells form tight junctions which resemble those of endothelial cells in vivo.

In the same laboratory, Sonoda et al. (page 195) used a COOH-terminal fragment of Clostridium perfringens enterotoxin, which was previously shown to bind specifically to claudins 3 and 4, to probe the function of claudins in tight junctions in L cells and MDCK I cells. The toxin caused the disappearance of claudin-3 and claudin-4 from the junctions and the concurrent disintegration of tight junction strands, suggesting that claudins are directly involved in the barrier function of tight junctions. Shoichiro Tsukita, senior author on both papers, says that the availability of specific antibody and toxin tools to probe claudin function should lead to new strategies for drug delivery and new animal models for pathological states such as edema and inflammation.

Signaling Pathways in Tumor Development

Role of uPAR and Integrins in Tumor Dormancy

The poorly understood transition of metastatic cancer cells from a dormant state to an active, invasive tumor is the subject of studies by Aguirre Ghiso et al. (page 89), who found that in human carcinoma cells, this transition involves the signaling of urokinase plasminogen activator receptor (uPAR) and α5β1 integrins.

Earlier studies have correlated low uPAR levels with tumor dormancy, but the signaling pathway responsible for this phenomenon remained unknown. In the new work, the researchers examined the involvement of uPAR in α5β1 integrin signaling, and found that in cells with high uPAR, the frequency of uPAR–α5β1 interaction was increased, leading to a higher avidity of α5β1 and increased adhesion of cells to fibronectin. Fibronectin adhesion, in turn, activates MEK1/ERK signaling, which promotes cell growth. Disrupting uPAR/α5β1 complexes in uPAR-rich cells reduces signaling and promotes dormancy. “Since a correlation between high uPAR level and shortened time to cancer recurrence has been shown in several types of cancer...our finding may be of more general importance,” says Liliana Ossowski, senior author on the paper, who adds that additional experiments will be needed to determine how widespread the mechanism is.

Sonic Hedgehog and Cell Cycle Arrest

Focusing on the ability of Sonic hedgehog (Shh) activation to produce the cardinal features of human basal cell carcinoma (BCC), Fan and Khavari (page 71) report that Shh promotes cell proliferation by opposing epithelial cell growth arrest. In contrast to the classical multi-step pathway of carcinogenesis, Shh activation alone appears to be sufficient to induce a condition identical to BCC in genetically engineered murine and human skin, and mutations in Shh pathway genes have been correlated with human BCC.

The researchers transduced human epidermal cells with a retroviral vector expressing Shh before grafting the cells onto immune-deficient mice. Cells in the Shh-expressing epidermis apparently fail to undergo cell cycle arrest in vivo. In culture, the cells are resistant to the exit from S and G2/M that normally occurs in response to elevated calcium concentrations, and Shh expression also opposes growth inhibition by p21Cip1, another signal of known importance in intact tissue.

The report is the first to show a primary effect of Shh on cell cycle progression, and the team is now examining the impact of Shh on downstream growth regulators. In addition to their relevance to the pathogenesis of BCC, the new findings may help illuminate the role of Shh in normal cell growth control during development.

Signaling in Dental Stem Cells

Taking advantage of the ability of rodent incisors to grow continuously, Harada et al. (page 105) have developed an organ culture system for murine teeth and identified signaling pathways involved in maintaining putative dental stem cells. Their findings support a model of stem cell maintenance which may also be applicable to other developing and regenerating tissues.

Though the mouse incisor system has been studied extensively, previous work has not focused on stem cells. In the new study, the researchers established an organ culture of the apical end of the mouse incisor, and used BrdU and DiI labeling to demonstrate that stem cells are present in the cervical loop epithelium. In situ hybridization and biochemical studies suggest that FGF-10 expressed in the mesenchyme stimulates the expression of lunatic fringe, which in turn modulates the function of Notch and controls stem cell proliferation and differentiation. Irma Thesleff, senior author on the paper, explains that the group plans to widen its search for regulatory factors in the system: “We are just starting with this, and there is no question that microarrays will provide fantastic possibilities in studies on whole genetic pathways regulating developmental processes.”
Cytoplasmic Dynein and MTOC Positioning

By injecting double-stranded RNA (RNAi) to selectively silence gene expression, Gönczy et al. (page 135) demonstrate that cytoplasmic dynein is required for positioning the microtubule organizing center (MTOC) in the one-cell stage of the C. elegans embryo. Whereas genetic studies have helped elucidate the role of cytoplasmic dynein in yeast, where the protein is not essential for viability, inactivation of dynein genes in higher eukaryotes is lethal. To avoid this difficulty, the researchers injected RNAi into the gonad of a mother worm, then analyzed the first cell division in the resulting embryo by time-lapse microscopy and indirect immunofluorescence.

The team found that the dynein heavy chain is required for pronuclear migration and centrosome separation in the one cell stage embryo, and that p150\(^{Glued}\) and p50/dynamitin, components of the dynactin complex involved in dynein activity, are also required for these processes. Cytoplasmic dynein also appears to be involved in maintaining the association between centrosomes and the male pronucleus. The results support a model in which centrosome separation is driven by cytoplasmic dynein anchored on the nucleus. Pierre Gönczy, first author on the paper, explains that the team is now using RNAi to study a variety of other cellular processes: “Thus far, we have screened through 2,000 genes on chromosome III [using RNAi], and have gotten a large zoo of phenotypes affecting fundamental cell division processes.”