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Signal Transduction Underlying Cell Morphogenesis: Editorial Overview

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Cells adopt various types of morphology depending on their environment. For example, they change shape in response to extracellular stimuli, during progression through the cell cycle, or on contact with or isolation from other cells in culture or in situ. Cell morphology is determined primarily by the cytoskeleton, especially the actin cytoskeleton and microtubules, and by adhesion contacts. Basic mechanisms for assembly of the cytoskeleton and for cell adhesion, as well as the signaling pathways and molecules that regulate these processes, have recently begun to be revealed. This Minireview series, consisting of 10 articles, is intended to highlight this recent progress in order to provide insight into the molecular events that underlie cell morphogenesis. It also addresses the prospects for future studies in this exciting field. This editorial overview provides some background to the articles that follow.

Actin dynamics and Rho GTPase signaling

Assembly of the actin cytoskeleton begins with the nucleation and polymerization of actin monomers. The resulting actin filaments are then either bundled, cross-linked, anchored, or severed by the action of various actin-binding proteins. All cell morphogenetic processes rely on a specific combination of these actin-binding proteins to achieve the specific type of actin cytoskeleton required. There are two types of actin filaments: branched filaments, which form an actin mesh, and non-branched filaments, which are long and straight. In the yeast *Saccharomyces cerevisiae*, these two types of actin filaments are formed selectively by the action of the Arp2/3 complex and Bni1p, respectively, and they serve as the basis for the formation of actin patches and actin cables, respectively (*1*). In animal cells, extracellular stimuli activate members of the Rho family of GTPases to induce the formation of specific types of actin cytoskeleton, resulting in the adoption of specific types of cell morphology. Rho, Rac, and Cdc42 thus induce the formation of actin stress fibers, the actin meshwork in lamellipodia, and bundled actin filaments in filopodia, respectively (*2*). These actions of Rho family members are mediated by downstream effectors that selectively bind to the active form of each GTPase. Members of the WASP (Wiskott-Aldrich syndrome protein) family of proteins are Cdc42 effectors, and mDia (mammalian homolog of *Drosophila* diaphanous), which is the ortholog of yeast Bni1p, functions as an effector of Rho. In this Minireview series, Miki and Takenawa (*3*) review both how WASP family proteins are activated by Cdc42 and by other signaling pathways, and how they induce the formation of actin filaments via Arp2/3. These authors also discuss the similar mechanism by which Rac activates the WASP homolog WAVE to induce the formation of the actin meshwork in lamellipodia. Bito (*4*) takes neuronal morphogenesis as an example to review the actions of two Rho effectors, mDia1 and ROCK (Rho-associated kinase), in Rho-regulated actin dynamics in mammalian cells. Hakoshima et al. (*5*) address the structural biology of Rho GTPases as well as that of their regulators and effectors, and they discuss how Rho GTPases work as molecular switches, including how they are activated and down-regulated and how they transmit signals to downstream effectors.

Microtubules: dynamic instability and selective stabilization

In addition to the actin cytoskeleton, microtubules play an important role in the organization of cell morphology. For example, migrating fibroblasts adopt a characteristic morphology to the direction of migration; disruption of microtubules by treatment with nocodazole, however, results in a loss of this cell polarity. Nocodazole treatment also disrupts the mitotic spindle, resulting both in the adoption by cells of a spherical morphology and in mitotic arrest. These observations have led to the suggestion that microtubules transmit a spatiotemporal signal for cell morphology (*6*).

Microtubules are long hollow tubes composed of 13 protofilaments, each consisting of alternating α- and β-tubulin monomers. This arrangement of monomers generates polarity of microtubules, with the β-tubulin end being known as the plus end and the α-tubulin end as the minus end. Microtubules grow out from the microtubule-organizing center, which is associated with the centrosome in animal cells, with the minus end embedded in the centrosome and growth occurring only at the plus end. Microtubules extend in all directions in the cell, and they undergo assembly and disassembly in a stochastic manner at the plus end, a phenomenon known as dynamic instability (*7*). This stochastic process has been suggested to be required for targeting of microtubules to specific sites in the cell cortex or to mitotic chromosomes.

The attachment to such target sites results in microtubule stabilization, and the stabilized microtubules are then thought to perform specific functions. The spindle microtubules are thus stabilized by attachment to the

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kinetochore and mediate chromosomal segregation. In interphase cells, a subset of microtubules directed to the direction of cell migration is stabilized and mediates the induction of cell polarity. Cdc42 also contributes to this microtubule-mediated cell polarization, and microtubule stabilization also occurs in a Rho-dependent manner (*8*, *9*). The review by Mimori-Kiyosue and Tsukita (*10*) in this issue focuses on the actions of proteins that bind to the plus end of microtubules and discusses possible mechanisms of microtubule stabilization. It is possible that targeted microtubules recruit motor proteins or deliver signaling molecules, structural proteins, or membrane vesicles to modulate cell morphology locally. Interaction of microtubules with the actin cytoskeleton also may play a role in microtubule targeting and stabilization. In *S. cerevisiae*, Bni1p accumulates at prospective microtubule target sites to nucleate actin and to mediate the extension of actin cables from these sites; microtubules captured by plus end–binding proteins are then recruited to such sites by delivery on the actin cables mediated by the action of the unconventional myosin motor protein Myo2p (*11*). In animal cells, microtubules are targeted to focal adhesions, which are actin-based cell-to-substrate adhesion sites, and modulate the assembly of these structures (*12*). It thus appears that bidirectional interactions occur between the actin cytoskeleton and microtubules. The possible role of mDia in this process is discussed by Bito (*4*) in relation to neuronal morphogenesis.

Septins: a fourth type of cytoskeleton

Although intermediate filaments, which form a third type of cytoskeleton, are not covered specifically by this Minireview series, septins are. Septins were discovered only relatively recently and constitute a family of GTP-binding proteins that form polymers in the cell. Kinoshita (*13*) describes the complexity of mammalian septins and the oligomers and higher-order structures, including rings, coils, and curved bundles, that they form in vitro. The in vivo relevance of these proteins is then discussed by comparison of septin structures found in yeast and *Drosophila* and those detected in mammalian cells. These structures associate with actin-based cellular components such as the contractile ring and stress fibers. Kinoshita concludes by pointing out some important issues in septin research that are likely to be addressed in the next few years.

Cell-substrate adhesion versus cell-cell adhesion

The actin cytoskeleton and microtubules determine cell morphology by several actions. One such important action is modulation of cell adhesion. Two types of cell adhesion, cell-to-substrate and cell-to-cell adhesion, contribute to cell morphology. Cell-substrate adhesion is mediated by the binding of integrins at the cell surface to proteins of the extracellular matrix (ECM). Integrins are expressed constitutively at the cell surface but, under resting conditions, are present in a low-affinity state. They must be activated in order to be able to bind ECM proteins with high affinity on the outside and to become tethered to the actin cytoskeleton inside the cell (*14*).

Integrin activation requires two signals: one provided as a result of cell activation by soluble ligands such as growth factors, and the other derived from an initial attachment of ECM proteins to the integrin molecules. The pathways by which these signals result in integrin activation have remained unclear. Recent studies indicate, however, that the small GTPase Rap1 works as a switch in integrin activation. Rap1 also exhibits complex effects on the activity of ERK (extracellular signal–regulated kinase) and cell proliferation. Hattori and Minato (*15*) review the functions of Rap1 in signal transduction. They also describe the phenotypes of mice transgenic for Rap1 or for regulatory molecules such as Rap GTPase-activating protein (GAP), and discuss them with regard to Rap1 functions identified in cultured cells. The GTP-bound form of Rap1 binds to AF-6 (also known as afadin), which also binds Rap1GAP and regulates the site and dynamics of Rap1 action. Hattori and Minato (*15*) suggest that regulation of Rap1 signaling by AF-6 may occur not only at sites of cell-substrate adhesion but also at cell-cell adhesions, because AF-6/afadin, together with the intercellular adhesion molecule nectin, contributes to cell-cell adhesion.The nectin system is reviewed by Shimizu and Takai (*16*) in this Minireview series.

Neither cell-substrate nor cell-cell adhesions are static structures but rather undergo dynamic reorganization. The turnover of these structures requires endocytosis of their components—integrins in the former and cadherins in the latter—and recycling of these molecules back to the plasma membrane. Sabe (*17*) reviews recent studies that suggest that the small GTPase Arf-6 plays a regulatory role in such membrane recycling events at both cell-substrate and cell-cell adhesions. It is likely that these events are coordinated during cellular processes such as migration of epithelial cells. Sabe further discusses the implication of such Arf-6–dependent regulation in cancer cell invasion.

Both cell-substrate and cell-cell adhesions contribute to the formation of epithelial cell monolayers. Epithelial cells form two major types of cell-cell adhesions: adherens junctions and tight junctions (*18*, *19*). Adherens junctions are formed as a result of the homophilic binding of cadherins expressed at the cell surface. The cytoplasmic portion of cadherin molecules binds catenins, which, in turn, bind to the actin cytoskeleton. Whereas adherens junctions permit the passage of solutes between the adhering cells, tight junctions, as their name implies, confer a tight seal between cells. Tight junctions are formed as a result of the binding of opposing strands of claudins that are expressed on neighboring cells. The cytoplasmic portion of claudins binds the protein ZO-1–3, which also tethers actin filaments.

As indicated above, Shimizu and Takai (*16*) review a third type of cell-cell adhesion, that mediated by nectin, as well as the relation of this system to other types of cell-cell adhesion. One consequence of nectin-nectin binding is activation of Rac and Cdc42. Given that these GTPases strengthen adherens junctions and induce apical-basal cell polarity (see below), they may constitute a link between the nectin-afadin system and other types of cell-cell adhesion. Among the three types of cell-cell adhesions discussed, tight junctions are located most apically at the cell-cell boundary, with adherens junctions being positioned below the tight junctions and the nectin system being associated with the adherens junctions. The

three junctional complexes are thus distributed asymmetrically along the apical-basal axis. Many other molecules in epithelial cells are also asymmetrically distributed along this axis. Molecular complexes responsible for the generation of this apical-basal polarity have recently been identified. Ohno (*20*) describes one of these complexes, Par6-aPKC-Par3, and its role not only in epithelial cell polarization but in asymmetric cell division. The activated form of Cdc42 binds to Par6 and activates the Par6-aPKC-Par3 complex. Cdc42 thus appears to contribute to establishment of the polarity of both migrating cells and cells in an epithelial monolayer. Ohno (*20*) also reviews studies on two other molecular complexes that govern the apical-basal polarity of epithelial cells, as well as discusses how cells deploy these various complexes to induce polarity.

Spatial cues for cell morphogenesis in tissues

In addition to apical-basal polarity, epithelia exhibit another type of polarity, that along the *x-y* axis, known as planar cell polarity (PCP). Cells in epithelia within the body do not organize cytoskeletal structures randomly or homogeneously at the cell periphery; they rather generate such structures at specific positions, which are likely determined by spatial cues. Uemura and Shimada (*21*) take the epidermis of the *Drosophila* wing as an example of PCP and review studies that have provided insight into its mechanism. Each epidermal cell of the *Drosophila* wing extends a hair from its surface. The hair itself is composed of actin bundles and its formation is induced by Rho-ROCK signaling, which typically triggers both the formation of stress fibers in cultured fibroblasts and the retraction of cell processes in neurons (*4*, *22*). Each wing epidermal cell extends the hair at its distalmost vertex, indicating that Rho-ROCK signaling is restricted specifically to this location in the cell. Two groups of proteins appear to be responsible for this pattern of hair formation: one that determines the hair location and another that prevents the production of multiple hairs. Uemura and Shimada (*21*) review recent progress in characterization of the biased distribution of the first group of proteins at cell-cell boundaries and propose a model for PCP. They also address the identity and mode of action of polarity cues before extending their discussion to molecular events that underlie the establishment of PCP in vertebrates.

Conclusions

Thus, this Minireview series covers several topics in cell morphogenesis. As seen in the overview, one of the characteristics in this field is that studies that developed separately have been integrated intensively in recent years to provide some insights into what occurs in vivo in several typical processes in cells and tissues. We will see more comprehensive views over many morphogeneic processes in the coming years.

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