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Cell regulation From protein dynamics to animal behavior: new insights into complex cell regulatory mechanisms

Editorial overview

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Abbreviations

7-TM	seven-transmembrane
ER	endoplasmic reticulum
GluR	glutamate receptor
miRNA	microRNA
per	period
PGC	primordial germ cell
tim	timeless
TRAP	transmembrane adaptor

A diversity of cell types, ranging from neurons to immune cells and unicellular organisms, are faced with the common challenge of modulating cellular functions in response to changing cues from neighboring cells or the external environment. The solutions to these problems have ancient origins; therefore, it is not surprising that many of the same basic modes of cell regulation have been usurped by cells as divergent as yeast, *Dictyostelium* and neurons in the *Drosophila* and human brain.

Dynamic regulation of cell function is controlled in large part by modulating protein activities; however, the diversity of specific strategies by which this is achieved is staggering. Nevertheless, many of the mechanisms for altering protein activities, such as those described in this issue of *Current Opinion in Cell Biology*, can be boiled down to variations of the following three basic themes: changes in protein or mRNA concentration, protein trafficking and/or retention, and post-translational modifications. During recent years, there have been many new insights into how these controls are accomplished, many of which have been surprising and even anti-dogmatic.

A recurring theme in many of the reviews in this issue is that once a new mode for regulating cell function is discovered in one organism, it is likely to be found throughout most of phylogeny. A second theme is that it is not uncommon for the same protein to regulate cell function through multiple mechanisms. These concepts are well illustrated in the review by Sun and Chen, who describe new roles for ubiquitination in signaling. Ubiquitination is best known for its role in protein turnover, which is mediated by attachment of the small polypeptide, ubiquitin, to one or more lysine residues in target proteins and subsequent conjugation of an additional ubiquitin to a lysine in ubiquitin itself. During the last few years, it has become clear that polyubiquitination is a widespread post-translation modification, which regulates the activities of target proteins in a degradation-independent manner. Monoubiquitination also serves a regulatory role and, like polyubiquitination, is a reversible modification that alters protein function in a manner somewhat comparable to protein phosphorylation.

The multifaceted roles of ubiquitination in cell regulation arise in many of the reviews in this issue.

Among the most surprising recent discoveries in cell biology is the recognition that small RNAs can regulate protein levels. Nakahara and Carthew review new insights into the activities and functions of one class of small RNAs, referred to as microRNAs (miRNAs). Although miRNAs were not recognized until recently, they correspond to 0.5–1.0% of all genes in worms, flies and humans. In fact, they represent a percentage of genes comparable to those encoding other gene regulatory factors, such as DNA-binding transcription factors. Regulation by miRNAs is accomplished through dual effects on translation and mRNA degradation. The biological roles of only a few miRNAs have been described, although it is clear that they are critical for development. In addition, there are indications that miRNAs may contribute to cell regulation in neurons by modulating local levels of protein expression in specialized regions, such as growth cones and near synaptic membranes.

An important mechanism for regulating the concentration of integral membrane proteins at synaptic membranes is controlled exit from the endoplasmic reticulum (ER). Regulated ER exit of membrane proteins is a well known mechanism in non-neuronal cells and in neurons may be the rate-limiting step controlling the concentration of glutamate receptors (GluRs) in dendrites and spines. In the review by Vandenberghe and Bredt, the authors describe recent insights into the mechanisms underlying ER export of both ionotropic and metabotropic GluRs. These findings may be relevant to understanding activity-dependent modulation of synaptic strength, which is affected by changes in the concentration of GluRs at postsynaptic membranes.

Once integral membrane proteins are inserted in specific regions of the plasma membrane, it is necessary to maintain their localized distribution by minimizing lateral mobility of the proteins in the membrane. One way of accomplishing this is via a set of integral membrane proteins, claudins, which form a barrier that prevents the mixing of proteins in the apical and lateral membranes. As reviewed by Anderson, Van Italic and Fanning, the claudins also comprise a permeability barrier in apical junctions (e.g. tight junctions), permitting the selective intercellular transfer of various solutes. In addition, claudins associate with at least two types of macromolecular assemblies, both of which contribute to cell polarity. One includes PAR3, PAR6 and aPKC whereas the other contains PAT-1, Pals-1 and Crb-3.

The central proteins in macromolecular complexes are adaptor proteins, which consist of protein–protein and protein–lipid interaction domains. The roles of various types of adaptor proteins in cell regulation are particularly

well characterized in immune cells and are the subject of the review by Veillette. These include transmembrane adaptors (TRAPs), which nucleate proteins that function in protein-tyrosine-kinase-mediated signaling at the plasma membrane, and cytoplasmic adaptors, such as SLP-76. Interestingly, while some adaptors are positive regulators, others contribute to inhibitory signals.

The spatial distributions of many signaling proteins present in supramolecular signaling complexes are not static. Dynamic changes in the localizations of signaling proteins contribute to many aspects of cell regulation. In the case of plasma membrane receptors, internalization through endocytosis leads to signal attenuation. The receptors are either recycled or are subsequently degraded. In the review by Polo, Pece and Di Fiore, the authors put forth the intriguing proposal that defects in endocytosis of those receptors, which control cell proliferation, may lead to certain types of cancers. Given that monoubiquitination of some endocytotic proteins serves as a signal for endocytosis and that polyubiquitination can lead to degradation of internalized receptors, the authors propose that mutations in genes that function in ubiquitination pathways could lead to cancers. Similarly, mutations in caveolin-1, which participates in a unique form of endocytosis, have been identified in certain breast cancers.

Endocytosis of cell surface receptors can be mediated by uncoated vesicles, such as caveolae, or by clathrin coated pits. A surprising finding that has emerged over the last few years is that arrestin, which was originally thought to function exclusively in desensitization of seven transmembrane (7-TM) receptors, plays important roles in clathrin-mediated endocytosis. As described by Lefkowitz and Whalen, various arrestins participate not only in the endocytosis of 7-TM receptors but also in the internalization of other types of receptors, such as TGF β . The central role of ubiquitination in cell regulation arises again in this review, as ubiquitination of arrestin is critical for endocytosis. In addition, arrestin serves as an adaptor to bring E3 ligases to the receptors, which in turn leads to receptor ubiquitination and sorting to lysosomes. The role of arrestin as a new adaptor functioning in a variety of signaling pathways, such as MAP kinase pathways, is also discussed. Most recently, there are several studies indicating that β -arrestins play roles in cell migration in response to chemoattractants; this latter function may be related to arrestin making a contribution to MAP kinase activation.

Movements of cells in response to attractive cues are of central importance during development and for the physiology of certain types of differentiated cells, such as those that function in the immune system. The review by Raz focuses on the migration of primordial germ cells (PGCs), which exhibit directional migration in response

to chemokines released by somatic cells. Despite differences in PGC migration in organisms ranging from *Drosophila* to zebrafish and the mouse, in each case the directed movements of these cells during embryogenesis is mediated by chemokines, which bind to 7-TM receptors. The movement of the PGCs involves transepithelial migration, and depends on the Rho1 GTPase. This latter observation is consistent with observations that in many types of motile cells dynamic changes in actin filaments at the leading edge are crucial for cell movements.

Several classes of proteins that control dendritic actin formation are described in the review by Vartiainen and Machesky. These include a complex comprised of two actin-related proteins, Arp2 and Arp3, which is thought to serve as a template for some actin filaments, especially those at the leading edges of cells. Among the many proteins that regulate Arp2/3 function, the proteins referred to as WASP and SCAR may be the most important. SCAR appears to play a general role in lamellipodium formation and migration, whereas WASP seems to function in specialized processes such as endocytosis and formation of the immunological synapse. A controversial issue concerns the role of the ARP2/3 complex in cytokinesis, as different requirements are observed in different organisms.

Cytokinesis is a mechanical process that culminates with the cleavage of the mother cell into two daughter cells. As outlined in the review by Robinson and Spudich, the basic steps involve a series of shape changes in which the cell rounds up and becomes cylindrical and a furrow forms, leading to the appearance of a bridge connecting the two future daughter cells, which ultimately is severed. The authors outline a 'balance of forces' hypothesis, which proposes that cytokinesis proceeds as a result of a balance between the stiffness of the cell and the force applied by the contractile ring. Both the cytoskeleton and molecular motors play critical roles during cytokinesis. For example, microtubules are important for the formation of a single central furrow. Though the role of the ARP2/3 complex in this process is unresolved, cytokinesis clearly involves actin polymerization at the poles and force generated by myosin II.

Myosin II, which was originally discovered in muscle cells, is a classic actin-based motor that moves toward the plus-ends of actin filaments. By contrast, myosin VI is a minus-ended motor, a feature that sets it apart from all other myosins. Though myosin VI has been considered to be a transport protein that carries protein and vesicular targets, Frank, Noguchi and Miller put forth an intriguing and provocative proposal that the known functions of myosin VI are not due to movement along actin filaments *per se*. Rather, the biophysical features of myosin VI are consistent with the possibility that the roles ascribed to myosin VI might be due to the contributions of this

unusual myosin to crosslinking, stabilizing and organizing actin networks. These ascribed roles include myosin VI's functions in cell migration, vesicle movement and the localization of proteins, such as Miranda. Myosin VI interacts with Miranda and this association may be important for the asymmetric localization of this protein, which contributes to asymmetric cell division in neuroblasts.

As outlined in a review by Roegiers and Jan, asymmetric cell division is a conserved mechanism that contributes to the establishment of cell fate and may be the primary mode for establishing cell fate during *Drosophila* neurogenesis. The authors also review the state of our understanding of how asymmetric cell divisions contribute to vertebrate neurogenesis, although the underlying mechanisms are not as well worked out as in *Drosophila*. Of central importance to asymmetric cell division during *Drosophila* neurogenesis is the localized distribution of Numb, Miranda and other proteins to the one side of the neural stem cells. In addition to the actin cytoskeleton and myosin VI, asymmetric protein distribution is dependent on the complex composed of PAR3/PAR6/aPKC, which was described in the review by Anderson, Van Italie and Fanning. This apical complex, as well as myosin VI, also contributes to asymmetric cell divisions by regulating the orientation of the mitotic spindle. A key role for Numb in asymmetric cell division is to inhibit the activity of the transmembrane receptor, Notch. Numb associates with a component of the endocytic pathway and, according to one model, might downregulate Notch in one daughter cell through an endocytosis-mediated mechanism. There is evidence that monoubiquitination of the Notch transmembrane ligand, Delta, results in an increase in endocytosis of Delta, which in turn upregulates Notch. G-protein signaling also appears to contribute to asymmetric cell division by regulating the localization of the PAR3/PAR6/aPKC complex, mitotic spindle morphology and differences in sizes between the daughter cells.

In addition to functioning in cell fate determination, cell movements and other aspects of development, G-protein signaling is of equal importance to a variety of regulatory mechanisms in differentiated cells, such as those are initiated by sensory input and a variety of hormones. Despite the fact that G proteins have been subjected to intense scrutiny, for many years their roles in receptor and effector recognition have been ascribed to the α subunit and it was thought that the $\beta\gamma$ subunit freely dissociates from the activated α subunit. Robishaw and Berlot review some of the recent studies demonstrating that the $\beta\gamma$ subunit also interacts with other signaling molecules and that the α and $\beta\gamma$ subunits may remain closely associated with the activated α subunit. Given that there are many more G-protein-coupled receptors than α and $\beta\gamma$ subunits, specificity may be achieved through compartmentalization of G proteins in microdomains, regulated expression and the assortment of different

combinations of subunits. Interestingly, there are suggestions that the G-protein subunits may be molecular scaffolds that serve to recruit a variety of signaling proteins to the plasma membrane.

The final review in the issue illustrates how phenomena as complex as animal behavior can be understood to a great extent by dissecting cell regulatory mechanisms at the molecular level. Among the best-understood animal behaviors are circadian behavioral rhythms in *Drosophila*. Critical to these behaviors are oscillations in the concentrations of the *period* (*per*) and *timeless* (*tim*) RNAs and proteins, which occur through an autoregulatory loop. When the two proteins are at high levels, they form a complex, which translocates to the nucleus and represses expression of CLOCK and CYCLE. These latter proteins are necessary for expression of PER and TIM. Consequently the *per* and *tim* RNA levels decrease.

Turnover of the TIM protein occurs through the proteasome and appears to be dependent on ubiquitination. In the review by Levine, the author describes how circadian oscillations in gene expression are not restricted to the central brain. There are also tissue-specific oscillators, which operate through similar but not identical mechanisms. In addition, studies in bees and fruitflies indicate that social interactions influence circadian rhythms.

In conclusion, the various reviews in this issue not only describe new molecular insights into cell regulation, but also highlight how similar mechanisms are used to regulate seemingly disparate processes throughout most of phylogeny. Moreover, the proteins described in these reviews play many more cell regulatory roles than initially envisioned. Finally, the reviews highlight insights into the consequences of perturbations in cell regulatory mechanisms and molecules on human disease.