PERSPECTIVES: TRANSCRIPTION

Translocating Tubby

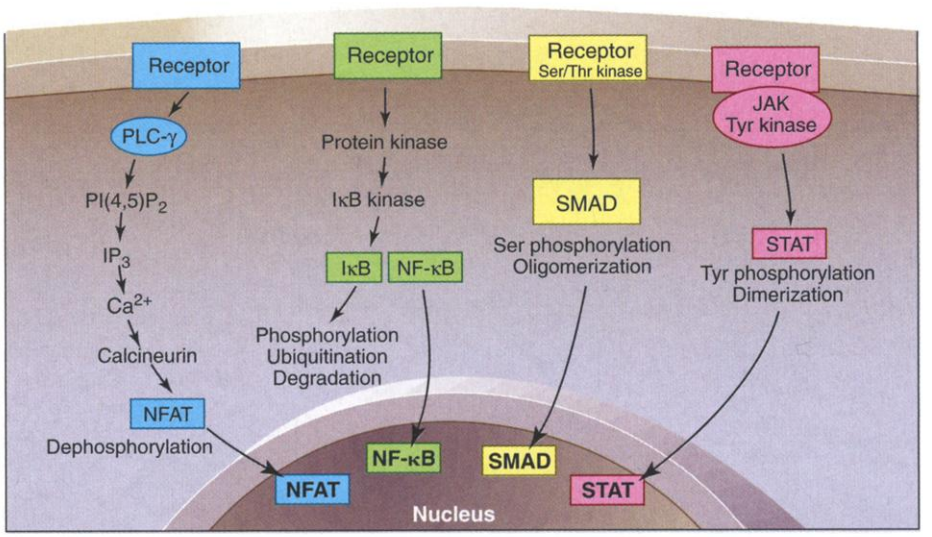
Lewis C. Cantley

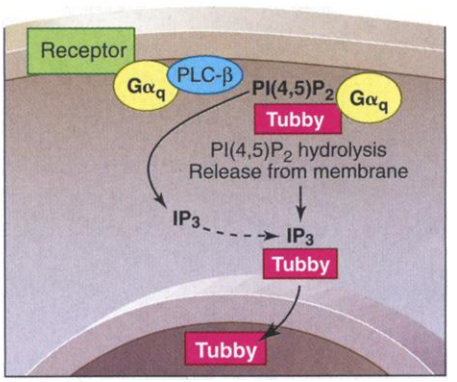
Once receptors in the plasma membranes of cells become activated through binding to their ligands (either hormones or growth factors), they initiate a cascade of signals that are transmitted to the nucleus where they switch on the expression of target genes. In many of these signal transduction pathways there is a key transcription factor "trapped" in the cell cytoplasm that becomes modified once the hormone or growth factor receptor is activated. The modified transcription factor is then free to escape from the cytoplasm and to enter the nucleus where it activates target genes. The signaling events that lead from receptor activation at the cell surface to the release of the transcription factor into the nucleus are still poorly understood for certain transcription factor families. A report by Santagata *et al.* (*1*) on page 2041 of this issue now reveals a new signaling pathway in which the putative transcription factor tubby is released from its association with plasma membrane phosphatidylinositol (PI) lipids and moves to the nucleus. The authors show that tubby—a defective version of which has been implicated in mature-onset obesity—is clipped from the plasma membrane by the enzyme phospholipase C–β, which breaks down (hydrolyzes) PI lipids following activation of G–protein coupled hormone receptors.

Some well-studied examples of transcription factors that move from the cytoplasm to the nucleus under the control of cell surface receptors include NFATs, NF-κB, SMADs, and STATs (see top figure). For these transcription factor families, the often convoluted signal transduction pathways that lead to their nuclear localization have been partly elucidated. Such pathways typically involve the phosphorylation or dephosphorylation (the addition or removal of phosphate groups) of the transcription factor or an associated protein. NFAT nuclear localization is regulated by the activated receptor stimulating PI turnover in the plasma membrane, resulting in elevated production of inositol-1,4,5-trisphosphate (IP_3). Then follows IP_3-mediated release of calcium ions from internal stores, activation of a secondary influx of calcium ions, calcium-dependent stimulation of a phosphoprotein phosphatase (calcineurin), and dephosphorylation of key sites on NFAT that expose a nuclear import signal (see top figure). Control of NF-κB is equally complex: activation of a serine/threonine kinase cascade results in phosphorylation of the inhibitor IκB to which NF-κB is bound. This phosphorylation marks IκB for degradation by a ubiquitin-dependent pathway and releases NF-κB, which moves to the nucleus. In contrast, the control of SMADs and STATs is more direct. The SMADs are phosphorylated by transforming growth factor–β/activin family receptors (which have serine/threonine kinase activity), resulting in the formation of multimeric SMAD complexes that enter the nucleus. Likewise, STATs are phosphorylated on tyrosine residues by receptor-associated tyrosine kinases, leading to intermolecular phosphotyrosine-SH2 domain interactions that expose nuclear import signals.

In their study, Santagata *et al.* (*1*) report a simple but unexpected pathway regulating the movement of tubby into the nucleus. Despite the genetic evidence in mice that loss of tubby results in obesity, insulin resistance, retinal degeneration, and hearing loss (*2–4*), it has been difficult to determine precisely what tubby does in the cell. The crystal structure of tubby reveals that it has two domains: an amino-terminal domain with similarity to the activation domains of transcription factors, and a carboxyl-terminal domain (the tubby domain) of 260 amino acids that binds to double-stranded DNA (*5*). When expressed at low levels, tubby is found primarily at the plasma membrane, but at higher levels it is also detected in the nucleus (*1*). The carboxyl terminus, in addition to binding DNA, is required for tubby to bind to the plasma membrane, whereas the amino terminus is required for tubby to reside in the nucleus.

While investigating how the tubby domain associates with the plasma membrane, Santagata *et al.* discovered that this domain binds with high specificity to





Getting to the nucleus. Signal transduction pathways initiated by activated receptors control translocation of transcription factors from the cytoplasm to the nucleus. (**Top**) Movement of the transcription factors NFAT, NF-κB, SMADs, and STATs is associated with the phosphorylation or dephosphorylation of signaling proteins by kinase and phosphatase enzymes. (**Bottom**) The putative transcription factor tubby is directly bound to $PI(4,5)P_2$, a phospholipid in the plasma membrane. Hydrolysis of this phospholipid by the enzyme phospholipase C–β, which is stimulated by activated G protein–coupled receptors, releases tubby (*1*). Tubby then moves to the nucleus and switches on target genes involved in the control of fat metabolism.

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bisphosphorylated phosphoinositides (containing phosphate at the 4-position of the inositol ring), such as $PI(4,5)P_2$ (phosphatidylinositol-4,5-bisphosphate), a phospholipid highly enriched within the plasma membrane. A cocrystal structure of the tubby domain with the head group of $PI(4,5)P_2$ revealed a basic pocket that is crucial for binding of tubby to this phospholipid. Furthermore, overexpression of an activated form of the heterotrimeric GTP-binding protein $G\alpha_q$—which stimulates activation of phospholipase C–β and the hydrolysis of $PI(4,5)P_2$—resulted in movement of tubby into the nucleus. In addition, expression of the serotonin receptor $5HT_{2c}$, which activates $G\alpha_q$, also resulted in relocation of tubby to the nucleus. A further twist to the story came with the finding that either activated or nonactivated forms of $G\alpha_q$ could bind to tubby in vivo, suggesting that $G\alpha_q$ helps to position tubby at plasma membrane locations where $PI(4,5)P_2$ hydrolysis occurs. This would ensure efficient release of tubby from the plasma membrane even when a relatively small fraction of total $PI(4,5)P_2$ is hydrolyzed. It is also possible that the water-soluble inositol polyphosphates, IP_3 and IP_4, that accumulate when $PI(4,5)P_2$ is broken down could contribute to the sustained dissociation of tubby from the membrane by competing with $PI(4,5)P_2$ for binding to tubby (see bottom figure).

The findings of Santagata *et al.* raise new questions about the involvement of PI turnover in cellular signaling. It has been assumed that the primary task of hormone-stimulated PI turnover is to increase cytosolic calcium, which then regulates a host of calmodulin-mediated events (such as calcineurin-dependent NFAT translocation). Calcium ions, however, do not appear to be primary players in tubby translocation because in experiments with a calcium ionophore (which allows calcium ions to flow into cells), tubby did not relocate to the nucleus. Interestingly, $PI(4,5)P_2$ in yeast can also be hydrolyzed by a phospholipase to generate IP_3. This pathway, however, does not appear to be linked to calcium ion regulation but rather is involved in the synthesis of more highly phosphorylated forms of inositol polyphosphates that are required for mRNA export and transcriptional regulation (*6*). Additionally, $PI(4,5)P_2$ is involved in the polymerization of actin filaments through its direct binding to a host of proteins that mediate this process. Thus, it is likely that $PI(4,5)P_2$ synthesis and its hydrolysis to IP_3 evolved for diverse purposes that are independent of calcium ion regulation. Moreover, IP_3-dependent increases in intracellular calcium are probably a relatively late (although still very important) adaptation of this pathway in higher eukaryotes.

There is much yet to learn about tubby. The high level of expression of both tubby and the $5HT_{2C}$ serotonin receptor in the paraventricular nucleus of the hypothalamus and the observation that $5HT_{2C}$-deficient mice (*7*) have mature-onset obesity and other characteristics similar to those of mice lacking tubby imply that tubby is controlled by this receptor in vivo. However, a host of other hormone receptors—including the bombesin, dopamine D1, melanocortin 4, and melanin concentrating hormone receptors—are coupled to $G\alpha_q$ and could regulate tubby in vivo. In addition, insulin has been shown to stimulate tyrosine phosphorylation of tubby, and this could impose an additional level of regulation (*8*). Finally, there is much yet to learn about the part that tubby plays in regulating downstream genes that control obesity. The study by Santagata *et al.* lays the foundation for future progress in this important area of research.

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PERSPECTIVES: CHEMISTRY

A New Twist on Chirality

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One of the great mysteries in science is the homochirality (single handedness) of the essential molecules of life. Natural sugars are almost exclusively right-handed; natural amino acids are almost exclusively left-handed. Current life forms could not exist without the uniform chirality of these monomers, which form the building blocks of polysaccharides and proteins. Uniform chirality is also essential for information storage and processing, as demonstrated by the supramolecular chirality of the DNA helix. But we still do not know the origin of this biomolecular homochirality.

Macroscopic chiral selection—which usually goes unnoticed in daily life, for instance, when one shakes hands or uses a corkscrew to open a bottle of wine—seems to bear no relation to chirality at the molecular level. But on page 2063 of this issue, Ribó *et al.* (*1*) report that simple stirring can lead to chiral selection. At first sight, these results seem hard to believe, but the authors provide strong support for their claim.

Pasteur tried, without success, to induce a preference for right- or left-handed molecules by performing reactions in a centrifuge and even by rotating growing plants to change the handedness of their natural products (*2*). Ever since, scientists have tried to generate excess left- or right-handed chiral molecules from achiral precursors without the intervention of any preexisting molecular chirality.

This problem, termed absolute asymmetric synthesis, has turned out to be a major challenge (*3*). Recent successful studies have used photochemistry with circularly polarized light (*4*), chiral selection based on the electroweak interaction (although disputed) (*5*), and the combination of a magnetic field and nonpolarized light (*6*). Numerous attempts have also been made to perform asymmetric synthesis by clockwise or counterclockwise rotation during the chemical conversion of an achiral compound. But the resulting indications of chiral selection are usually discarded as irreproducible or as artifacts. These failures are not unexpected if one realizes that the applied external chiral force must directly exert a polarizing effect on the reaction path, a condition not satisfied by bulk rotation (*3*).

Nevertheless, the vortex motion used by Ribó *et al.* acts as a true chiral force (*7*). How can it lead to chiral selection? In Ribó *et al.*'s system (*1*), stirring does not act on a chemical reaction but rather on an aggregation process. The assembly of identical achiral molecules into large chiral fiber-type structures under the direct influence of the external force created by stirring is biased to a particular handedness.

Ribó *et al.* use achiral disklike porphyrins that have a zwitterionic structure, that is, they contain both negatively and positively charged moieties (see the figure). These properties allow the molecules to aggregate through electrostatic interactions and hydrogen-bonding. A dilute solution of the porphyrin is slowly concentrated while being stirred, thereby stimulating the aggregation. The disk-shaped molecules stack side by side to form strands (called J-aggregates), which combine to form fibers and bundles of fibers.

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