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mologs of cotton CelA, and that a number of other sequences, while not completely related to CelA, share significant identity to one or more of the suspected UDP-Glc–binding domains.

Why plants have so many different *CelA* and related genes is now a focus of investigation in a number of laboratories. The glucan chains in the cellulose microfibrils made in primary and secondary cell walls are different in their degree of polymerization, possibly as a result of different modes of catalysis and organization into paracrystalline arrays. Of the 40 or so cell types that plants make, almost all can be identified by unique features of their cell wall. The family of *CelA* genes may be required to encode the diverse cellulose synthases needed to build these specialized walls. For example, another *Arabidopsis* mutant was described in which the water-conducting cells form incomplete secondary walls (6), apparently as a result of defective cell-specific cellulose deposition. In addition, some of the related genes may well encode other glycosyltransferases, such as those that synthesize β-xylans, mannans, the backbone of xyloglucan, mixed-linkage β-glucans, or callose.

As the *CelA* genes were sequenced and the multiple binding sites for the substrate UDP-Glc were deduced, researchers began to rethink the biochemical mechanism of cellulose synthesis. The catalysis of this polymerization must overcome a steric problem because the β(1→4) linkage requires each glucose unit to be flipped nearly 180° with respect to its neighbors (see figure). To make such a linkage by addition of one sugar unit at a time, either the synthase or the growing chain must rotate 180°, or the sugar must be added in a constrained position and flip or flop alternately into the proper orientation by some other factor associated with the synthase. Because a cellulose microfibril is composed of several dozen chains, each arising from a synthase, such a drastic reorientation of enzyme or substrate would be unlikely. Saxena *et al.* (3) revived an old idea that the unit of addition is cellobiose, but with a new twist. With multiple UDP-Glc binding sites, Saxena and colleagues reasoned that two UDP-Glc–binding domains positioned 180° from each other make two simultaneous glycosyl transfers that add cellobiose units to the growing chain, circumventing the need for reorientation of synthase or chain. As it had not been established whether the glucosyl units were added to the reducing or nonreducing end of the chain, they also reasoned that retention of the UDP moiety at the reducing end kept the growing end of the chain activated. A similar two-site model was proposed by Carpita *et al.* (7), but in this model the two glucosyl units are simultaneously polymerized at the nonreducing end. Another version of this model of cellulose synthesis at the nonreducing end was presented recently, with the additional proposal that the glycosyl units form covalent intermediates with serine or threonine in the active site (8). However, Koyama *et al.* (9) showed by direct staining of the reducing ends and by microdiffraction electron crystallographic analysis that the glucose units are indeed added to the nonreducing end. They also invoke the two-site model for addition of glucosyl units, but the C-4 hydroxyl of the sugar to be added is activated not by a hydroxyl amino acid, but by aspartic acid—the very amino acid that hydrophobic cluster analysis of the original *CelA* genes had predicted to be essential in three of the four highly conserved domains (see figure).

What other polypeptides are involved in the synthesis of cellulose? The identification of a Zn-binding domain in the NH_2-terminal region of CelA (*10*) not only indicates that the polypeptide interacts with another protein, but also provides a molecular means to identify a ligand. Although there is much yet to be learned about polysaccharide synthesis in plants, the long decades of disappointment are over, and the discovery of the first synthase genes marks a new age of progress in learning how plants make their cell walls.

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SIGNAL TRANSDUCTION

Lipid-Regulated Kinases: Some Common Themes at Last

Julian Downward

Kinases are enzymes that add phosphates to small molecules or other enzymes, creating active signaling molecules or turning enzymes on or off. Cells use this device over and over again to change their internal biochemistry in response to signals from the outside. One class of these kinases [phosphoinositide 3-kinases (PI 3-kinases)] phosphorylate lipids to form the second messenger phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3] in response to a very wide range of extracellular stimuli. PtdIns(3,4,5)P_3 acts on pathways that control cell proliferation, cell survival, and metabolic changes—often through two different protein kinases, p70 ribosomal protein S6 kinases ($p70^{s6k}$) and protein kinase B (PKB). Until now the way PtdIns(3,4,5)P_3 regulates these two kinases has appeared to be very different, with each new advance adding to the impression that they had very little in common. However, two reports in this issue of *Science* on pages 707 and 710 (*1*, *2*) and two recent reports in *Current Biology* (*3*, *4*) add considerably to the picture of how these kinases are regulated, and in so doing reveal surprising similarities in their control mechanisms. The same regulatory themes likely apply to many protein kinases, significantly simplifying the task of understanding their regulation.

The ability of PKB, also known as Akt, to phosphorylate its substrates is increased after the cell is exposed to growth factors and insulin, and this increase occurs via a pathway that includes PI 3-kinase. In addition to its kinase domain, PKB contains an amino-terminal pleckstrin homology (PH) domain, to which the PtdIns(3,4,5)P_3 signaling molecule binds (*5*). In addition, PKB itself is phosphorylated when PtdIns(3,4,5)P_3 is present by two different protein kinases, 3-phosphoinositide-dependent protein kinase 1 (PDK1), which

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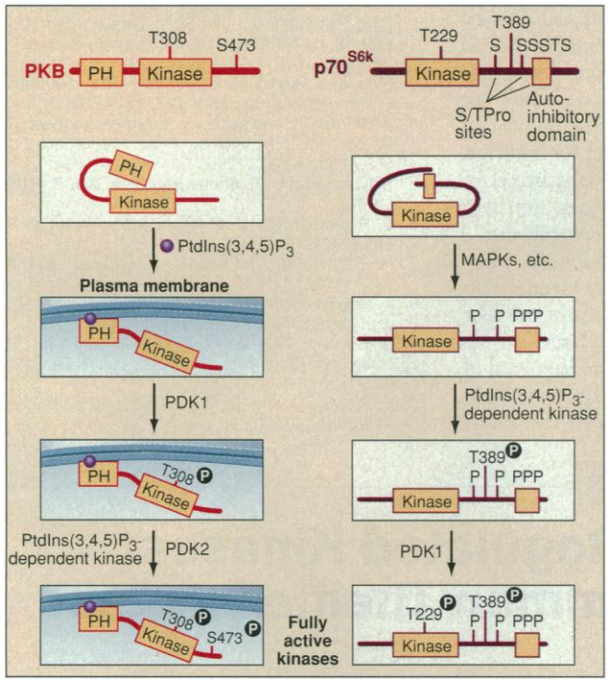
phosphorylates Thr^{308} in the activation loop of the kinase domain of PKB, and PDK2, which phosphorylates Ser^{473} near the carboxyl-terminal (*6*, *7*). Phosphorylation of these two sites is sufficient to fully activate PKB.

Stephens *et al.* (*2*) and Alessi *et al.* (*3*) now report the cloning of PDK1 and clarification of its function. PDK1 has a kinase domain that is distantly related to that of PKB and, like PKB, also contains a PH domain that binds to PtdIns(3,4,5)P_3 very tightly. However, unlike PKB, the kinase activity of PDK1 is not influenced by binding to PtdIns(3,4,5)P_3 in vitro, nor is it dependent on PI 3-kinase activity in the cell: Surprisingly, PDK1 is a constitutively active kinase that is neither stimulated by insulin nor inhibited by chemical inhibitors of PI 3-kinase. The dependence of PKB phosphorylation at Thr^{308} (and hence its activation) on PI 3-kinase activity lies at least in part in the binding of PtdIns(3,4,5)P_3 to the PH domain of PKB, not to that of PDK1. PDK1 will only phosphorylate PtdIns(3,4,5)P_3-bound PKB, suggesting that the PH domain of PKB normally restricts access of PDK1 to Thr^{308} but is displaced upon binding PtdIns(3,4,5)P_3, allowing phosphorylation to occur. PI 3-kinase is necessary for other steps in the process—translocation of PKB to the plasma membrane (*8*) and the phosphorylation of Ser^{473} by PDK2 (*9*), which remains uncloned.

How do these reactions fit together to regulate PKB? A likely model is as follows: PtdIns(3,4,5)P_3 binds to the PH domain of PKB, forcing its translocation to the plasma membrane and exposing Thr^{308} for phosphorylation by PDK1, which we now know is constitutively active. Serine-473 of PKB is then phosphorylated by PDK2, which is probably regulated by PtdIns(3,4,5)P_3 and likely located at the plasma membrane, at least in its activated form. PDK1 itself appears to be localized to a large extent in the cytosol, but some of the enzyme undoubtedly will be at the plasma membrane as a result of its binding to PtdIns(3,4,5)P_3. Concentration of all three of the kinases—PKB, PDK1, and PDK2—at the plasma membrane would facilitate the interactions of the upstream kinases with PKB. After its phosphorylation at both sites, PKB can detach from the membrane and phosphorylate its targets within the cell. PKB influences metabolism through phosphorylation of glycogen synthase kinase–3 and phosphofructokinase, as well as transmitting a potent survival signal. In part, this is by phosphorylation and inactivation of Bad, a pro-apoptotic Bcl-2 family member (*10*, *11*), although other targets are likely.



Similarities revealed for two key kinases, PKB and p70^{s6k}. Upon binding the messenger PtdIns(3,4,5)P_3, PKB is translocated to the plasma membrane, and PH domain masking of Thr^{308} is relieved. PKB is then sequentially phosphorylated at Thr^{308} by PDK1, which is constitutively active, and at Ser^{473} by PDK2, which is PtdIns(3,4,5)P_3 dependent, to yield a fully activated kinase. p70^{s6k} is phosphorylated initially by proline-directed kinases including MAP kinase in the autoinhibitory domain, then by a PtdIns(3,4,5)P_3-dependent kinase at Thr^{389} to generate a conformation where Thr^{229} is accessible for phosphorylation by PDK1 to yield a fully active enzyme.

In contrast, p70^{s6k} participates in the translational control of mRNA transcripts containing 5′ polypyrimidine tracts. Regulation of p70^{s6k} is also through PI 3-kinase, but it has appeared to be considerably more complex than that of PKB, involving phosphorylation of many more sites (*12*). However, the two kinases are distantly related, and p70^{s6k} is phosphorylated at Thr^{229} and Thr^{389}, sites that are analogous to Thr^{308} and Ser^{473} of PKB, the targets of PDK1 and PDK2. Pullen *et al.* (*1*) and Alessi *et al.* (*4*), therefore, tested whether PDK1 could phosphorylate p70^{s6k} at Thr^{229} and found that indeed it could. Furthermore, a dominant negative form of PDK1 blocked activation of p70^{s6k} in cells. This work represents the culmination of a huge effort that has led to the following model of p70^{s6k} regulation: Initially proline-directed kinases such as mitogen-activated protein kinases phosphorylate a number of neighboring sites in the carboxyl-terminal autoinhibitory domain. This relieves a conformation inhibition that may result from interaction of the amino- and carboxyl-terminal portions of the protein. Thr^{389} is then phosphorylated by an unidentified PtdIns(3,4,5)-P_3-dependent kinase. Both of these steps are required to induce a conformational change that transforms Thr^{229} into a good substrate site for the constitutively active PDK1. As is the case for PKB and Thr^{308} and Ser^{473}, phosphorylation of both sites causes a strongly synergistic activation of the kinase activity of p70^{s6k}. As yet the identity of the kinase for Thr^{389} of p70^{s6k} is unknown, but a sequence similarity with Ser^{473} of PKB suggests that PDK2 must be a good candidate. Phosphorylation of this site is PI 3-kinase dependent and reversed by a phosphatase indirectly activated by rapamycin.

The similarities in the regulation of these two distantly related kinases are quite striking. When inactive, both are in a conformation where the constitutively active PDK1 cannot phosphorylate its target site. In the case of PKB, a conformational change is elicited by binding of PtdIns(3,4,5)P_3 to its PH domain, whereas for p70^{s6k} this requires phosphorylation of the autoinhibitory domain by proline-directed kinases and phosphorylation of Thr^{389} by a PtdIns(3,4,5)P_3-dependent kinase. After these changes, PDK1 can phosphorylate its target site. For PKB there may not be an obligatory order for the phosphorylation of the PDK1 and PDK2 sites. These findings neatly show how regulatory mechanisms have been conserved between distantly related members of the same family of kinases, and yet have been adapted to fit the special regulatory needs of each kinase. Because many more distantly related kinases have sites related to Thr^{308} of PKB, a family of PDK1-related enzymes could exist that control the activity of a great many kinases that can only be phosphorylated after a priming conformation changes.

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