Taken with the gravity data and awesome imagery, the Europan ocean hypothesis is on solid ground (or beneath solid salt and ice). Before the Voyager flybys, theoretical evidence suggested that Europa's icy crust might be warm and perhaps even melted at its base, and those suggestions were made even before the discovery of tidal heating of Europa (*10*–*12*). This is an amazing confluence of observations and interpretations that rarely happens so neatly in planetary science.

We look forward to continued scrutiny during the Galileo Extended Mission. Ultimately, we will need to take closeup images and other measurements from orbit, land on and chemically analyze Europa's surface, geophysically probe the interior by surface instruments, and eventually take a dip in the ocean before we can conclude with certainty that the ocean exists. But I haven't heard any recent bets against the ocean.

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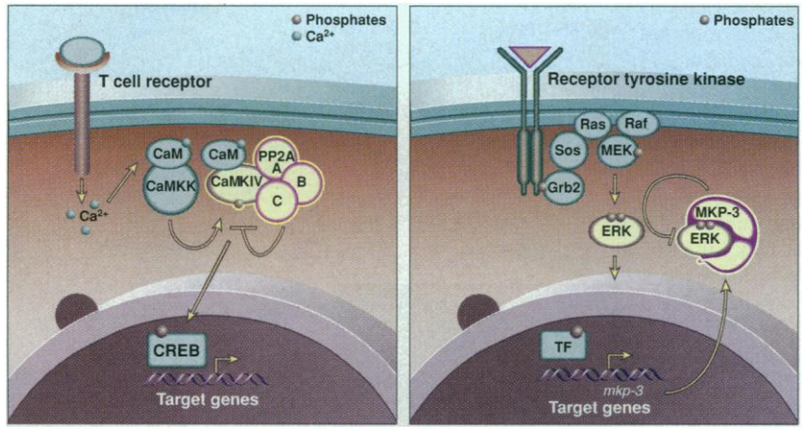
SIGNAL TRANSDUCTION

Kinases and Phosphatases—A Marriage Is Consummated

Ernst Hafen

Successful marriages, according to the age-old wisdom, often result from the union of opposites. Each partner counterbalances the excesses of the other. Within cells, such excesses on the part of some signal transduction molecules can have dire consequences. Runaway activity of protein kinases (enzymes that phosphorylate proteins), for example, can lead to uncontrolled cell growth and tumorigenesis. But kinases have prospective partners—the wide variety of protein phosphatases that keep the kinases in check by performing the opposite operation, dephosphorylation of the substrate. As two reports published on pages 1258 and 1262 of this issue (*1*, *2*) now demonstrate, kinases and phosphatases are indeed joined in a physical union.



A marriage of opposites. Tight regulation of kinases occurs by physical association with their respective phosphatases. (**Left**) Ca^{2+}/calmodulin-dependent kinase IV is constitutively associated with phosphatase 2A (PP2A), resulting in the kinase's rapid inactivation even in the presence of high intracellular Ca^{2+} (*1*). (**Right**) ERK2 activation triggers synthesis of MKP-3. Binding of MKP-3 ERK2 stimulates its catalytic activity and inactivates ERK (*2*).

The characterization of oncogenes in vertebrates and the systematic genetic analysis of signal transduction processes in model systems like yeast, *Caenorhabditis elegans*, and *Drosophila* have identified many more positively acting kinases than negatively acting phosphatases (*3*, *4*). This finding has led to the view that phosphatases may act constitutively by a hit-and-run mechanism, with little specificity for distinct kinases. Now, however, the number of molecularly characterized phosphatases is rapidly increasing. Some are highly specific for their substrate, and their transcription is regulated in a complex way (*5*). Indeed, the two new studies by the groups of Wadzinski and of Arkinstall point to an intricate regulation of kinases and phosphatases. Westphal *et al.* (*1*) examine the mechanism by which persistent, high concentrations of intracellular Ca^{2+} cause only a transient activation of CREB-dependent transcription. This sudden turning off of the response in spite of the continued presence of the activating signal occurs because, shortly after Ca^{2+}-calmodulin–dependent kinase IV (CaMKIV) activates CREB by phosphorylation, the kinase is inactivated by dephosphorylation via phosphatase 2A (PP2A) (see the figure, left panel). The authors show that CaMKIV and the PP2A heterotrimeric holoenzyme can be isolated as a stable complex from cultured cells and from brain extracts. Formation of this complex can occur without the phosphorylation of CaMKIV by its upstream activating kinase, CaMKK, but does require the CaMKIV kinase domain. PP2A function is required for the transitory activation of CREB in response to prolonged high intracellular Ca^{2+} because inhibition of PP2A by SV40 small t antigen results in the prolonged activation of a CREB-dependent reporter construct in Jurkat T cells.

This permanent association of a kinase with its phosphatase allows tight control of the activity of the corresponding kinase. It also raises a question, however. How can this kinase ever be activated sufficiently to increase CREB activity if the phosphatase is always present to undo the kinase's work? It is possible that phosphorylation of CaMKIV by CaMKK transiently outpaces the inactivation by PP2A. Alternatively, PP2A activity may be altered by posttranslational modification or by the activation of CaMKIV itself. Posttranslational activation of a phosphatase by its own substrate is described by Camps *et al.* in the second report (*2*).

Mitogen-activated protein (MAP) kinases, also known as extracellular signal–

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regulated kinases (ERKs), act at the end of a kinase cascade that is activated in response to a variety of extracellular signals via receptor tyrosine kinases (6) (see the figure, right panel). MEK (MAP or ERK kinase) is a dual-specificity kinase that phosphorylates ERK on a threonine and a tyrosine residue in the catalytic domain. Upon activation, ERK translocates into the nucleus and phosphorylates transcription factors of the ETS protein family (7). Signaling by ERK is important for a variety of developmental and physiological processes. More and more dual-specificity phosphatases are being identified that inactivate ERK and other ERK-related kinases (5). MAP kinase phosphatase–3 (MKP-3) is unique because it is highly specific for ERK and does not inactivate the related stress-activated protein kinases (SAPKs or p38). MKP-3 gene expression is induced as an immediate-early response to ERK activation and the protein is exclusively located in the cytoplasm (8). Camps *et al.* show that MKP-3 binds ERK2 via its noncatalytic amino-terminus. This interaction activates phosphatase activity of MKP-3 up to 30-fold. As in the case of PP2A and CaMKIV, binding of the phosphatase to its substrate is independent of the activity state of the kinase.

The association of MKP-3 and ERK2 provides an elegant mechanism for signal modulation and down-regulation. *mkp-3* gene expression is induced by ERK activation. In the cytoplasm, the newly synthesized MKP-3 phosphatase binds ERK with its amino-terminal domain and inactivates the ERK kinase activity with its carboxyl-terminal catalytic domain. It appears that the divergent amino-terminal domains of the dual-specificity phosphatases determine the phosphatases' binding specificity for the substrate kinases.

The importance of the tight control of ERK activity by associated phosphatases is emphasized by genetic studies in *Drosophila*. Activation of the *Drosophila* homolog of ERK, Rolled, is required for a number of developmental processes including the specification of terminal structures in the embryo, formation of wing veins, and the specification of photoreceptor cell fate in the developing eye (9). A gain-of-function mutation in *rolled*, *rolled*^{Sem}, was identified in a genetic screen for mutations that result in the formation of R7-type photoreceptor cells in the absence of one of the upstream signals. The *rolled*^{Sem} mutation causes the substitution of Asn for Asp^{334} in the kinase domain. In cell culture, the corresponding substitution (D319N) in mammalian ERK does not affect ERK's basal activity but rather increases its resistance to a variety of MAPK phosphatases after activation by the upstream kinase MEK (*10*, *11*). Camps *et al.* show that the resistance of D319N ERK2 to MKP-3 is due to substantially reduced binding of MKP-3 to the mutant ERK2. Thus, by analogy it appears that in *Drosophila*, failure to inactivate ERK in the *rl*^{Sem} mutants causes phenotypes similar to those observed by the ectopic, constitutive activation of the receptor tyrosine kinases controlling ERK. Thus, negative regulation by this phosphatase is a critical element of these essential pathways.

The demonstration that CaMKIV and ERK2 form stable complexes with their corresponding phosphatases suggests a tight coupling of activators and inactivators. Indeed, PP2A also forms stable complexes with p70S6 kinase and p21 activating kinase (PAK1) (*12*). From this work comes an emerging theme in cell signaling: Each kinase is complexed with its phosphatase. From a practical point of view, this association may permit the rapid identification of the specific phosphatase or phosphatases for a given kinase by biochemical purification or in a yeast two-hybrid system. The physical union of such opposites—protein kinases and phosphatases—allows each to keep the other in check and thereby guarantees the fidelity of the signal transduction process.

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NOTA BENE: SUPERFLUIDS

Quantum Trick Shots

Suppose in a game of billiards, you attempted an angle shot only to have the cue ball reverse its momentum after hitting the cushion and retrace its path. Such seemingly impossible events occur in the peculiar world of superfluids and the phenomenon known as Andreev reflection (*1*). Although observed in superconductors since the 1950s (*2*), this process of quantum "retroreflection" has now been seen at the free surface of superfluid helium-3 in a direct measurement by Okuda *et al.* (3) of the University of Tokyo.

The cue balls in this kind of billiards are quasiparticles: combinations of entities that act as single quantum particles. In superconductivity, the quasiparticles are pairs of electrons; in superfluids, the quasiparticles are pairs of helium atoms. And just as electrons in normal materials can coexist with objects called "holes" (a localized absence of an electron), quasiparticles go hand in hand with "quasiholes". In Andreev scattering, when a quasiparticle hits a boundary between superconductor (or superfluid) and normal material (nonsuperconductor or nonsuperfluid) it is converted into a quasihole. Momentum conservation dictates that the quasihole return along the same path.

This retroreflection has been directly observed in superconductors (4), but superfluids are perhaps a more interesting case: because helium atoms are electrically neutral, and the mechanism of pairing is quite different, novel types of scattering behavior can occur. Okuda *et al.* use a clever blackbody radiator design (5) conceived by Cousins *et al.* for Andreev measurements in mixed superfluid systems (6). The radiator emits a beam of quasiparticles that hit the helium liquid surface at an angle, like bullets from a gun turret. Only quasiparticles that are retroreflected can re-enter the narrow orifice of the turret and be counted, and this is what Okuda *et al.* have observed. Although such phenomena cannot exist in the world of billiards, these recent measurements reveal the continuing surprises offered by superfluids.

–David Voss

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