PERSPECTIVES: SIGNAL TRANSDUCTION

Signaling Specificity—a Complex Affair

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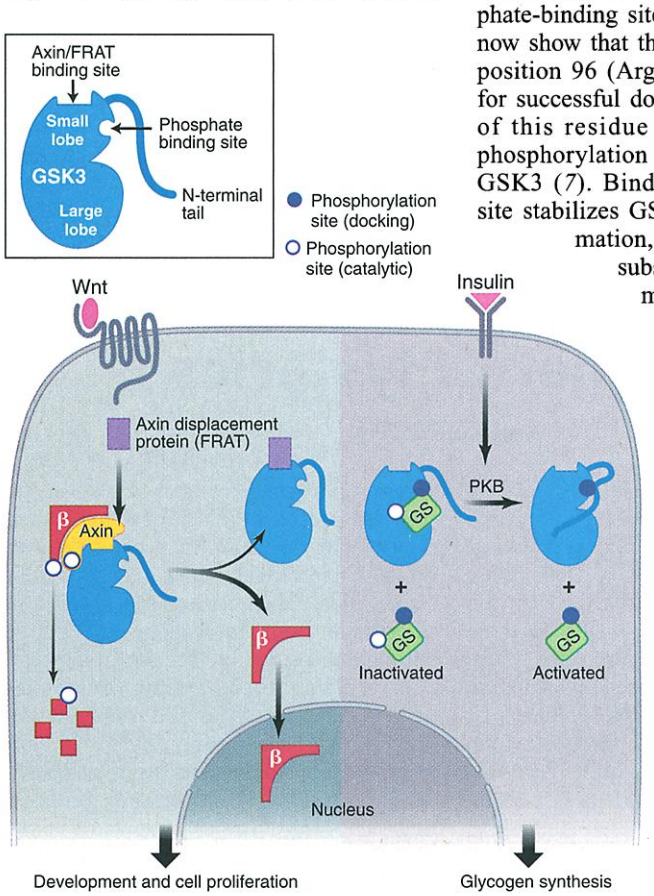
Extracellular stimuli such as hormones, growth factors, and cytokines bind to and activate their receptors at the cell surface. Signals from these receptors are then relayed, amplified, and integrated, resulting in the expression of target genes in the nucleus and subsequent biological responses. In principle, each receptor could activate a unique signaling pathway, but this does not appear to happen. Indeed, from the human genome sequence we know that about 5% of genes encode receptors, whereas fewer than 3% encode crucial enzymes of signaling pathways called kinases (which regulate other signaling components by attaching phosphate groups to them) (*1, 2*). This revelation implies that individual kinases transmit signals from multiple receptors and that the cell must have ways to strictly regulate the specificity of kinase signaling.

Several kinases implicated in multiple signaling pathways elicit conflicting responses depending on the cellular context. One such kinase is glycogen synthase kinase 3 (GSK3), a cytoplasmic serine-threonine kinase that is involved in insulin signaling and metabolic regulation, as well as in Wnt signaling and the specification of cell fates during embryonic development (*3*). GSK3 appears in two highly homologous and ubiquitously expressed forms, GSK3α and GSK3β (*4*). The insulin and Wnt signaling pathways differentially regulate GSK3α/β, resulting in distinct downstream events (*5*), but how they accomplish this is not clear. The recent report of the crystal structure of GSK3β by Dajani and colleagues in *Cell* (*6*), together with a biochemical study of GSK3β by Frame *et al.* in *Molecular Cell* (*7*), reveal how GSK3 selectively regulates different downstream targets according to which signaling pathway is activated.

GSK3 is unusual among kinases in that its normal activity in the cytoplasm is blocked by activation of signaling pathways (see the figure). For example, in response to insulin signaling, protein kinase B (PKB, also called Akt) directly phosphorylates and hence inhibits GSK3 activity, ultimately leading to conversion of glucose to glycogen (*3*). Also an important component of the Wnt signaling pathway, GSK3 is essential for normal development of the embryo and for regulation of cell proliferation in the adult. Wnt signaling inhibits GSK3, resulting in the dephosphorylation of β-catenin, which then moves to the nucleus where it engages transcription factors such as LEF (*3*). During Wnt signaling, GSK3 is incorporated into a multiprotein complex comprising β-catenin, axin, and the adenomatous polyposis coli gene product, APC (*3*). The formation of this complex regulates the phosphorylation of β-catenin by GSK3 and may prevent cross-talk between the insulin and Wnt signaling pathways. GSK3 therefore participates in two very different signal transduction pathways (insulin and Wnt) that regulate distinct biological processes. How is the fidelity of signal transduction by GSK3 enforced? Insight comes from the observation that GSK3 phosphorylates two types of protein substrates: "primed" and "nonprimed."

Metabolic regulation by insulin signaling and GSK3 involves the phosphorylation of a diverse range of substrates, including the enzyme glycogen synthase (see the figure). Many of these substrates must be prephosphorylated or "primed" before they can dock with a GSK3 phosphate-binding site. Frame and co-workers now show that the amino acid arginine at position 96 (Arg^{96}) of GSK3β is critical for successful docking, and that mutation of this residue markedly inhibits the phosphorylation of primed substrates by GSK3 (*7*). Binding of substrate to this site stabilizes GSK3 in an active conformation, thereby directly linking substrate specificity to enzymatic activity (*6*). Once the substrate is bound, GSK3 sequentially phosphorylates the substrate at clustered serines spaced at 4–amino acid intervals (*3*). For example, the phosphorylation of glycogen synthase at Ser^{656} by casein kinase II primes this substrate for sequential phosphorylations at Ser^{640}, Ser^{644}, Ser^{648}, and Ser^{652} by GSK3 (*3*). During insulin signaling, PKB phosphorylates GSK3β at Ser^{9} (*8*). Dajani and colleagues now demonstrate that this amino-terminal phosphorylation blocks the access of substrates to the GSK3 phosphate-binding site, thereby blocking GSK3's activity (*6*). Insulin therefore selectively inhibits the phosphorylation (and inactivation) of primed substrates by GSK3.



Regulating GSK3. (Left) After Wnt binds to its receptor at the cell surface, proteins such as FRAT displace axin from GSK3, resulting in an accumulation of β-catenin in the nucleus and transcription of Wnt target genes. **(Right)** Binding of insulin to its receptor results in activation of the insulin signaling pathway, which induces PKB to phosphorylate GSK3 at Ser^{9} in the amino terminus. The phosphorylated amino terminus behaves as a pseudosubstrate, competing with "primed" substrates such as glycogen synthase (GS) for GSK3's catalytic site, effectively preventing GSK3 substrates from becoming phosphorylated. GSK3 bound to axin is not available for phosphorylation on Ser^{9} in response to insulin, thereby restricting the effects of insulin to a specific subset of GSK3 substrates.

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GSK3 also phosphorylates substrates in a manner that does not require prephosphorylation ("nonprimed" substrates). These substrates include components of the Wnt signal transduction pathway, such as β-catenin and axin. Interestingly, mutation of Arg^{96} in the phosphate-binding site of GSK3β does not alter phosphorylation of these nonprimed substrates (*7*). Frame and colleagues provide evidence that axin binds to GSK3 at a site distinct from the phosphate-binding site; this binding appears to inhibit GSK3 phosphorylation at Ser^9 in response to insulin (*7*), thereby restricting the effects of insulin to a specific subset of GSK3 substrates. Thus, PKB inhibits GSK3's phosphorylation of primed substrates (such as glycogen synthase, a target of the insulin pathway) without affecting nonprimed substrates (such as β-catenin, a target of the Wnt pathway) (*3*). In this manner, GSK3 is able to selectively regulate primed and nonprimed substrates through phosphorylation, thereby inducing distinct cellular responses.

Is this phenomenon applicable to the differential regulation of signaling pathways by other protein kinases? Mitogen-activated protein kinases (MAPKs) have docking sites (CD and ED domains) that are similar to the Arg^{96} binding site of GSK3 and contribute to substrate specificity (*9*). Whether these docking sites are required for the phosphorylation of a particular subset of substrates but not others requires further investigation. However, the transcription factor Elk-1 contains a targeting domain that is required for phosphorylation by the extracellular signal–regulated kinase and c-Jun amino-terminal kinase groups of MAPKs, but not by p38 MAPK (*10*). This implies that other protein kinases may strictly control the selective activation of downstream substrates in a similar way to GSK3.

Selective substrate phosphorylation has broad implications for drug discovery. Diseases associated with elevated GSK3 activity include non–insulin-dependent (type II) diabetes mellitus, Alzheimer's disease, and depression (*3, 11*), whereas mutant inactive forms of GSK3 are associated with certain solid tumors. GSK3 inhibitors such as lithium and small-molecule drugs that compete with adenosine triphosphate can mimic the effects of both the insulin and Wnt signaling pathways (*11, 12*). Thus, prolonged use of such drugs for the treatment of one disease (for example, diabetes) could induce other diseases (such as cancer) that may arise through the activation of Wnt signaling. In contrast, a drug that interacts with the phosphate-binding site of GSK3 may selectively inhibit the phosphorylation of "primed" GSK3 substrates, such as the insulin target glycogen synthase, without affecting the phosphorylation of "nonprimed" substrates, such as the Wnt pathway targets axin and β-catenin. This provides an opportunity for the rational design of specific inhibitors of GSK3 that are selective for different groups of target molecules of this multifunctional kinase. The presence of substrate docking sites on other protein kinases suggests that this strategy may be generally applicable to the design of selective inhibitors of signal transduction.

References

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PERSPECTIVES: COSMOLOGY

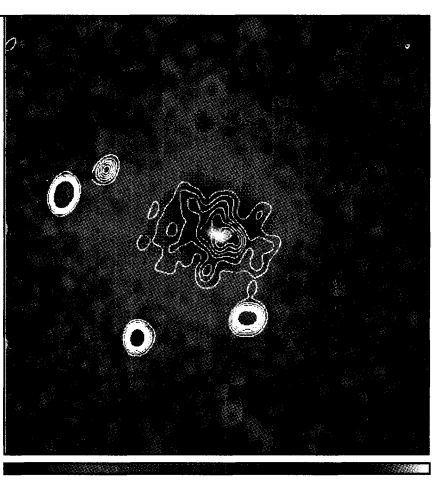
Magnetic Mysteries

Axel Brandenburg

At present—some 14 billion years after the Big Bang—magnetic fields of appreciable strength are found in virtually all galaxies and also in galaxy clusters. Although weak compared with the fields at Earth's or the Sun's surface, these fields are enormous considering the scales involved and may influence the formation of stars and galaxies, the dynamics of galaxy clusters, and energy transport within galaxy clusters. Even 1 to 2 billion years after the Big Bang, such fields must already have existed at about the same strength as today (*1*). How did these fields arise? And did primordial magnetic fields exist in the early universe? Answers to these questions remain speculative, but upcoming space missions promise exciting insights.

Galactic magnetic fields are usually inferred through the presence of polarized (synchroton) emission at radio and shorter (down to submillimeter) wavelengths. Most galaxies show synchrotron emission, but not all galaxy clusters. However, this may be a result of a lack of relativistically fast electrons, which emit such radiation under the influence of a magnetic field, rather than a lack of the field itself. Abell 2163 is an example of a cluster with very strong radio emission (see the first figure).

According to one leading theory, magnetic fields in galaxies and galaxy clusters may have arisen through battery mechanisms in ionization fronts just after the first stars formed (*2*, *3*). Differential forces acting on opposite charges generated a relative drift between them. The resulting field was amplified exponentially through gas motions (*4*). Such "dynamo" processes are certainly possible in principle but cannot easily explain why in some galaxy clusters, the fields are very coherent over several galactic radii (*5*). According to another theory, the ejecta of starburst galaxies may have magnetized galaxy clusters (*6*). In both cases, the field strength may have been boosted by mergers and collisions among clusters, but simulations indicate that the scale of the fields would remain small (*7*).



Strong emission. Radio synchroton emission contours are superimposed on a color-coded x-ray image of the galaxy cluster Abell 2163. The strongest radio emission comes from the center. Cluster diameter, ~2 megaparsecs.

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In contrast, if a magnetic field on the scale of several galactic radii already existed at the time of galaxy formation, this would provide an important clue to the origin of fields on large scales. New missions may soon enable detection of very early magnetic fields through measuring the temperature and polarization anisotropies of the cosmic microwave background (CMB) (*8*). The PLANCK space tele-

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