Trying on a New Pair of SH2s

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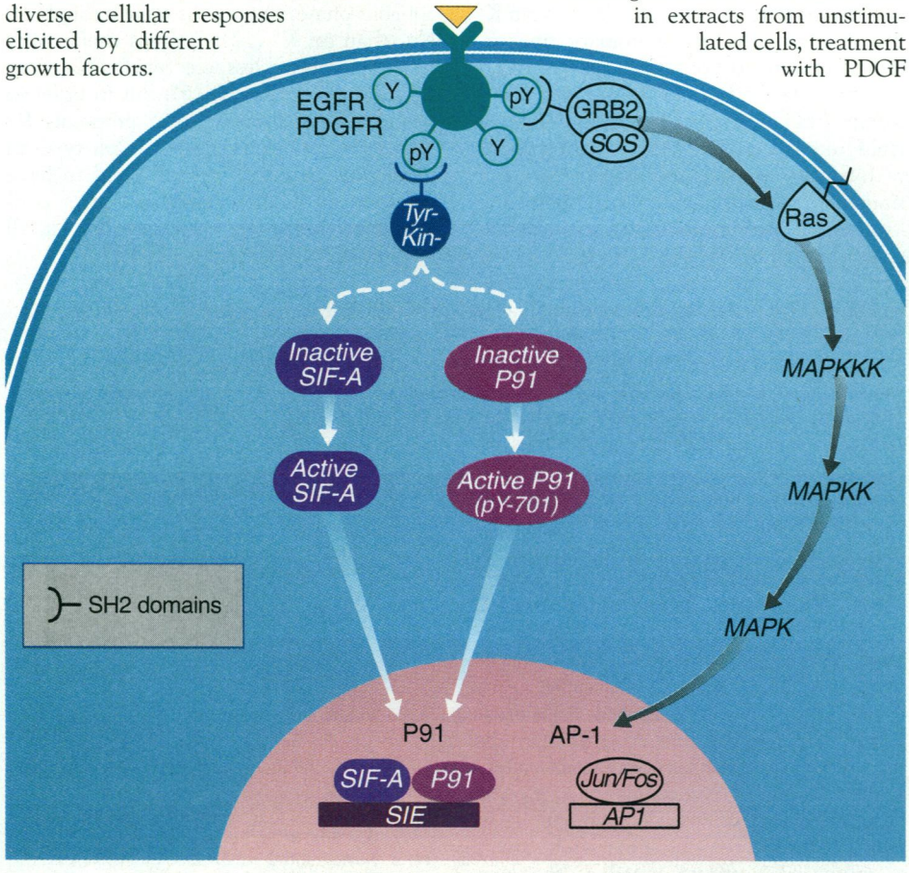
How can we know the dancer from the dance? In the field of growth factors, key intermediates in a signal transduction pathway are often identified by their association with activated receptors and subsequent phosphorylation on tyrosine residues (*1*). But the steps leading from growth factors to the nucleus have proven far more elusive—this despite clear evidence that growth factors activate distinct genetic programs in their target cells (*2*). Until recently, researchers trying to dissect these nuclear signal transduction pathways could only point to a convergent road leading to Ras and ultimately to transcription factor AP-1. This convergent road of action to the nucleus was difficult to reconcile with the diverse cellular responses elicited by different growth factors.

Several reports in this issue of *Science* (*3–7*) now characterize a more direct signaling pathway that involves the interferon-responsive factor p91 as a key intermediate in growth factor–dependent transcription.

Some of the earliest evidence for specific growth factor pathways emerged from studies showing that conditioned medium from v-*sis* transformed NRK cells could rapidly induce expression of the c-*fos* proto-oncogene (*8*). This conditioned medium, which was later shown to contain high concentrations of platelet-derived growth factor (PDGF), could stimulate c-*fos* transcription through a specific *sis*-inducible element termed the SIE2. Although no SIE binding activities were detectable in extracts from unstimulated cells, treatment with PDGF led to the rapid accumulation of an SIE binding activity termed SIF (*sis*-inducible factor) even in the absence of new protein synthesis. Remarkably, epidermal growth factor (EGF) could also stimulate SIF, indicating that multiple factors might converge on a similar signaling pathway to the nucleus. Phosphorylation appeared to be a likely mechanism by which SIF might be activated. Phosphorylation has been shown to regulate a number of transcription factors at several levels including nuclear translocation, DNA binding activity, and transactivation potential.



Pathways for growth factor–activated transcription of specific genes. Binding of ligands, such as EGF or PDGF, to their receptors activates autophosphorylation of the receptor on tyrosine residues (pY). Cytoplasmic proteins containing SH2 domains bind to these phosphorylated residues. GRB2 brings the guanine nucleotide exchange factor SOS to the receptor and thus results in sequential activation of Ras, a series of protein kinases (mitogen-acitvated protein kinase, MAPK; MAP kinase kinase, MAPKK; and MAP kinase kinase kinase, MAPKKK), and the transcription factor AP-1. A more direct pathway to the nucleus is now suggested in which the latent cytoplasmic proteins p91 and SIF-A become phosphorylated on tyrosine in stimulated cells—possibly by one or more soluble tyrosine kinases associated through SH2 domains with the receptor—and are then translocated to the nucleus where complexes containing such proteins bind to specific DNA sequences like the *sis*-inducible element (SIE).

In cells treated with EGF, SIF activity appears first in the cytoplasm, suggesting that in quiescent cells SIF might reside in the cytoplasm and venture into the nucleus only in response to growth factor stimulation. Indeed, Sadowski and Gilman (*10*) demonstrated that SIF DNA binding activity could be reconstituted in vitro when cytosolic fractions from unstimulated cells were incubated with membranes and then treated with EGF. These results suggested that EGF may stimulate both translocation of SIF and its DNA binding activity.

Enter p91. In earlier studies, interferon (IFN)-α had been shown to stimulate transcription of target genes through the factor ISGF-3 (*9,11,12*), a multimeric complex of cytoplasmic proteins that become tyrosine phosphorylated and translocated to the nucleus in response to IFNs. In response to IFN-γ, only one subunit of ISGF-3, termed p91, is so activated. The p91 protein then binds to the IFN-γ–activated site (GAS) of IFN-γ–responsive genes. A single phosphorylation site on p91, Tyr^{701}, appears to be both necessary and sufficient to mediate nuclear translocation, DNA binding activity, and transcriptional activation by IFN-γ (*7*). But the IFN-γ receptor does not possess intrinsic tyrosine kinase activity, suggesting that a cytoplasmic kinase must be transiently recruited to the membrane following receptor activation. In this respect, the IFN receptors have a distant resemblance to the growth hormone and hematopoeitin receptors, which have recently been shown to bind JAK2, a member of the Janus family of tyrosine kinases (*13*). Indeed, JAK2 appears to reconstitute IFN-γ–responsive transcription when expressed in a non-inducible cell line (*5, 6*), suggesting that this or a related kinase may directly phosphorylate p91 and thereby stimulate transcription of appropriate genes.

An unexpected twist in this story came as investigators noticed that growth factors induced SIF in much the same way that

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IFN-γ stimulated p91 (*4*–*6*). Sequence similarities between SIE and GAS elements suggested that these two biologically divergent pathways might actually converge on similar if not identical transcription factors. Now, p91 has been detected with specific antisera in protein complexes that bind to particular DNA elements in cells stimulated by an array of growth factors including EGF, PDGF, colony-stimulating factor–1 (CSF-1), and interleukin-10 (IL-10) (*3*–*6*). The DNA binding activity of p91 is apparently induced by phosphorylation of the same site (Tyr^{701}) that is phosphorylated in response to IFN-γ (*5*). Other cytokines including IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) can similarly activate complexes of DNA binding proteins, but these complexes appear not to contain p91 (*3*).

How does p91 become phosphorylated by many different growth factor pathways? Unlike the interferon receptors, most growth factor receptors do have intrinsic tyrosine kinase activity (*1*). In response to ligand, these receptors dimerize and become autophosphorylated on multiple tyrosine residues within their cytoplasmic extensions. These phosphotyrosine residues, in turn, allow the activated receptor to associate with other proteins involved in growth factor–dependent signaling. Interaction between most signaling proteins and the activated receptor occurs through conserved domains, termed SH2 for Src Homology 2, which are about 100 amino acids in length and specifically recognize phosphotyrosine residues with nanomolar affinity. Sequences flanking each phosphotyrosine residue further specify which SH2-containing proteins will interact with the activated receptor. Phosphorylation of the PDGF receptor, for example, recruits GRB2 to the cell membrane through its SH2 domain. GRB2 carries with it the guanine nucleotide exchange factor SOS, which can then activate Ras.

The presence of an SH2 domain on p91 suggests that this protein may interact directly with the receptor. And two lines of evidence support this contention. First, activation of p91 by EGF is very rapid (seconds) and occurs even when cells are placed at 4°C (*5*, *6*). Second, activation of p91 in vitro can be blocked by incubation with the SH2 domain of GRB2 suggesting that an SH2 interaction is indeed important in this event (*10*). But the problem comes in explaining how p91 would be released from the cytoplasmic membrane and translocated into the nucleus after phosphorylation has occurred. That would require either dephosphorylation of the receptor or a conformational change in p91 that disengaged the protein from its binding site. Moreover, several growth factors appear to induce p91 phosphorylation, and that would necessitate rather nonspecific SH2 interactions with each receptor. Alternatively, a simpler mechanism might involve the activation of a cytoplasmic kinase after its recruitment to the membrane by various growth factor receptors.

If the SH2 domain on p91 is not important for recruitment to activated receptor sites, where might it function? Data presented by Sadowski *et al.* (*6*) indicate that SH2 interactions may be important for the formation of inter-subunit contacts between p91 monomers. In this regard, addition of phosphotyrosine can inhibit p91 binding activity in stimulated extracts, suggesting that the SH2 domain may participate in DNA recognition. Furthermore, the palindromic nature of both the GAS and SIE sites suggests that p91 may bind these elements as a dimer.

If different growth factors and cytokines can stimulate common factors in the nuclear transduction pathway, where does the specificity come into account for the diversity in cellular responses to these polypeptides? In the case of INF-α and INF-γ, different sets of genes appear to be activated depending upon the subunit composition of the activated ISGF3 factor (*12*). These multimeric complexes have distinct DNA binding activities that can differentiate between IFN-α– and IFN-γ–responsive genes. In their reports, Sadowski *et al.* (*6*) and Ruff-Jamison *et al.* (*4*) show that SIF activity can actually be resolved into three different protein-DNA complexes termed SIF A, B, and C. SIF B and C complexes appear to contain p91. But SIF A, which is most heavily induced by EGF, appears to represent a different and as yet uncharacterized tyrosine phosphorylated protein that may correspond to the 92-kD EGF-induced factor described by Ruff-Jamison *et al.* (*4*). By contrast IFN-γ can activate only p91 (SIF B and C) and does not induce SIF A. These results imply that each growth factor may regulate both overlapping and unique sets of genes by stimulating the phosphorylation of both shared and distinct factors that assemble into multimeric complexes.

The transcriptional activities associated with each growth factor–inducible complex (SIF A, B, C) are as yet uncharacterized. Clearly, the *fos* gene may not be ideal for these functional studies because additional promoter sequences besides the SIE are required for induction by PDGF or EGF (*2*). The identification of additional target genes for growth factors will allow investigators to test whether different growth factor response elements can indeed be distinguished by specific affinities for various protein complexes. The shared activation of p91 by an increasing number of growth factors and cytokines suggests, however, that this protein may be a common subunit that escorts different proteins to relevant target sites; which goes to show that in the field of signal transduction, as in politics, you dance with the one who brought you.

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