possible to modify the free amino group in the vancosamine sugar moiety with hydrophobic substituents, such as biphenyls, and to show that this modification increased activity against vancomycin-resistant enterococci (*10*). Now Ge *et al.* have gone further, using their expertise in carbohydrate chemistry to obtain unanticipated findings about vancomycin analogs.

Their first intriguing discovery was the retention of substantial antibacterial activity in a chlorobiphenyl vancomycin derivative that was missing the first (leucyl) residue. This meant that recognition of the D-Ala-D-Ala terminus by the analog was abrogated, ruling out the conventional high-affinity interaction between the antibiotic and the peptidoglycan as the mode of drug action. The investigators further pruned the vancomycin skeleton down to the modified vancosamine-glucose disaccharide and found, to their amazement, that the disaccharide alone retained powerful antibiotic activity. They used permeabilized *Escherichia coli* bacteria to determine which step in the peptidoglycan synthesis pathway was blocked by the disaccharide. In contrast to vancomycin, which primarily abrogates transpeptidation, the vancomycin analog and its disaccharide fragment alone selectively blocked the transglycosylation step of peptidoglycan synthesis.

It is thought that vancomycin binds to D-Ala-D-Ala termini in the non–cross-linked mature peptidoglycan, and also in the lipid-disaccharide pentapeptide precursor molecules that are substrates for incorporation into expanding peptidoglycan chains. It now appears that the vancomycin analog and its disaccharide fragment directly interact with one or more of the transglycosylases involved in oligomerization of the glycan strands. The discovery that these enzymes are targets for the modified sugars of vancomycin reveals simple strategies for defining which transglycosylases are involved in bacterial cell wall synthesis and for designing a new class of antibiotics by manipulation of vancosamine and glucose rings. To what extent the dimethoxyphenyl ring component (mimicking residue 4 of vancomycin) of the disaccharide fragment is crucial for anchoring the disaccharide in the bacterial cell membrane and for the fragment's antibacterial activity will also need to be explored.

All in all, the deconstructionist approach to vancomycin appears to have struck gold with the discovery of a disaccharide fragment of a vancomycin analog that is more powerful than vancomycin itself. These results may accelerate the discovery and development of simple sugar-based fragments that are equally adept at killing vancomycin-sensitive and vancomycin-resistant pathogenic bacteria by targeting not the transpeptidation step but the transglycosylation step of peptidoglycan synthesis.

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PERSPECTIVES: SIGNAL TRANSDUCTION

Nuclear Fusion of Signaling Pathways

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Extracellular factors that regulate cell growth and differentiation often bind to receptors at the cell surface. Signals are then transduced to the nucleus where they activate specific transcription factors that elicit changes in the pattern of gene transcription. We know of many linear, intracellular signal transduction pathways, and often there is cross talk between them. But how and where they intersect and whether this cross talk results in an enhanced signal (synergy) or a reduced signal (antagonism) is not known. One answer to this conundrum entails the homologous transcriptional coactivator molecules p300 and CREB-binding protein (CBP). These huge nuclear proteins interact with numerous transcription factors through distinct domains (see the figure). They are thought to be bridges that connect individual transcription factors to the basal transcription machinery, thus helping to activate transcription (*1*). However, what remains unclear is whether p300 or CBP interacts simultaneously with more than one transcription factor in a promoter complex. Studies in mice deficient in p300 or CBP show that these molecules are essential and are in limited supply within the cell. Thus, competition for p300 and CBP may explain negative interference between different signaling pathways (*2*).

Now, on page 479 of this issue, Nakashima *et al.* (*3*) report on an alternative way in which signaling pathways can communicate with each other. They show that p300 facilitates synergistic cross talk between two different signaling pathways—activated by the cytokines LIF (leukemia inhibitory factor) and BMP2 (bone morphogenetic protein 2)—which together stimulate the differentiation of fetal neuroepithelial cells into astrocytes. LIF is a survival and differentiation factor for neurons, and a deficiency in LIF or its receptor results in changes in astrocytes in the central nervous system (*4*). Similarly, the BMP2 protein is expressed throughout neural development and promotes the differentiation of neural progenitor cells into astrocytes that have structural, trophic, and immunomodulatory functions in the brain (*5*).

The LIF and BMP2 cytokines induce distinct signal transduction pathways that each activate a different transcription factor (STAT3 and Smad1, respectively) at the cytoplasmic face of the plasma membrane (see the figure). LIF binds to and induces the heterodimerization of transmembrane receptor subunits, resulting in the activation of the JAK family of protein tyrosine kinases. The activated JAKs then phosphorylate the transcription factor STAT3 on a tyrosine residue. Phosphorylated STAT3 dimerizes, translocates to the cell nucleus, binds to DNA, and activates the transcription of target genes (*6*). In the same way, dimers of BMP2 bind to and induce tetramerization of the two types of BMP receptor, which have serine-threonine kinase activity. This results in the phosphorylation of the Smad1 transcription factor, which associates with Smad4, moves to the nucleus, and stimulates expression of target genes (*7*).

As both STAT and Smad family proteins have been shown to bind to p300 (*8*), Nakashima *et al.* investigated the possible interactions between Smad1, STAT3, and p300 (*3*). Upon phosphorylation by BMP receptors, Smad1 interacted with p300; STAT3 also bound to p300 but independently of tyrosine phosphorylation, suggesting that the phosphorylation-induced dimerization that is essential for the binding of STAT3 to DNA is not required for

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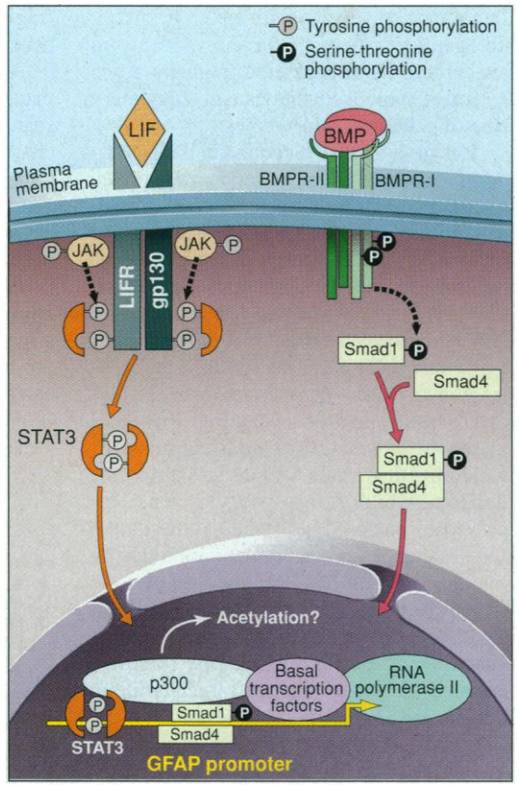

p300 as nuclear signal integrator. Synergistic integration of the signaling pathways for the cytokines LIF (leukemia inhibitory factor) and BMP2 (bone morphogenetic protein 2) is achieved by recruitment of the nuclear transcriptional coactivator p300. LIF induces the heterodimerization of the LIF receptor (LIFR) with gp130, leading to the activation of the JAK family of protein tyrosine kinases. Subsequent tyrosine phosphorylation of LIFR and gp130 generates docking sites for the transcription factor STAT3, which is phosphorylated by JAKs after docking. Dimerized STAT3 moves to the nucleus and interacts with the promoter for glial fibrillary acidic protein (GFAP), a marker of astrocyte differentiation. Dimers of the cytokine BMP induce the tetramerization of the BMPR-I and BMPR-II receptors, which have serine-threonine kinase activity. BMPR-II then phosphorylates and activates BMPR-I, which in turn phosphorylates Smad1. Phosphorylated Smad1 oligomerizes with Smad4, and this oligomer (of unknown stoichiometry) probably binds directly to the GFAP promoter. Synergistic integration of LIF and BMP2 signaling is achieved by the joint recruitment of p300 by STAT3 and Smad1. The basal transcription machinery is then activated by p300, possibly through its ability to acetylate histone proteins and thus loosen chromatin structure.

its interaction with p300. The pivotal question, however, is whether STAT3 and Smad1 can bind simultaneously to p300. Indeed, STAT3 and phosphorylated Smad1 were found to coprecipitate from transfected cells only when p300 was coexpressed with both transcription factors. Consistent with the finding that a complex between these three molecules exists, STAT3 and Smad1 bind to different regions within p300: STAT3 primarily binds to the amino-terminal half, and Smad1 to the carboxyl-terminal region.

But does the formation of a STAT3-Smad1-p300 complex activate gene transcription? In order to prove this, Nakashima and colleagues studied the GFAP (glial fibrillary acidic protein) promoter, which is turned on during astrocyte differentiation. LIF and BMP2, each in their own right, induce GFAP promoter activity, and the combination of the two cytokines results in synergistic activation of the promoter. The STAT3 protein binds to the GFAP promoter (*9*), and deletion of the STAT3 binding site or expression of a dominant-negative STAT3 molecule reduces the responsiveness of the GFAP promoter to LIF. In addition, expression of Smad6 and Smad7 (inhibitors of Smad1) suppresses induction of the GFAP promoter by BMP2 but not by LIF. So far, there is no evidence that BMP2 induction of GFAP involves direct binding of DNA by Smad1, but this seems likely to be the case. Thus, LIF-activated STAT3 and BMP2-activated Smad1 bind to different DNA sites within the GFAP promoter. Physically, the two transcription factors would then be bridged by a p300 (or CBP) molecule, which would interact with basal transcription factors and lead to initiation of transcription (see the figure). Indeed, overexpression of p300 promotes GFAP transcription in response to LIF, BMP2, or both cytokines together. And conversely, amino- and carboxyl-terminal fragments of p300 (which are decoys for STAT3 and Smad1, respectively) suppress transcription, clearly indicating the functional synergy between STAT3, Smad1, and p300 (*3*).

Several interesting questions arise from this work. How does p300 promote gene transcription induced by STAT3 and Smad1? Is it simply by bridging these factors and connecting them to the basal transcription machinery? Alternatively, might the crucial step be changes in local chromatin structure? This could very well be the case because p300 possesses (and can also recruit proteins with) histone acetyltransferase activity, and acetylation of histones loosens the chromatin structure facilitating gene transcription (*1*). As p300 is also capable of modulating transcription factors by acetylation (*10*), acetylation and thereby activation of STAT3 and Smad1 may represent another mechanism though which p300 promotes gene transcription.

Is the p300-facilitated synergism between the LIF and BMP2 signals necessary for astrocyte differentiation? Maybe not, because differentiation can be induced by LIF or BMP2 alone in vitro, albeit at a slower pace (*3*). However, the concentration of LIF or BMP2 in vivo may not be high enough to induce astrocyte differentiation, thus necessitating synergistic signals. Or could it even be that LIF induces BMP2 production, and vice versa, resulting in a synergistic interdependence that is regulated by p300 (*10*)? As short-term in vitro culture of astrocyte progenitor cells might alter their differentiation potential, it will also be important to determine whether multipotent neuronal stem cells react to LIF and BMP2 in the same fashion as more committed progenitor cells. New techniques to directly isolate neuronal stem cells by flow cytometry may be helpful in this regard (*10*).

The emergence of p300 as a synergistic integrator of different signal transduction pathways may not be limited to LIF and BMP2, or to related cytokines that use the same receptor components. Given that p300 (and CBP) accommodates binding to so many transcription factors (*1*), various signals (such as growth factors or steroid hormones) may converge on this nuclear protein, and we predict that this new paradigm of signal integration will become a research topic as hot as nuclear fusion.

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