100 YEARS AGO

It is well known that the shipments of rum from Demerara, especially during the past year, have been "faulty," and very great pecuniary loss has resulted to the colony. Through the kindness of a friend and the courtesy of the Excise authorities, we received certain samples direct from a bonded warehouse; we were informed that the spirit had been returned at 42 per cent. over proof, equivalent to 74.6 per cent. alcohol by weight. On microscopical examination of a sediment at the bottom of the samples, using a magnification of 1200 diameters, we found chains of small cocci; after the spirit had been kept for some days the cocci were seen to be surrounded with a gelatinous envelope, and after a further interval of time the cocci were found disseminated throughout the liquid, and were rapidly developing and multiplying .... the observation of the existence and multiplication of any micro-organism in a spirit of such alcoholic strength appears to be of so much scientific interest, and the problem of its presence of such technical importance, that we send this note as a preliminary communication.

From *Nature* 1 July 1897.

50 YEARS AGO

Mr. P. B. Medawar has been elected to the chair of zoology in the University of Birmingham. Not yet thirty-three, he has had a brilliant career at Oxford ... and his research lies along two very different lines. In one he is developing the mathematical treatment of animal form and the process of growth and ageing; in this he carries forward the pioneer work of Sir D'Arcy Thompson. [In the other he is studying] the differences between individuals. Here come his valuable researches on mammalian skin grafting, so important in their human application; his approach to the problem is both immunological and genetical. A recent development has been his discovery and investigation of the curious phenomenon of an induced spread of pigmentation when pieces of black skin are grafted into white areas of a piebald guinea-pig. Prof. Medawar has a fertile imagination combined with an ability for putting his ideas for research into practice. We look forward with confidence to the development of his Birmingham school of zoology.

From *Nature* 5 July 1947.

Signal transduction

Mad about SMADs

Jeff Wrana and Tony Pawson

The transforming growth factor-β (TGF-β) family of signalling molecules can regulate the proliferation of many cell types. Central to TGF-β-mediated signalling in vertebrates are the so-called SMAD proteins and, on pages 82 and 87 of this issue, Hata *et al.*[1] and Shi *et al.*[2] provide our first glimpse of how the structure of these signalling molecules might regulate their function. Moreover, the new results give an insight into the different ways in which mutations in the SMAD proteins can disrupt their activity in development and human cancer.

For some time, analysis of signalling by TGF-β concentrated on the activation of serine/threonine kinase receptors at the cell surface. TGF-β binds a type-II receptor kinase, which phosphorylates (and activates) a type-I receptor kinase that then initiates downstream signalling. But the discovery of the MAD-related family of signal transducers has strikingly increased our understanding of intracellular events in the TGF-β pathway. The family now includes the prototypic *Drosophila* gene, *Mothers against dpp* (*Mad*), three genes from *Caenorhabditis elegans* (*sma-2, sma-3* and *sma-4*) and seven vertebrate members (*Smad1–7*). The Smad2 and Smad4 proteins are tumour suppressors, and they are involved in TGF-β-mediated signalling[3–7].

Considerable work on the SMAD proteins has fleshed out a general framework to describe how this unique signalling pathway functions. In the case of TGF-β, the activated TGF-β receptor recognizes Smad2 — and probably Smad3 — and phosphorylates them at carboxy-terminal serine residues[5,7]. Phosphorylated Smad2 then forms a heteromeric complex with another MAD-related protein, Smad4, and accumulates in the nucleus[6,7]. In the nucleus, Smad2–Smad4 complexes can regulate transcriptional responses by specifically interacting with DNA-binding proteins, such as FAST1 (ref. 8).

An analogous pathway has been described for bone morphogenetic proteins. This pathway involves the phosphorylation of Smad1 and, probably, Smad5, followed by association with Smad4 and nuclear accumulation[6,9]. So the receptor-regulated SMADs (that is, Smads1, 2, 3 and 5) appear to specify the biological response to TGF-β or bone morphogenetic proteins, whereas their partner — Smad4 — is common to both pathways. The phosphorylation-dependent activation of SMADs provides a simple and direct means by which cell-surface receptors can regulate gene expression.

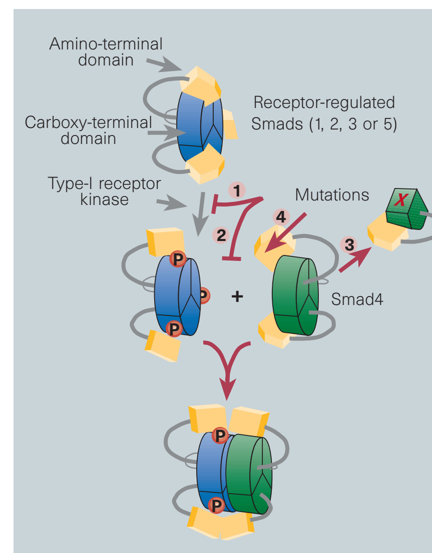

Figure 1 **SMAD proteins are involved in TGF-β signalling, and new studies show how the structure of SMADs could regulate their function. The trimeric carboxy-terminal domain of Smads1, 2, 3 or 5 is shown, based on the crystallographic studies of Shi *et al.*[2]. The amino-terminal domain autoinhibits the carboxy-terminal region and Hata *et al.*[1] suggest that phosphorylation of receptor-regulated SMADs (blue) may relieve this inhibition. This will allow formation of a hexameric complex with Smad4 (green), and this complex can interact with DNA-binding proteins to activate transcription. Mutations (red) identified in the carboxy-terminal domain can prevent phosphorylation (1), heteromeric association (2), or trimer formation (3), whereas mutations in the amino-terminal domain may lead to a**

It is generally recognized that the amino- and carboxy-terminal regions of SMADs act as regulatory and effector domains, respectively. Hata *et al.*[1] and Shi *et al.*[2] now suggest potential mechanisms as to how these domains control SMAD function. In their crystallographic studies, Shi *et al.* have solved the structure of the Smad4 carboxy-terminal domain. The monomer is composed of a β-sheet sandwich, capped at one end with a group of three large loops and an α-helix (the loop/helix domain), and at the other by a triple α-helix structure. Interestingly, these monomers assemble to form a trimeric structure in the crystal, with the loop/helix domain of one monomer interacting with the triple helix of the next. So, the overall structure resembles a disk, with the amino termini extending from one face of the disk and the carboxy termini exposed on the side. On the face of the disk opposite to

the amino-terminal side, the third loop from the loop/helix cap is exposed on the surface (Fig. 1). The authors propose that this loop is critical in mediating the formation of a hexameric complex between Smad4 trimers and trimers of phosphorylated, receptor-regulated SMADs.

If TGF-β-mediated signalling occurs through the ligand-dependent assembly of SMAD hetero-oligomers, how is the formation of these complexes regulated? Hata *et al.* provide biochemical evidence that the amino-terminal domain of either Smad2 or Smad4 physically interacts with the carboxy-terminal domain, to prevent the formation of a Smad2–Smad4 complex. Although several models are consistent with these data, the amino-terminal domain could, perhaps, fold back on the carboxy-terminal domain to control its activity, possibly by altering the conformation or sterically hindering the accessibility of the third loop (Fig. 1).

Most of the mutations that affect *Mad*-related genes in cancer cells or in invertebrate development map to the carboxy-terminal domain. Based on structural and functional criteria, Shi *et al.* have now defined three types of substitutions that affect SMAD function. The first class of mutations cause amino-acid substitutions that map to the interface regions between the Smad4 monomers, thereby destabilizing the trimeric complex. The second class map to the third loop on the face of the disk, and these mutations may disrupt the formation of heteromeric complexes. The third class map to the hydrophobic core, and such mutations may destabilize the structure of the protein. But mutant proteins from any of these classes will disrupt the formation of hexameric complexes — so, presumably, they block the transmission of a TGF-β signal.

In contrast to the carboxy-terminal domain, relatively few of the mutations that are associated with a cancerous phenotype have been mapped to the amino-terminal domains of MAD-related proteins. However, two mutations that affect the same amino-terminal arginine residue have been found in both Smad2 and Smad4. Hata *et al.* have characterized these mutations, and they show that they may increase the affinity of the amino-terminal domain for the carboxy terminus. In essence, this locks the regulatory domain onto the effector domain and so prevents signalling of events that would presumably lead to the suppression of cell growth. Such a gain of autoinhibitory activity would represent an unusual mechanism by which to inactivate a tumour suppressor, and it could explain why relatively few missense mutations have been identified in the amino-terminal region of SMADs.

Smad4 does not possess carboxy-terminal serine residues, and it is not regulated by phosphorylation. Yet serine phosphorylation is critical to the function of Smads1, 2, 3 and 5. So what structural role does phosphorylation play in controlling the function of these receptor-regulated SMADs? By analogy with the Smad4 structure, the carboxy-terminal serines of these SMADs are likely to be accessible to the type-I receptor serine/threonine kinase. Phosphorylation at the tail could thus relieve autoinhibition by the amino-terminal domain, revealing the third loop and allowing the formation of hetero-oligomers (Fig. 1).

Interestingly, many of the mutations characterized within the context of the Smad4 structure were originally identified in receptor-regulated SMADs, and they interfere with their phosphorylation[4,9]. Because phosphorylation is required for the formation of Smad2–Smad4 complexes, the main defect in these mutants could be in phosphorylation, in the interaction with Smad4, or a combination of both. Solving the structure of full-length SMAD proteins should provide some answers, as well as insights into how the structure of these signalling molecules regulates their function. But remaining issues include the molecular basis for recognition of specific SMADs by individual receptors, the basis for nuclear accumulation of phosphorylated SMADs, and the precise mechanisms by which SMADs control gene expression. □

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Semiconductors

Do the twist to get fit

Pauline Rigby

The substrate problem is a big one for the optoelectronics industry. It is only possible to grow good-quality material, without any defects that would prevent light emission, if a substrate can be found with an almost identical atomic spacing to the layer grown on it. This is lattice matching. For some material systems, such as gallium nitride (GaN), there is no lattice-matched substrate available, so it proved extremely difficult to grow defect-free material, and the development of GaN for blue light emission was delayed for 20 years. To magnify the problem, dozens of different materials are required to cover the wavelength ranges of all optoelectronics applications, and each needs a lattice-matched substrate. But now Ejeckam *et al.*[1] have invented a substrate that doesn't require lattice matching — a universal substrate.

When an epitaxial layer is grown on a substrate with a different lattice parameter, at first the growing layer elastically distorts itself to fit the crystal matrix so that the lattice planes are continuous across the interface. When a certain critical thickness[2] is exceeded, the elastic energy is released by plastic deformation, in the form of dislocations which nucleate at the surface and then travel in to the interface. A dislocation is a boundary between part of a crystal that has slipped and part that hasn't (Fig. 1). As it moves through the crystal it displaces one row of atoms at a time; on reaching the far side it has displaced an entire layer. The energy barrier to this process is much lower than it would be for all the rows of atoms to move at once.

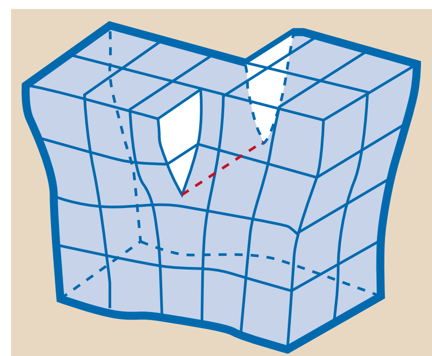

Figure 1 **A screw dislocation in a simple cubic lattice.**

One approach to the problem is to make the substrate very thin, so extending the critical thickness by sharing the strain between the substrate and overlayer[3]. As one side of the structure is in tension and the other in compression, the structure bends like a bimetallic strip. These structures are extremely fragile, too fragile to withstand normal wafer handling and processing.

Dislocations are usually detrimental to device performance, but Ejeckam *et al.* have put them to work, to make a stretchy universal substrate. A film of gallium arsenide (GaAs) only 30 Å thick is grown on an ordinary GaAs substrate (with an aluminium arsenide (AlAs) spacer layer in between), then the thin film is bonded to another substrate, essentially by pressing the surfaces