weaker than a simple scaling of GRB970228 and GRB970508 suggests (J. van Paradijs, Univs Amsterdam and Alabama, Huntsville).

Fortunately, there may be a simple explanation. The X-ray spectrum obtained with the ASCA satellite (T. Murakami, ISAS) indicated the presence of a large amount of absorbing gas between us and the GRB, much more than expected from our own Galaxy. Assuming a standard gas-to-dust ratio, and assuming that the burst was at a moderate redshift, $z = 1$, the implication is that there was so much dust extinction that the optical afterglow should be too dim to be detectable.

The GRB field has now shifted to new wavelengths. The major players today are the X-ray, optical and radio observatories, as well as the γ-ray ones. After a small pause over the summer, BeppoSAX is up and running again. Two other options are now also available, and can provide new targets. First, there is the combination of the Burst And Transient Source Experiment (BATSE), on board the Compton Gamma Ray Observatory, with the Rossi X-ray Timing Explorer (RXTE) and the Ulysses satellite. BATSE detects a burst and defines a relatively small region in the sky where the source should be located. RXTE scans this region for X-ray afterglows; the triangulation of the arrival times of a burst between Ulysses and BATSE provides rings in the sky which further narrow the search area. So far, out of several follow-ups, one X-ray source was detected in this manner. Second, the All Sky Monitor on RXTE recently detected (D. Smith, MIT) two GRBs (GRB970815, GRB970828), providing fast, accurate locations that have increased our sample of X-ray afterglows.

Finally, the high gas-column density detected in the X-ray spectrum of GRB970828 may be indicative of the association between the GRB and a dense interstellar cloud, possibly a star-forming region. The evidence is inconclusive, but this may be the first hint of a relation between the GRBs and the star-forming regions, as one of us (B.P.) proposed at the Huntsville meeting. However, we may have to wait for the detection of perhaps 100 afterglows before the GRB enigma is finally sorted out (K. Hurley, Univ. California, Berkeley). That should take a lot less than 30 years. □

Bohdan Paczyński is in the Department of Astrophysical Sciences, Princeton University, Princeton, New Jersey 08544, USA.
e-mail: bp@astro.princeton.edu
Chryssa Kouveliotou is with the Universities Space Research Association at NASA/Marshall Space Flight Center, ES-84, Huntsville, Alabama 35812, USA.
e-mail: kouveliotou@msfc.nasa.gov

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Signal transduction

Feedback from inhibitory SMADs

Malcolm Whitman

Members of the transforming growth factor-β (TGF-β) superfamily are implicated in biological processes ranging from inhibition of cell proliferation in somatic tissues, to specification of cell fate during embryogenesis. Over the past two years, understanding of the intracellular pathways by which TGF-β signals are mediated has been spurred by studies of the SMAD family of signal transducers[1]. Discovered through genetic screens in *Drosophila melanogaster* and *Caenorhabditis elegans*, the biochemistry and cell biology of vertebrate SMADs has implicated them as regulators of the cell-type specific transcriptional responses that are induced by TGF-β ligands. Now, papers in this issue by Imamura *et al.*[2], Tsuneizumi *et al.*[3] and Nakao *et al.*[4] (pages 622, 627 and 631), along with the work of Hayashi *et al.*[5] in *Cell*, expose a new aspect of SMADs — some of them inhibit, rather than mediate, TGF-β signalling. These inhibitory SMADs are, themselves, induced by TGF-β stimulation, suggesting that there is an intracellular negative-feedback loop that regulates TGF-β signals.

Smads 1 to 5 — the canonical signal-transducing SMADs — act downstream of a family of transmembrane serine–threonine kinase receptors for the TGF-β superfamily ligands. Smad1 and, probably, Smad5 mainly act downstream of the bone-morphogenetic-protein (BMP) subset of the TGF-β superfamily. Smad2, and possibly Smad3, act downstream of several other ligands, including TGF-β itself[1] (Fig. 1, overleaf). When their cognate upstream receptors are stimulated by binding of the appropriate ligand, these pathway-specific signal-transducing SMADs are directly phosphorylated at a carboxy-terminal SS(V/M)S consensus site by type I receptors. Phosphorylated (that is, activated), pathway-specific SMADs can each form a stable complex with Smad4, which is found in the signalling pathways of all the different classes of TGF-β superfamily

100 YEARS AGO

Mr. H. Savage Landor, who left England in March last, commissioned by Mr. Harmsworth, the proprietor of the *Daily Mail*, to endeavour to enter the sacred city of Lhassa, in Tibet, has not been successful in his undertaking. News has just been received that a few days after crossing the frontier of Tibet, disguised as a Chinese pilgrim, all except two of Mr. Landor's men abandoned him. In spite of this, Mr. Landor continued on his journey, but eventually he lost all his provisions, and by an act of treachery was made a prisoner by the Tibetans. He was sentenced to be beheaded, but at the last moment the Grand Lama stopped the executioner, and commuted the sentence of decapitation to the torture of the stretching log – a kind of rack upon which Mr. Landor was chained for eight days – after which he was released. Mr. Landor has now returned to India, suffering from the effects of the torture to which he was subjected, and which he half anticipated before he set out upon his hazardous journey.
From *Nature* 7 October 1897.

50 YEARS AGO

From the obituary of Hans Fischer, "to whom we owe most of our knowledge of the chemistry of hæmin, chlorophyll and the bile-pigments". Fischer, who won a Nobel prize in 1930, died on 31 March 1945, but* Nature *only became aware of his death several months later.

There was an atmosphere of unusual jollity in [his] laboratory, directed in its legitimate expression by Herr Paulus, the store-keeper, and controlled, in its more outrageous excesses, by Fischer's tact and humour. A fledgling 'doctor', returning from his oral examination – a formality – found his bench littered with the starting materials for a celebration and a large blackboard, on which a cartoon and some lines of doggerel reminded him that he was mortal. More rarely, there were occasions in the cellars, and at Christmas the supply of 5-litre flasks was exhausted as all undertook the preparation of 'christmas-pyrrole' by a process supposed to render denatured alcohol potable.

● We regret also to announce the death of Prof. Max Planck, For.Mem.R.S., on October 4, aged eighty-nine.
From *Nature* 11 October 1947.

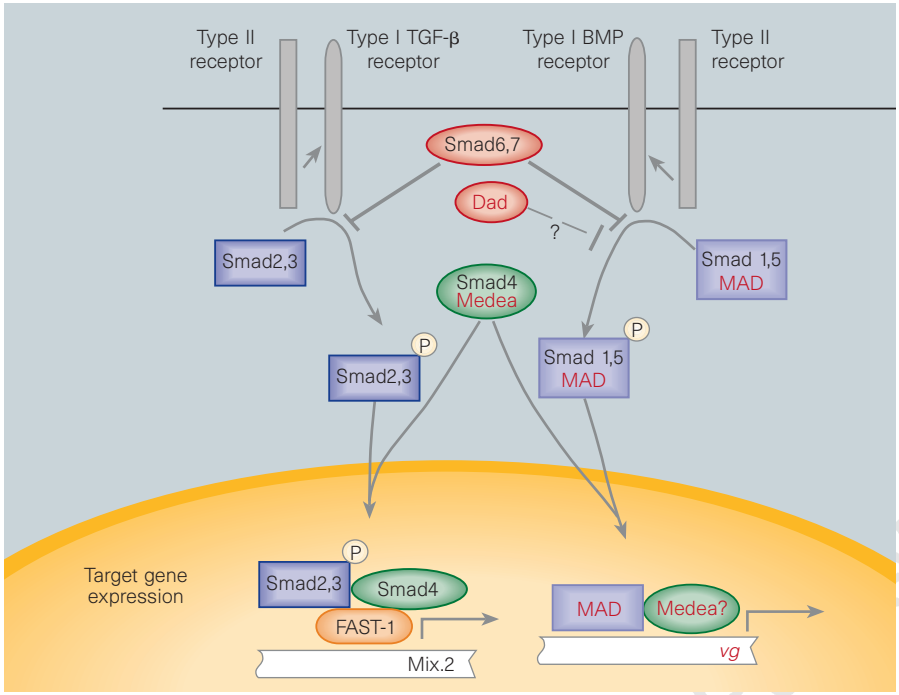

Figure 1 **SMADs involved in the transmission and inhibition of transforming growth factor-β (TGF-β) superfamily signals. Ligand-binding type II receptors associate with, phosphorylate and activate type I receptors (for TGF-β or bone morphogenetic protein; BMP). The type I receptors phosphorylate pathway-specific SMADs (Smads 1, 2, 3 and 5). These activated SMADs then associate with Smad4, and translocate to the nucleus where they may regulate transcription either by associating with nuclear transcription factors, as in the case of the Mix.2 promoter[9], or by binding directly to DNA, as in the case of the *vestigial* (*vg*) promoter[10]. The inhibitory SMADs described by Imamura *et al.*[2], Tsuneizumi *et al.*[3], Nakao *et al.*[4] and Hayashi *et al.*[5] target the first step in the intracellular transduction pathway — type I receptor phosphorylation of pathway-specific SMADs. For simplicity, additional homomeric interactions among receptors and SMADs are not shown. *Drosophila* proteins are shown in red. Medea is a *Drosophila* homologue of Smad4 (ref. 11), and the association of Medea with the *vestigial* promoter is hypothetical.**

ligands. This complex then translocates to the nucleus, where it regulates transcriptional responses to TGF-βs[1]. The pathway-specific SMADs (Smads 1, 2, 3 and 5) all associate with type I TGF-β-superfamily receptors and are phosphorylated at the conserved carboxy-terminal site; Smad4 functions through association with these pathway-specific SMADs.

Now, Imamura *et al.*[2], Nakao *et al.*[4] and Hayashi *et al.*[5] report that Smad6 and Smad7 inhibit the vertebrate SMAD-based signalling pathway described above. Moreover, Tsuneizumi *et al.*[3] show that, in a homologous signalling pathway in *Drosophila*, the product of the *Daughters against decapentaplegic (Dad)* gene is an inhibitory SMAD. Overexpression of these newly discovered SMADs inhibits intracellular signalling by members of the TGF-β superfamily. Smad6 and Smad7 can inhibit both TGF-β and BMP signalling in cultured cells or in frog embryos[2,4,5]. The *Dad* gene inhibits patterning by *decapentaplegic*, a *Drosophila* BMP homologue, in the *Drosophila* imaginal wing disk[3]. Like Smad4, these inhibitory SMADs lack the carboxy-terminal phosphorylation site that is found in each of the pathway-specific SMADs. But unlike Smad4, Smads 6 and 7 do interact with type I receptors — and they probably interact more stably than do the pathway-specific SMADs. Presumably, this is because Smad6 and Smad7 lack the carboxy-terminal phosphorylation site, so they are not phosphorylated and released from activated receptor kinase.

So, competitive inhibition of the SMAD binding site on the type I receptor kinase may explain how Smad6 and Smad7 inhibit signalling by members of the TGF-β superfamily. Overexpression of Smad6 or Smad7 inhibits ligand-stimulated phosphorylation of pathway-specific SMADs[2,4,5], indicating that these inhibitory SMADs can block signalling at this initial step in the intracellular transduction pathway. In theory, an inhibitory SMAD might work by sequestering signalling SMADs or downstream effectors, as well as by tying up receptors. Intriguingly, Topper *et al.*[6] have identified a truncated form of Smad6 that is co-induced with Smad7 by shear stress in vascular endothelial cells. The truncated Smad6 stably associates with Smad1, Smad2 and Smad4, as well as with Smad7. So, some inhibitory SMADs might also act by sequestration of signalling SMADs.

Defining the specificities of inhibitory SMADs with respect to upstream ligands and receptors, and downstream signalling SMADs, will be critical for understanding their biological functions. Overexpression of Smad7 inhibits phosphorylation of Smad2 and Smad3 by activated type I TGF-β receptor, and prevents phosphorylation of Smad1 by activated BMP type I receptors. Smad7 could, therefore, be a generalized inhibitor of signalling by the TGF-β superfamily[4]. Smad6 is more complicated — it inhibits phosphorylation of Smad2 but not Smad3. Moreover, it inhibits phosphorylation of Smad1 by the BMP type IB receptor, but not by the BMP type IA receptor. Analysing the specificity of inhibitors by overexpression is a tricky business, because inhibition *in vivo* would be expected to be a function of the endogenous concentrations of type I receptors, signalling SMADs and inhibitory SMADs. But inhibitory SMADs that distinguish between different ligands or receptor subtypes of the TGF-β superfamily ligands would provide considerable flexibility to a cell's repertoire of responses to TGF-β stimuli.

The question surrounding specificity of the inhibitory SMADs is closely tied to understanding their role in feedback-inhibition of TGF-β signals. If each inhibitory SMAD targets the full range of type I receptors in the TGF-β superfamily, feedback-inhibition from one ligand will reduce responsiveness to the larger superfamily of ligands. Alternatively, if inhibitory SMADs are specific for distinct receptors or signalling SMADs, they may provide a mechanism by which cells can independently regulate either sensitivity to distinct ligands or specific downstream responses. The timing for induction of inhibitory SMADs may also be an important aspect of feedback control — Smad7 is induced much more rapidly than the product of the TGF-β-responsive *PAI-1* gene. So we need to know how inhibitory SMADs modulate not only the magnitude but also the duration of a TGF-β signal.

Although SMADs have become a focus of research, other signalling pathways have been implicated in TGF-β-mediated signal transduction[7]. If inhibitory SMADs specifically target the SMAD component, it is possible that feedback-regulation by inhibitory SMADs does not downregulate TGF-β signalling entirely. The effects of inhibitory SMADs on a variety of transcriptional and phenotypic responses to TGF-βs are reported in the new papers, but a more exhaustive examination of biological responses to TGF-β will be necessary to determine whether inhibitory SMADs block some or all components of TGF-β signalling.

Ligands of the TGF-β superfamily are morphogens in both vertebrate and invertebrate[8] embryos. This suggests that the spatially and temporally complex regulation of responses to TGF-βs across developing tissues is fundamental to embryonic patterning. Now, the discovery that inhibitory

SMADs are feedback regulators of TGF-β signalling opens up the question of how the expression and regulation of these molecules participates in the patterning of TGF-β responses in developing tissues. □

Malcolm Whitman is in the Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, USA.
e-mail: mwhitman@warren.med.harvard.edu

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Telomerase

Cancer and the knockout mouse

David Wynford-Thomas and David Kipling

Telomeres are specialized structures at the ends of linear chromosomes. They usually consist of tandem arrays of a short DNA sequence (TTAGGG in vertebrates) and associated proteins, and they are thought to have at least two essential functions[1]. First, they stop natural chromosome ends behaving as random breaks, which might otherwise generate inter-chromosomal fusions and activate DNA-damage-induced cell-cycle arrest. Second, they provide the structural basis for solving the end-replication problem[2] — the inability of DNA polymerases to completely replicate the end of a DNA duplex (see box).

In the germ line, telomeres are maintained by the compensatory addition of TTAGGG repeats to chromosome ends. These repeats are synthesized by an enzyme called telomerase (Fig. 1, overleaf), and a lack of telomerase activity — such as occurs physiologically in most adult human somatic cells — leads to progressive telomere shortening with every cell cycle[3]. If such cells are forced to grow for long enough, they eventually lose telomere function leading to end-to-end chromosome fusions and cell death. What, then, might be the effect of a constitutional (germline) deficiency of telomerase? An intriguing (although still partial) answer is provided in the latest issue of *Cell*, where Blasco *et al.*[4] report their studies of a telomerase-deficient mouse.

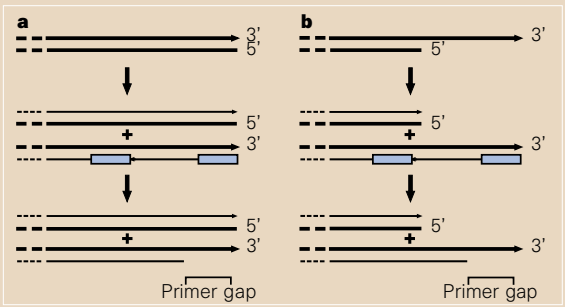
Telomerase contains an essential RNA component that provides the template for the specific synthesis of TTAGGG repeats. Blasco *et al.* used transgenic technology to create a germline deletion of the *mTR* gene, which encodes the RNA component of mouse telomerase. The authors bred homozygous, null $mTR^{-/-}$ mice which turned out to be both viable and fertile, despite having no detectable telomerase activity. Moreover, the mice have now been maintained for six generations (G1–6). The absence of any initial phenotype is striking. So is the fact that the next few generations have also shown no symptoms, suggesting that the telomerase inhibitors that have been envisaged for cancer therapy will not have any acute toxicity. But telomere shortening is occurring: by the latest generation studied (G6), there is clear evidence of telomere erosion, with around five per cent of chromosomes in embryonic fibroblasts lacking detectable TTAGGG, accompanied by increasingly frequent chromosome fusions.

This 'delay' in manifestation of the phenotype almost certainly reflects the unusually long telomere-repeat arrays in the germ line of this mouse species. These repeats are not exhausted until many generations have elapsed — a human 'knockout' would be predicted to show a much more rapid onset. Yet, despite reaching what seems to be a critical state of erosion, cells from generation G6 do not show an obvious reduction in growth capacity in culture, or any reduction in the ability to generate tumours when oncogene-transformed cells are injected into nude (immunodeficient) mice.

It has been suggested that progressive erosion of telomeres in somatic cells (which have physiologically repressed telomerase) represents a barrier to indefinite cell proliferation. Tumour cells are thought to overcome this block by reactivating telomerase. But the apparently undiminished tumorigenic ability of oncogene-transformed $mTR^{-/-}$ cells suggests that telomerase may not be necessary for tumorigenesis. Data showing that, in mice, telomerase can be up-regulated at an early stage in tumour development[5] — before any significant telomere erosion has occurred — leads the authors to hint that the current dogma may be wrong. In other words, telomerase may be nothing more than a passive bystander, rather than facilitating tumour growth[4]. If so, this would have considerable implications for telomerase as a target for cancer therapy (although it does not necessarily detract from its value as a diagnostic marker).

There are, however, good reasons for exercising caution when extrapolating from the mouse model. The main limitation is that the phenotype is probably not yet completely developed. Although telomere failure is occurring at G6, most of the chromosomes retain detectable TTAGGG. The very fact that viable G6 animals exist suggests

The end-replication problem



For lagging-strand DNA replication, short RNA primers (blue) are made by RNA primase. These are then extended by DNA polymerase to form Okazaki fragments. When these RNA primers are removed, there is no way to synthesize lagging-strand sequence that is complementary to the small region at the end of the chromosome (which is at least as large as an RNA primer). So, with continuing cell division, sequence is lost from the ends of linear chromosomes. Some blunt-ended daughter molecules are produced by this scheme, irrespective of whether the starting terminus is blunt-ended (a) or has a 3' extension (b).

Why are such natural blunt ends not recognized as DNA damage? One possibility is that this is because unnatural ends have a slightly different chemical structure; for example, radiation-induced blunt ends can have terminal 3' phosphoglycolate residues whereas physiological ends do not. Human cells may have an additional mechanism to ensure that natural chromosome ends are even more different. This involves a degradative pathway which, even in telomerase-deficient human cells, results in 3' extensions for most chromosome ends[9]. This may be analogous to a degradative pathway that has been described in budding yeast, involving Cdc13p and other proteins[10]. **D.W.-T. & D.K.**