Mechanism of activation of the TGF-β receptor

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Transforming growth factor-β (TGF-β) signals by contacting two distantly related transmembrane serine/threonine kinases called receptors I and II. The role of these molecules in signalling has now been determined. TGF-β binds directly to receptor II, which is a constitutively active kinase. Bound TGF-β is then recognized by receptor I which is recruited into the complex and becomes phosphorylated by receptor II. Phosphorylation allows receptor I to propagate the signal to downstream substrates. This provides a mechanism by which a cytokine can generate the first step of a signalling cascade.

THE TGF-β superfamily is a large group of cytokines which are among the most versatile carriers of growth and differentiation signals[1-6]. Members of this family participate in setting up the basic body plan during embryogenesis in mammals, frogs and flies; control formation of neural tube, limbs, cartilage, bone and sexual organs; suppress epithelial cell growth, promote wound repair, and influence important immune and endocrine functions[1-6]. Alterations in the activity of these factors in humans have been implicated in fibrosis, immunosuppression, cancer and other disorders[3,7].

Progress towards elucidating the mode of action of these factors has been made with the identification of their receptors[8-21]. TGF-β and related factors bind to sets of two membrane proteins called receptor types I and II[1,2]. Genetic evidence from cell mutants resistant to TGF-β action suggests that TGF-β binding to receptor I requires the presence of receptor II and, furthermore, that both receptors are required for signalling of any response[1,4,18,20-26]. These properties appear to be shared by receptors for other TGF-β family members[14,17,26,27].

Molecular cloning of types I and II receptors for TGF-β and the related activins and bone morphogenetic proteins (BMPs) has shown that they are a family of proteins with a small extracellular region, a single transmembrane segment, and a cytoplasmic region with a serine/threonine kinase domain[1,2]. The type I receptor kinase domains are more similar to each other than they are to the type II receptor kinase domains, with which they show less than 40% amino-acid sequence identity. The type I receptors share various other structural features not found in the type II receptors, including the spacing of cysteines in the extracellular region and a highly conserved serine- and glycine-rich segment adjacent to the amino-terminal boundary of the kinase domain.[21]

Studies with the cloned TGF-β and activin type I receptors have confirmed their inability to bind ligand in the absence of type II receptors[16-21], and the failure of the latter to signal in the absence of type I receptors[23,26]. Another feature of this system is the ability of type II receptors to interact with different type I receptor isoforms. Thus, the TGF-β type II receptor, TβR-II, can interact with at least two type I receptors, called TβR-I (also known as ALK5 (ref. 18) or R4 (ref. 20) and TSR-I[17], whereas the activin type II receptors ActR-II and ActR-IIB can interact with at least three distinct activin type I receptors[17,19,28,29]. The specificity of the biological response to ligand in a given cell type appears to be defined by the particular type I receptor engaged in the complex, thus providing a basis for the multifunctional nature of these cytokines[26].

The basis for the requirement of TGF-β and related factors to interact with two distinct transmembrane kinases in order to signal has remained unsolved. This requirement is in contrast to the well-characterized tyrosine kinase receptors which act as ligand-activated homodimeric kinases, or dimers of highly related kinases, which autophosphorylate on tyrosine residues forming sites for association with various signal-transducing proteins[30,31]. The pathways initiated in this manner, as those initiated by G-protein-coupled receptors, often lead to cytoplasmic serine/threonine kinases that act in sequence, with one phosphorylating and activating the next[32]. Here we describe the early events triggered by TGF-β binding to its receptors, and demonstrate that receptor II is a constitutively active kinase which uses the ligand to recruit, phosphorylate and signal through receptor I, thus generating the first step of a TGF-β signalling pathway.

TGF-β receptor II is constitutively active

Because ligand-stimulated autophosphorylation of tyrosine kinase receptors initiates their signalling process, we asked if the same would occur with the TGF-β type II receptor which binds ligand on its own. We examined the phosphorylation state of TβR-II in Mv1Lu cells, a mink lung epithelial cell line whose TGF-β receptors and responses are well characterized[33,34]. Human TβR-II constructs tagged at the carboxy terminus with an influenza virus haemagglutinin epitope (HA) were used. The functional properties of these and other tagged receptors used were similar to those of unmodified receptors, as determined by their ability to restore TGF-β binding and responsiveness when expressed in receptor-defective mutant Mv1Lu cells (refs 23, 26, and results not shown).

Immunoprecipitation of the transfected TβR-II from [^{32}P]phosphate-labelled cells with anti-HA antibody showed that this receptor was highly phosphorylated in the absence of TGF-β addition (Fig. 1*a*). Phosphorylation occurred on multiple serine residues, as determined by receptor phosphoamino-acid analysis and tryptic phosphopeptide mapping (Fig. 1*b*, *c*). Tryptic digestion of the receptor and analysis by two-dimensional thin layer electrophoresis and chromatography yielded eight phosphopeptides of similar labelling intensity and a ninth phosphopeptide (spot 1) which was more intensely labelled. The latter probably corresponds to the serine-rich C-terminal tail of TβR-II because it was missing in phosphopeptide maps (not

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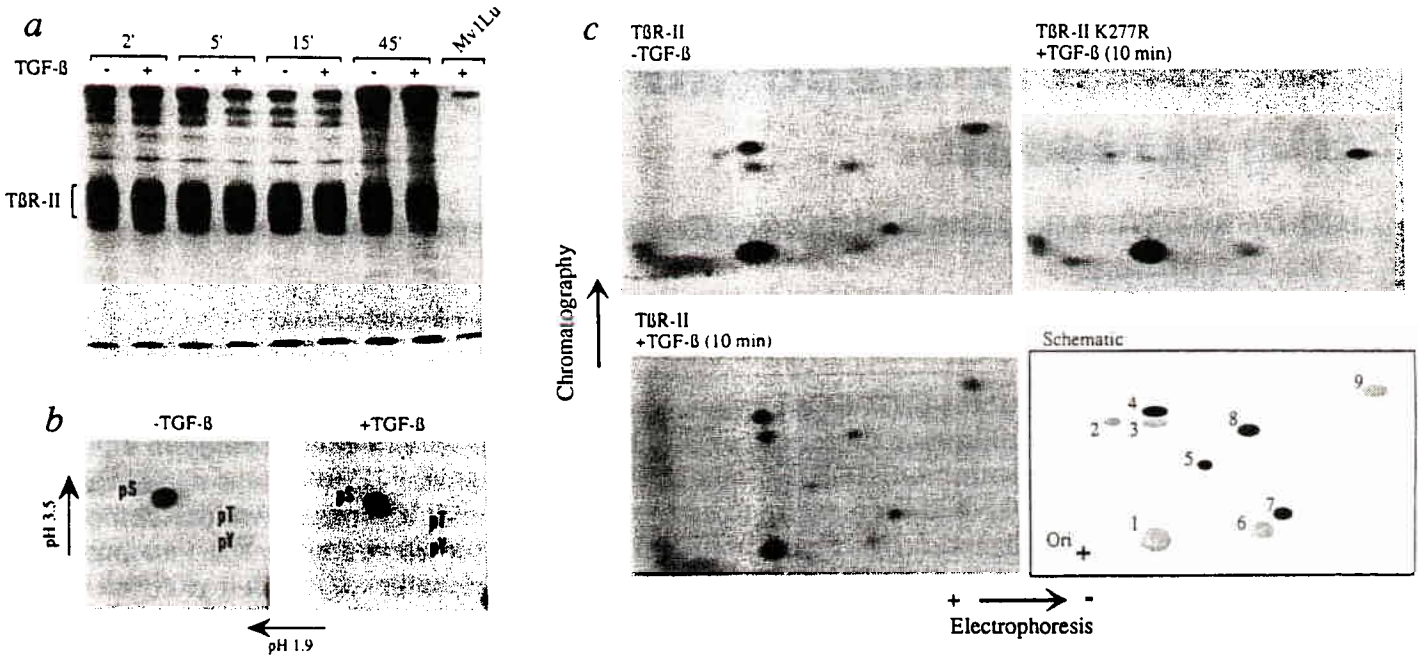

FIG. 1 *a*, Phosphorylation of TβR-II. Mv1Lu cells stably transfected with TβR-II tagged with an HA-epitope (TβR-II/HA; ref. 23) or parental Mv1Lu (Mv1Lu lane) were [^{32}P]phosphate-labelled and then incubated in the presence (+) or absence (−) of TGF-β for the indicated times. Cell lysates were immunoprecipitated with anti-HA antibody and analysed by SDS–PAGE and autoradiography. *b*, Phosphoamino-acid analysis of TβR-II. [^{32}P]phosphate-labelled TβR-II/HA transfectants were incubated in the presence or absence of TGF-β, and TβR-II/HA was purified by immunoprecipitation, isolated by SDS–PAGE, and subjected to acid hydrolysis and phosphoamino-acid analysis[41]. pS, Phosphoserine; pT, phosphothreonine; pY, phosphotyrosine. *c*, Tryptic phosphopeptide mapping of TβR-II. R-1B cells transiently transfected with TβR-II/HA or kinase-defective TβR-II/HA(K277R) cDNAs in the pCMV5 expression vector[23,26], were [^{32}P]phosphate-labelled in the presence or absence of TGF-β and isolated by immunoprecipitation. Tryptic digests of gel-purified TβR-II were resolved in two dimensions with electrophoresis in pH 1.9 buffer and chromatography in phosphochromatography buffer[41]. A schematic drawing of the tryptic phosphopeptides is shown with autophosphorylated peptides (black spots) and location of sample application (Ori+) indicated.

METHODS. Transiently transfected L17 cells, a highly transfectable clone of R1-B cells[23,26], were incubated in phosphate-free minimal essential media containing 1 mCi ml^{-1} [^{32}P]phosphate for 2 h followed by 10 min with or without 1 nM TGF-β, washed once in ice-cold PBS and lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% (v/v) Triton X-100, 50 mM NaF, 10 mM sodium-pyrophosphate and 1 mM sodium-orthovanadate). Receptors were immunoprecipitated with anti-HA monoclonal antibody (12CA5; BAbCo)[23]. Phosphoamino-acid analysis and tryptic-phosphopeptide mapping were done using the HTLE-7000 system (CBS Scientific Co.)[41].

shown) of a mutant receptor, TβR-II(Δtail), that lacks this tail yet has undiminished signalling activity[35].

Exposure of cells to TGF-β1 (the TGF-β isoform used throughout these studies) for 2–45 min did not alter the overall phosphorylation level of TβR-II or the pattern of tryptic phosphopeptides (Fig. 1*a–c*). However, mutation of lysine 277 to arginine in the putative ATP binding site of TβR-II, which destroys the kinase and signalling activities of this receptor[23,25], caused loss of receptor phosphorylation. Specifically, four phosphopeptides (spots 4, 5, 7 and 8) that were present in tryptic digests of wild-type TβR-II were missing in the mutant receptor (Fig. 1*c*). The high level of expression of TβR-II in these cell lines precluded analysis of phosphorylation events that may occur because of complexing between TβR-II and the endogenous type I receptor. Nevertheless, these results suggest that TβR-II, in the absence of TβR-I, is constitutively phosphorylated by cellular kinases at multiple sites and by itself at additional sites, and that its autophosphorylating activity is not regulated by ligand binding.

TGF-β recruits receptor I

To investigate why TGF-β type I receptors only bind ligand in the presence of TβR-II, we focused on TβR-I which with TβR-II signals cell-cycle arrest[26] and expression of various extracellular matrix proteins[18,26]. Although TβR-I does not bind TGF-β when expressed alone, biotinylation of intact COS-1 cells transfected with TβR-I showed that this receptor was synthesized and reached the cell surface as efficiently as TβR-II (Fig. 2*a*). Therefore, the inability of TβR-I to bind ligand when expressed in the absence of TβR-II was not due to a failure of TβR-I to reach the cell surface but to its inability to recognize free TGF-β.

When expressed together, TβR-I and TβR-II form a ternary complex with TGF-β[23]. To determine whether this complex preexists or is induced by the ligand, complementary DNA vectors encoding HA-tagged TβR-I (TβR-I/HA) and hexahistidine-tagged TβR-II (TβR-II/His) were expressed at high levels by transient transfection into the mutant Mv1Lu cell clone R-1B which is defective in TβR-I[26,36]. Both receptors are expressed at roughly equal levels which are much higher than the endogenous TGF-β receptors, thus allowing for the analysis of transfected receptor complexes with minimal endogenous receptor background[26]. To measure complex formation between transiently expressed TβR-I/HA and TβR-II/His, proteins were isolated under non-denaturing conditions from lysates of metabolically labelled cells through a two-step procedure using Ni-NTA-agarose that binds polyhistidine sequences followed by immunoprecipitation with anti-HA antibody and protein A–Sepharose (Fig. 2*b*). The results demonstrate that the association of TβR-I with TβR-II was highly dependent on ligand and occurred at physiological temperatures or in the cold within minutes of TGF-β addition to the cells (Fig. 2*c*). This complex was highly stable and could not be dissociated by exposure of purified complex to 4 M urea, 0.5 M LiCl, 1% SDS at room temperature, or pH as low as 2.5 but was disrupted by boiling in 1% SDS (data not shown). Receptors I and II transiently expressed at high level in these cells consistently displayed a trace amount of complex in the absence of ligand (Fig. 2*c*, lane 2), suggesting that these receptors may have a low intrinsic affinity

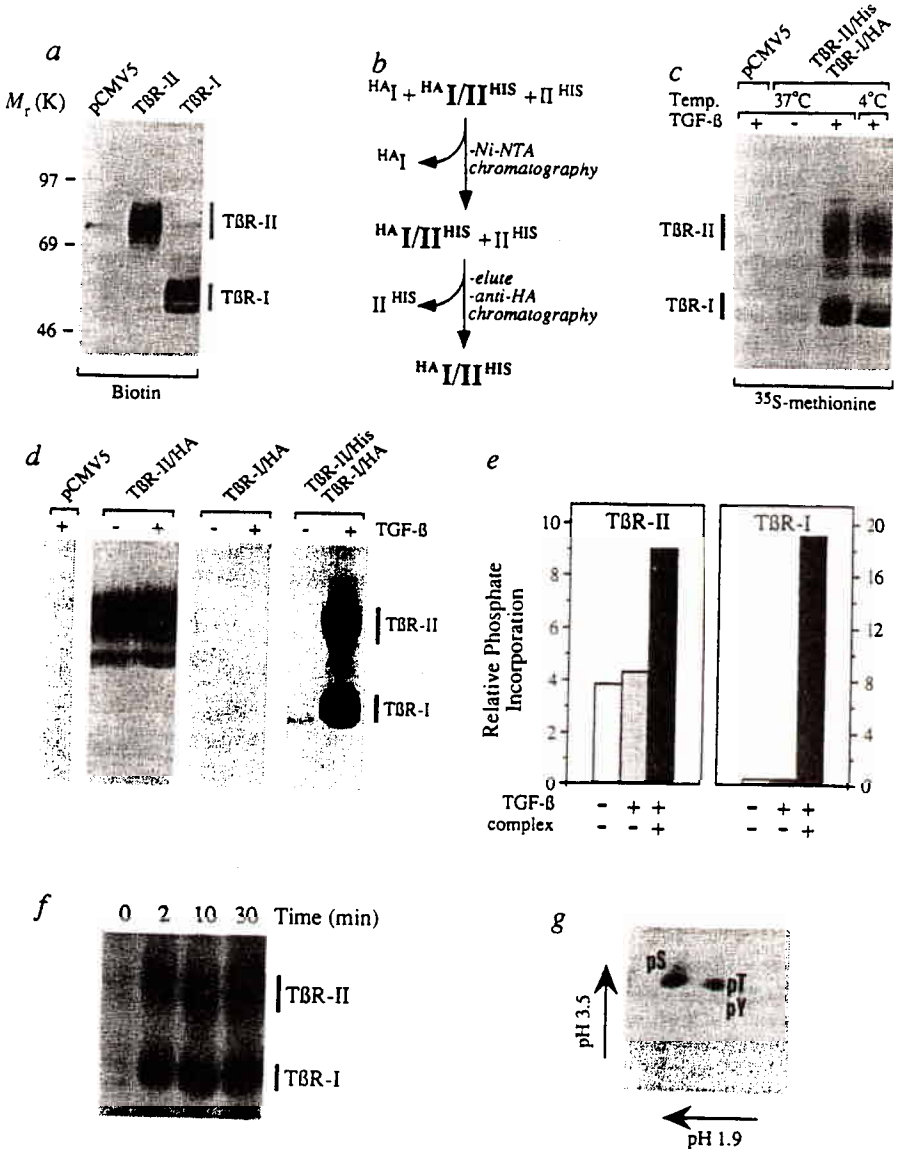

FIG. 2 *a*, Cell-surface expression of TβR-II and TβR-I in transiently transfected COS-1 cells was detected by cell-surface labelling with biotin as previously described[42]. *b*, TβR-I and TβR-II tagged at the C terminus with an HA-epitope[26] and a hexahistidine (HIS) sequence[17], respectively, were isolated as a heteromeric receptor complex by sequential chromatography over Ni-NTA agarose and an anti-HA affinity column. *c*, Ligand-dependent complex formation. Transfected R-IB cells were labelled with [^{35}S]methionine and treated with (+) or without (−) TGF-β for 10 min at 37 °C or 30 min at 4 °C, and receptor complexes isolated. *d*, Receptor phosphorylation. Transfected cells were labelled with [^{32}P]phosphate and incubated with (+) or without (−) TGF-β. Receptors from lysates of cells singly transfected were isolated with anti-HA antibodies, whereas in double transfectants, receptor complexes were purified. Autoradiographic exposure times of single versus double precipitations were adjusted according to the signal from [^{35}S]methionine-labelled receptors isolated from parallel samples (not shown). *e*, The relative phosphorylation level of each receptor from *d* is plotted as the ratio of [^{32}P]phosphate to [^{35}S]methionine incorporation. Cells singly transfected were treated with (grey bars) or without (white bars) TGF-β. Receptors I and II from TGF-β-treated double transfectants (black bars) were quantified separately. *f*, Time course of complex formation and phosphorylation of TβR-I in [^{32}P]phosphate-labelled R-IB cells transfected with TβR-II/His and TβR-I/HA incubated with 1 nM TGF-β for the indicated times. *g*, Phosphoamino-acid analysis of TβR-I. Phosphorylated receptor complexes were separated by SDS–PAGE and TβR-I subjected to phosphoamino-acid analysis as described for TβR-II (Fig. 1*b*).

METHODS. For metabolic labelling, cells were incubated for 2 h in 50 μCi ml^{-1} [^{35}S]translabel (ICN) in methionine-free media and lysed as in Fig. 1. For purification of receptor complexes, cells were lysed in lysis buffer without EDTA and containing 25 mM imidazole, receptors collected by Ni-NTA-chro-matography, eluted with 250 mM imidazole[17] and complexes purified by binding to anti-HA antibody beads.

for each other. TβR-II binds ligand independently of TβR-I, suggesting that TβR-I recognizes TGF-β bound to TβR-II but not free in the medium or bound to other membrane proteins. Therefore, one function of the ligand is to help recruit TβR-I into a stable complex with TβR-II.

Receptor I phosphorylation

TβR-I isolated as a complex with TβR-II showed a heterogeneous electrophoretic migration pattern common in phosphorylated proteins: TβR-I from complexes formed by incubation of cells with TGF-β1 at 4 °C migrated as a single band on sodium dodecyl sulphate electrophoresis gels whereas TβR-I from receptor complexes formed at 37 °C consistently migrated with slower mobility (Fig. 2*c*).

Analysis of receptors isolated from [^{32}P]phosphate-labelled cells confirmed that TβR-I was phosphorylated when it was in a complex with TβR-II. TβR-II transfected alone showed a high level of phosphorylation, but this level was not increased on incubation of cells with TGF-β (Fig. 2*d*, *e*). Similarly, phosphorylation of TβR-I expressed alone did not increase on TGF-β addition to the medium, although the basal level of phosphorylation was very low (Fig. 2*d*, *e*). However, when TβR-I was isolated as part of a ligand-induced complex with TβR-II from cells cotransfected with both receptors, phosphorylation was almost 50-fold higher than when it was expressed alone (Fig. 2*d*, *e*). Ligand-induced phosphorylation of TβR-I in receptor complexes was near-maximal 2 min after TGF-β addition to the cells (Fig. 2*f*), and occurred on serine and threonine residues at a ratio of about 3:1 (Fig. 2*g*). In contrast, the phosphorylation level of TβR-II in ligand-induced complexes was increased less than twofold compared with TβR-II expressed alone (Fig. 2*d*, *e*). This increase was quantitative because no new phosphopeptides were observed (see below). This modest increase could represent protection of phosphates against phosphatase activity in the complex or may represent differences in the [^{32}P]phosphate/[^{35}S]methionine ratios of the cell-surface receptor pool (isolated in the complex) versus the entire receptor pool (isolated in the anti-HA immunoprecipitate).

Receptor II phosphorylates receptor I

To determine whether ligand-induced phosphorylation of TβR-I was catalysed by its own kinase activity or by the kinase activity of TβR-II, we compared the phosphorylation state of these receptors when cotransfected with wild-type or kinase-defective

mutants of each other. When TβR-I was coexpressed with the kinase-defective receptor TβR-II(K277R), it bound ligand and formed a complex with this mutant receptor as efficiently as it did with wild-type TβR-II, but did not become phosphorylated (Fig. 3*a*). Mutation of lysine 232 to arginine in the putative ATP binding site of TβR-I destroys the kinase and signalling activities of this receptor[20,26]. When this construct, TβR-I(K232R), was coexpressed with TβR-II, it bound ligand, formed a complex and became phosphorylated in a manner similar to wild-type TβR-I (Fig. 3*a*). These results suggest that the kinase activity of TβR-II is required for TβR-I phosphorylation.

This conclusion was supported by the results of receptor phosphopeptide mapping. Tryptic digestion of [^{32}P]phosphate-labelled TβR-I derived from ligand-induced receptor complexes yielded one major phosphopeptide and five minor ones (Fig. 3*c*). The major phosphopeptide contained phosphoserine and phosphothreonine in a 3:1 ratio as determined by phosphoamino-acid analysis (data not shown). This phosphopeptide was absent from TβR-I derived from complexes formed with TβR-II(K277R) (Fig. 3*c*). Furthermore, tryptic digestion of TβR-I(K232R) yielded a phosphopeptide map identical to that of TβR-I (Fig. 3*c*), indicating that TβR-I is not an autophosphorylating kinase in intact cells even when it participates in a receptor complex. Thus, ligand-induced phosphorylation of TβR-I within the receptor complex was dependent on the kinase activity of TβR-II.

Analysis of the tryptic phosphopeptide map of TβR-II isolated from complexes with TβR-I showed that it was identical to that of TβR-II in complex with TβR-I(K232R) (Fig. 3*b*). Furthermore, the pattern of tryptic phosphopeptide maps obtained from TβR-II in the complex and TβR-II isolated in the absence of TβR-I were the same (compare Figs 1*b* and 3*b*). Together these data indicate that TβR-II is not a substrate of TβR-I and that its phosphorylation is not qualitatively altered as a consequence of ligand-induced complex formation. Because the kinase activity of TβR-I is essential for signalling, but neither autophosphorylates nor phosphorylates TβR-II, these results collectively suggest that ligand-bound TβR-II recruits and phosphorylates TβR-I which then phosphorylates downstream substrates.

To obtain direct evidence that TβR-I is phosphorylated by the kinase activity of TβR-II, we did *in vitro* kinase assays using ligand-induced complexes formed in intact cells. To generate receptor complexes containing a minimal level of phosphorylation, cells were incubated with TGF-β in the cold. The receptors were purified from cell lysates using Ni-NTA-agarose beads and phosphorylation was allowed to proceed in the presence of [γ-^{32}P]ATP. Phosphorylated receptors were eluted by boiling in SDS and type I receptors collected by immunoprecipitation with anti-HA antibodies. Phosphorylation of TβR-I was observed when coexpressed with wild-type TβR-II receptor but not the kinase-defective version, TβR-II(K227R). Both wild-type and

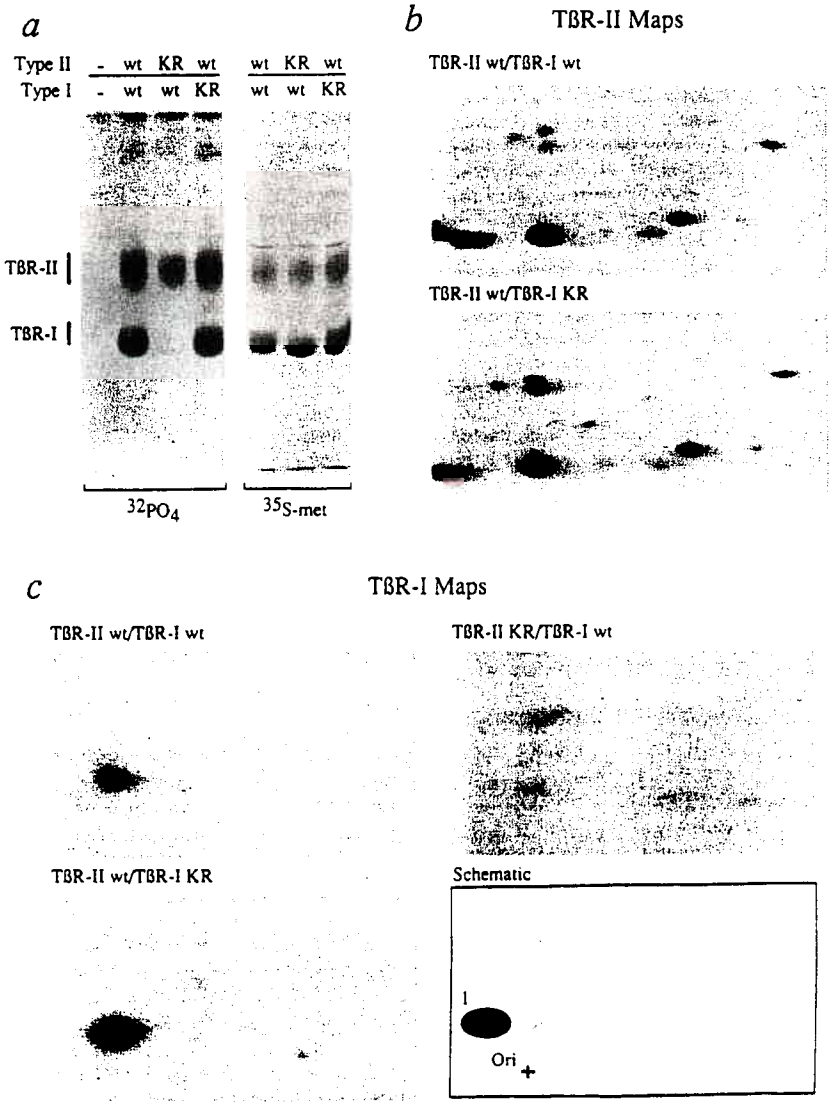

FIG. 3 *a*, Phosphorylation of TβR-II and TβR-I in complexes formed between wild-type and kinase-deficient receptors. R-1B cells were transiently transfected with the indicated combinations of wild-type (wt) TβR-II/His and TβR-I/HA or their kinase-deficient (KR) derivatives TβR-II/His(K277R) and TβR-I/HA(K232R). Cells were labelled with either [^{32}P]phosphate or [^{35}S]methionine, and incubated with TGF-β for 10 min at 37 °C. Receptor complexes were then purified, resolved by SDS–PAGE and receptors visualized by autoradiography or fluorography. The similar methionine content in receptor II and receptor I allows direct comparison of labelling intensities to assess the amounts of receptor proteins. *b*, *c*, Tryptic phosphopeptide mapping of TβR-II and TβR-I isolated from ligand-dependent complexes. [^{32}P]phosphate-labelled complexes between the indicated receptor combinations were generated as described and shown in *a*. Tryptic phosphopeptides from TβR-II (*b*) or TβR-I (*c*) were resolved in two dimensions[41]. A schematic diagram of the phosphotryptic peptides for TβR-I is shown with the TβR-II kinase-dependent peptide (spot 1) and the location of sample application (Ori +) indicated.

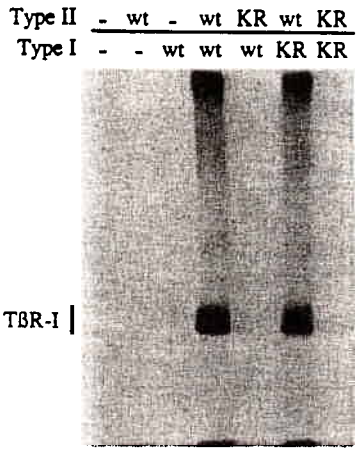

FIG. 4 Phosphorylation of TβR-I by TβR-II *in vitro*. R-1B cells were transiently transfected with wild-type (wt) TβR-II/His and TβR-I/HA or their kinase-deficient (KR) derivatives TβR-II/His(K277R) and TβR-I/HA(K232R), either singly or in combination, as indicated. Cells were incubated with 1 nM TGF-β for 15 min at 4 °C, lysed and receptors collected by binding to Ni-NTA-agarose. These samples were incubated in kinase assay buffer containing 50 μCi [γ-^{32}P]ATP[26]. To separate receptor I from receptor II and unincorporated [γ-^{32}P]ATP, complexes were eluted from Ni-NTA-agarose by boiling in 1% SDS, diluted 10-fold in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% (v/v) Triton X-100, 1% Na deoxycholate (see Fig. 5) and TβR-I isolated by immunoprecipitation with anti-HA antibody.

kinase-defective TβR-I(K232R) were similarly phosphorylated (Fig. 4). Tryptic phosphopeptide maps of TβR-I phosphorylated *in vitro* were similar to those of TβR-I phosphorylated in intact cells except that the ratio of incorporated phosphate varied, with the relative level of spot 1 being lower than in TβR-I phosphorylated in intact cells (data not shown). Thus receptor I in the TGF-β receptor complex is directly phosphorylated by receptor II.

Receptor complexes [^{32}P]-labelled *In vivo*

To identify the regions of TβR-I phosphorylated by TβR-II, [^{32}P]-labelled receptor complexes *in vivo* were purified by Ni-NTA chromatography, eluted by boiling in SDS to separate the two receptors, and TβR-I was collected by immunoprecipitation. Purified TβR-I was subjected to digestion with chymotrypsin and V8 protease with each protease generating a single phosphopeptide fragment with an approximate relative molecular mass (M_r) of 3,500 and 2,800 (3.5K and 2.8K), respectively (Fig. 5*b*). Analysis of the sequence for TβR-I revealed that only the GS domain, a region highly conserved in type I receptors from *Drosophila* through to humans[1,21,29] could yield both chymotryptic and V8 fragments with these mobilities (Fig. 5*a*). To determine the effect of phosphorylation on receptor I function, we constructed a mutant form of TβR-I(TβR-I(TS185-204VA)) in which the threonine and serine residues encompassed by the V8 digestion sites were mutated to valine and alanine, respectively. TβR-I(TS185-204VA) was essentially equal to TβR-I in its ability to be expressed, bind TGF-β with TβR-II and form a complex with this receptor (data not shown). However, analysis of receptor complexes purified from [^{32}P]phosphate-labelled cells showed that TβR-I(TS185-204VA) was unable to undergo ligand-induced phosphorylation (Fig. 5*c*). Together with the chymotryptic and V8 protease maps these results suggest that the GS domain is the site of phosphorylation by TβR-II.

To test the signalling capacity of TβR-I(TS185-204VA), we used the TGF-β resistant R-1B cells which lack functional TGF-β type I receptors. Cotransfection of TβR-I and a luciferase reporter gene under the control of the TGF-β-responsive promoter 3TP[23] restored the responsiveness of these cells to TGF-β (Fig. 5*d*) as previously described[20,26]. In contrast, cotransfection of the reporter with TβR-I(TS185-204VA) failed to restore TGF-β-inducible gene expression (Fig. 5*d*). Similar results were obtained when two other TGF-β responses, elevation of plasminogen activator inhibitor-I and fibronectin expression[26], were analysed (data not shown). To assess the antiproliferative signalling capacity of these receptors, R-1B cells stably expressing either TβR-I or TβR-I(TS185-204VA) were generated. TβR-I restored the full antiproliferative responsiveness of R-1B cells whereas TβR-I(TS185-204VA) did not and cells transfected with this mutant receptor continued to proliferate in the presence of TGF-β (Fig. 5*e*). Thus, phosphorylation of receptor I by receptor II is an essential step in the TGF-β signalling pathway.

Discussion

Receptor-mediated signal transduction often involves ligand-dependent activation of multisubunit receptor complexes[30,31,37]. The intracellular domains of these receptors, activated by the binding of ligand, then control the recruitment and activation of mediators of the intracellular signalling pathways. These pathways often lead to cytosolic kinase cascades. Here we describe the activation mechanism of the TGF-β receptor, a representative of the transmembrance Ser/Thr kinase class of receptors, and provide evidence for a novel mode of signal transduction involving two transmembrane kinases that act in sequence (Fig. 6).

The TGF-β type II receptor binds ligand free in the medium which, by analogy with the tyrosine kinase receptors, raises the possibility that ligand binding activates its kinase domain. However, we show that TβR-II is constitutively autophosphorylated and ligand occupancy causes no appreciable change in this activity. Activin type II receptors also display ligand-independent autophosphorylation activity[38], suggesting that this is a general feature of type II Ser/Thr kinase receptors.

Unlike TGF-β type II receptors, receptor I at the cell surface is unable to bind ligand from the medium. However, receptor I can recognize ligand bound to receptor II and form a very stable ternary complex. The formation of this complex is tightly correlated with signalling[23]. Cells defective in one or the other receptor are refractory to any TGF-β effects[18,20,23,24,26,34,36]. The ability to label receptor I by crosslinking to ^{125}I-TGF-β using an 11-Å bifunctional reagent[39] indicates that receptor I contacts the ligand. Whether receptor I recognizes receptor II-induced conformational alterations in TGF-β or a TGF-β/receptor II protein interface is not yet known. Nevertheless, these data suggest that in the TGF-β receptor and presumably other Ser/Thr kinase receptors, the ligand does not function to activate the kinase domain of the primary receptor (receptor II) but rather functions to recruit type I receptors into a complex with type II receptors.

Recruitment of receptor I into the complex results in receptor I phosphorylation. This phosphorylation occurs on Ser and Thr residues and is not an autophosphorylation but rather a phosphorylation by receptor II. Proteolytic mapping of receptor I suggests that the GS domain is the site of phosphorylation. Receptor complexes in which receptor I is unphosphorylated either because of a mutation in the receptor II kinase domain or mutations in the GS domain, are unable to transmit signals. Thus, phosphorylation of receptor I by receptor II is essential for the propagation of TGF-β signals.

Functional analysis of TβR-I and TβR-II has shown that both kinase domains are essential for signalling all TGF-β responses[20,26]. Because receptor I neither autophosphorylates nor phosphorylates receptor II, but is required for signalling, we conclude that receptor II functions to phosphorylate receptor I which propagates the signal to substrates downstream of the TGF-β receptor complex. Consistent with this model, the nature of the biological response to ligand in both the TGF-β and activin systems is specified primarily by the type I receptor isoform that is engaged in the complex[17,26].

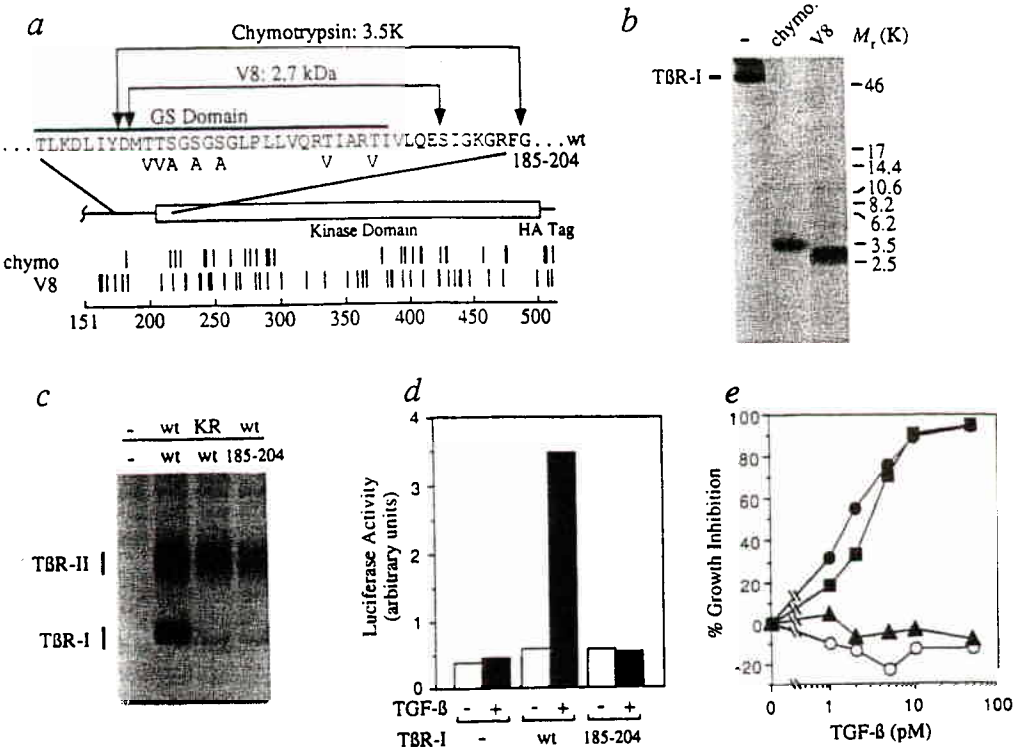

FIG. 5 *a*, The primary sequence of the wild-type (wt) GS domain (overline) and the changes introduced into the mutant TβR-I(TS185-204VA) (185–204) is indicated. The chymotryptic and V8 protease fragments generated from the GS domain (arrow brackets) and all the predicted chymotrypsin and V8 protease sites within the intracellular domain (vertical lines) are shown. The approximate amino-acid residue numbers of TβR-I are indicated on the scale at bottom. *b*, [^{32}P]phosphate-labelled TβR-I was incubated with chymotrypsin (chymo), V8 protease (V8) or no additions (−) and the resultant phosphopeptides analysed on Tris-Tricine gels[43]. *c*, Receptor complexes were purified from [^{32}P]phosphate-labelled R-1B cells transiently transfected with the indicated TβR-II receptor (top row) and either wild-type (wt) or the GS domain mutant, TβR-I/HA(TS185-204VA) (185–204) (bottom row). *d*, *e*, TβR-I(TS185-204VA) does not restore TGF-β responses in receptor I defective cells. R-1B cells transiently transfected with the indicated TβR-I receptor and a TGF-β inducible luciferase reporter construct (3TP-Lux[23,26]) were incubated with (+) or without (−) 250 pM TGF-β and cell lysates assayed in *d* as previously described[23,26]. *e*, The TGF-β antiproliferative responses of Mv1Lu cells (filled circle), R-1B cells (empty circle) and R-1B cells stably expressing either TβR-I (filled square) or TβR-I(TS185-204VA) (filled triangle) were measured as previously described[26] and are plotted in *e* as the percentage decrease in [^{125}I]deoxyuridine incorporation relative to untreated cultures[23].

METHODS. Purified [^{32}P]phosphate-labelled TβR-I/HA was isolated from receptor complexes collected on Ni-NTA-agarose as described in Fig. 4. Phosphorylated TβR-I/HA was digested for 1 h at 37 °C in 50 µl 50 mM ammonium bicarbonate containing 0.1 µg of chymotrypsin 1 µg V8 protease and the products were resolved on 17.5% Tris-Tricine gels[43]. M_r standards are peptide fragments derived from myoglobin (Sigma).

The phosphorylation of the GS domain in type I receptors could function in a number of ways to activate signalling by the kinase domain of type I receptors. Motifs in the intracellular domain of many different classes of receptors mediate signalling by regulating the association of signalling molecules with the activated receptor. By analogy, phosphorylation of the GS domain of receptor I could fulfil a similar function, acting as a docking site to bring substrates to the type I receptor. However, the GS domain of type I receptors that signal different biological responses are highly conserved[1,21]. Thus, an alternative function for this region may be to act as a kinase activation domain analogous to the phosphorylated 'lip' identified in a number of cytosolic kinases[40]. Suitable receptor substrates that could be used to distinguish between these possibilities have not yet been found. Thus, resolution of this issue awaits the identification of downstream targets of the type I receptors.

We have described the molecular events involved in activation of the TGF-β receptor. Whether every aspect of this mechanism will apply to all the Ser/Thr kinase receptors for the TGF-β superfamily awaits further study. However, the results suggest a general model for signal transduction by this class of receptors (Fig. 6), in which the ligand is directly involved in mediating the association of two distinct transmembrane kinases, which, with one phosphorylating the other, generates the first step in a signalling pathway. □

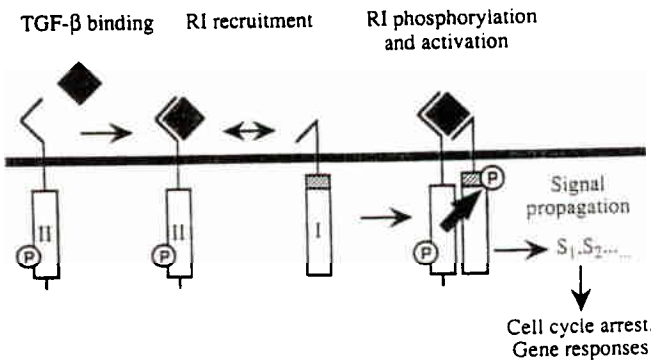

FIG. 6 A general model for the initiation of signalling by the TGF-β receptor. Receptor II is the primary TGF-β receptor and is a constitutively active serine/threonine kinase that recruits receptor I by means of bound TGF-β (diamond). Subsequent phosphorylation of the GS domain (striped box) by receptor II allows the receptor I kinase to propagate the signal to downstream substrates that mediate antiproliferative as well as gene responses.

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LETTERS TO NATURE

Detection of a large mass of dust in a radio galaxy at redshift $z = 3.8$

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ELLIPTICAL galaxies are thought to have formed most of their stars in a rapid burst in the early Universe[1], but an unambiguous example of a 'primaeval' elliptical galaxy (one undergoing its first major burst of star formation) has yet to be discovered. High-redshift radio galaxies are among the most promising candidates[2,3], because their low-redshift counterparts are identified exclusively with ellipticals, but the presence of an active nucleus complicates the analysis of their evolutionary state from optical–infrared observations[3–5]. The failure of optical searches to detect primaeval ellipticals[6–9] suggests that they may be very dusty, prompting us to search for thermal emission from the dust, which will be redshifted to submillimetre wavelengths in our reference frame. Our detection of submillimetre emission from the radio galaxy 4C41.17, reported here, suggests that it contains a large mass of dust, probably located in a dust lane obscuring the centre of the galaxy[10–14]. The observations are consistent with the recent occurrence of a massive burst of star formation, but probably not the first such episode. We conclude that this galaxy was already in the final stages of its formation at a look-back time of 12–15 billion years.

Building on the discovery[15] of far-infrared luminous starburst galaxies by the Infrared Astronomy Satellite (IRAS), we have embarked on a programme (D.H.H., J.S.D. and S.R., manuscript in preparation) to search for rest-frame far-infrared emission from high-redshift ($z > 2$) radio galaxies by observing that emission in the submillimetre range.

We observed 4C41.17, the most distant known galaxy[10] (that is, the most distant known object with a spatially resolved optical continuum potentially dominated by starlight), as part of this programme on 30 September 1993. During exceptionally dry and stable conditions we were able to integrate continuously for three hours with the ^{3}He bolometer UKT14 (ref. 16) on the James Clerk Maxwell Telescope (JCMT) through the 800-μm continuum filter. The observation was made using a beam size of 16.5 arcsec (equivalent to a linear size of 110–230 kpc, for cosmological density parameter $\Omega_0 = 1$–0, and Hubble constant $H_0 = 50$ km s^{-1} Mpc^{-1}), chopping at 7.81 Hz with an east–west throw of 60 arcsec. The result was a flux-density measurement of $S_{800\,\mu m} = 17.4 \pm 3.1$ mJy, the only unambiguous detection in a pilot study of six $z > 2$ radio galaxies. Further observations were made at 450 μm in December 1993 (again in excellent conditions), resulting in a sensitive 3σ limit, $S_{450\,\mu m} < 56$ mJy, after a further three hours of integration.

On the basis of its rather blue ultraviolet–optical spectral energy distribution (SED) and complex multi-component morphology, it has been claimed that 4C41.17 may be a genuine example of a primaeval elliptical galaxy[10]. The importance of our 800-μm detection is that it allows us to make a completely independent assessment of the 'evolutionary state' of 4C41.17.

Figure 1 shows the radio–ultraviolet SED of 4C41.17. Neither an extrapolation of the steep radio-lobe spectrum nor the much flatter spectrum of the recently discovered radio core ($\alpha = -0.23$, where α is the spectral index for the flux density, $f_\nu \propto \nu^\alpha$) can account for the observed 800-μm flux density. Our detection can thus only be explained by thermal emission from dust or by non-thermal emission from an additional synchrotron component. Although our data do not allow us to exclude the second of these possibilities on the basis of the submillimetre spectral index ($\alpha > 2.5$) it is highly implausible that the far-infrared emission of 4C41.17 is due to a synchrotron component. To avoid exceeding the observed flux-density of the radio-core at the observing wavelength $\lambda_{obs} = 2$ cm, this component would need to become self-absorbed at the rest-frame wavelength $\lambda_{rest} < 510$ μm; such compact (<0.015 pc) and energetic synchrotron components have only ever been observed in the flaring 'blazar' class of active galactic nuclei, where they are always accompanied by a series of components with progressively lower turnover frequencies, producing the characteristic flat radio spectrum. It is therefore hard to sustain a synchrotron interpretation of our 800-μm data, as 4C41.17 would then be unique in containing, in isolation, such an energetic and compact emission region.

It is therefore reasonable to assume that the rest-frame far-infrared emission in 4C41.17 is dominated by optically thin thermal emission from dust, particularly as this assumption has been validated for IRAS-detected galaxies (such as F10214+4724; refs 17, 18) and radio-quiet quasars[19,20], which, like 4C41.17, have far-infrared peaks in their rest-frame SED. The addition of a 450-μm limit to the 800-μm detection restricts the rest-frame dust temperature to $\leqslant 43$ K (for $\beta = 2$) or $\leqslant 67$ K (for $\beta = 1$), where β is the grain emissivity index. Adopting a dust temperature $T_{dust} = 50$ K, $\Omega_0 = 1$, $H_0 = 50$ km s^{-1} Mpc^{-1} and an average dust opacity (at a rest-frame wavelength of 167 μm) of $\kappa_d = 3.5$ m^2 kg^{-1} (refs 21, 22), yields a dust mass $M_{dust} \approx 3 \times 10^8 M_\odot$.