Fig. 1 and Scheme 1 give conceivable assignments that could lead to the behaviour seen. As all those who have investigated the spectra are agreed, a group $-AH$ greatly stabilizes the $-PO_3^{2-}$ form; the change in group pK postulated here is from 5.8 to 2.1 when $-A^-$ is converted into $-AH$ (the value of the lower pK is comparatively unimportant). These assignments, with a value of 6.4 for $-AH$ when the phospho group is $-PO_3^{2-}$, would lead to a fifth of the phospho group titrating with a pK of 6.5. The same conclusions can be reached without explicit use of the fact that each group has fractions that possess the different titration pK values of the molecule; thus Gorenstein *et al.* (1976) proposed essentially the same model for the interaction of the phospho group of nucleotides with ribonuclease.

No attempt is made to assign actual values from the published data. The change in chemical shift for model compounds in solution as $-PO_3H^-$ is titrated to $-PO_3^{2-}$ depends greatly on the medium (Korpela *et al.*, 1987a,b), so it is not possible to say what fraction of the phospho group might titrate at the observed pK . Indeed, all assignments of group constants are based on assumptions (Dixon, 1976), although these can have a firm rational basis (e.g. Metzler *et al.*, 1973; Gorenstein *et al.*, 1976).

Fig. 1 is not meant to imply that the actual curves shown could be followed by the enzyme in the lower part of the pH range given; denaturation due to hydronation of many carboxylate groups would follow taking the pH so low. The region 0–5 is shown only to illustrate the meaning of the pK values discussed.

This demonstration that the observed shift may be due to titration of part of the phospho group does not mean that this is the true explanation. It is still perfectly conceivable that a nearly ionization changes the environment of the phosphorus atom in some other way. Even if the shift is indeed due to titration of a small fraction of the phospho group, this alters the previous conclusions rather little. The conclusions that the phospho group is dianionic over the whole pH range 5–8 would be valid for most of the group [as Sanchez-Ruiz *et al.* (1991) stated], just as the conclusion that the ionization of pK 6.5 must be elsewhere would be valid for most of that ionization. The conclusion that the $-PO_3^{2-}$ form of the phospho group is stabilized in the enzyme by a peculiar environment would also remain justified. The ratio between the forms $-A-H \cdots O-PO_2^-$ and $-A^- \cdots H-O-PO_2^-$ may merely reflect the relative sojourn times of a rapidly mobile hydron.

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A common pattern between the TGF- β family and glutaredoxin

Transforming growth factors- β (TGF- β 's) constitute a superfamily of polypeptide factors involved in the control of cell growth and differentiation. The superfamily includes a variety of short proteins whose functions, although only partially characterized, are diverse and even antagonistic (Massagué, 1987; Sporn & Roberts, 1990). Three regions of sequence conservation can be identified in the C-terminal domain of the mature TGF- β polypeptides (Wozney *et al.*, 1988; see Fig. 1a). The first region is statistically the most significant. We have found that, with the exception of a large insertion, this region is very similar to a region in animal glutaredoxins (Fig. 1a). We have derived an amino acid class covering pattern (Smith & Smith, 1990) from the sequence of animal glutaredoxins and TGF- β 's in this region (see Fig. 1b) and found its information content equal to 9.14. That is, the similarity implied by this pattern is statistically equivalent to sharing more than nine identical amino acids. Such a degree of similarity between animal glutaredoxin and TGF- β 's is significant, since the overall degree of conservation within TGF- β 's in this region is statistically equivalent to sharing no more than 13 amino acids. In other words, of 13 conserved amino acid equivalences among TGF- β 's in this region, nine of them are also conserved in glutaredoxins. We have observed that a pattern's information content correlates with its functional specificity (Guigó *et al.*, 1991). In general, patterns with information content greater than 8 approach 100% specificity, while those with information content below 6 show little functional specificity. In fact, the pattern is not present in any protein in the SWISS-PROT database, release 13 (EMBL, 1990), other than TGF- β 's and animal glutaredoxins.

Glutaredoxins (or thioltransferases) have been proposed to exert a general thiol redox control on proteins by acting as effective thiol–disulphide oxidoreductases (Holmgren, 1985; Ziegler, 1985), a function they share with thioredoxins. Interestingly, the aforementioned pattern contains the animal glutaredoxin second dithiol site, which is missing from *Escherichia coli* and yeast glutaredoxin. It is still unknown whether this second dithiol site is essential for enzyme activity. However, a similar arrangement of thiols has been observed in thioredoxins, where microbial thioredoxins have only one dithiol, whereas animal thioredoxins contain an additional one (Johnson *et al.*, 1988). The fact that this configuration is not present in bacterial thioredoxin and glutaredoxin, but has been retained in both proteins of animal origin, suggests that the second dithiol may have an important function (Hopper *et al.*, 1989).

Dithiol active groups seem to play a role in hormone–receptor interactions (Robillard & Konings, 1982; Grippo *et al.*, 1985). Glutaredoxin, for example, has been shown to enhance the binding of L-tri-iodothyronine (T3) to its receptor by preserving the T3 receptor in a reduced state (Takagi *et al.*, 1989). Thiol-reducing activity has been recently discovered in the gonadotropin hormones themselves. In this case, a dithiol site in gonadotropins would be responsible for disulphide cross-linking, leading to the formation of an irreversible hormone–receptor complex (Boniface & Reichert, 1990). These observations have suggested the possible existence of an structural motif common to the dithiol sites of thioredoxin, glutaredoxin, disulphide isomerases and gonadotropins (L. Ellis, personal communication).

The mechanism of activation of the TGF- β receptor is still unknown and, as has been pointed out in a recent review (Massagué *et al.*, 1990), attempts to determine whether TGF- β is acting through any of the known signalling pathways (those involving tyrosine protein kinases, G proteins, ion channels or phosphatidylinositol phospholipases/C kinases) have generally

(a)

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BMPASHUMAN  --SSCKRHP--LYVDF--SDVGWMDIWA-----PPGYHAEFYCHGECPP--PLADHLNSTNHAIVQTLVNSVN--S-KIPKACC--VPTLSAISMLYLDENEKVVVK--NYQDMVVEGCGCR
BMPBSHUMAN  --KNCRRH--LYVDF--SDVGWMDIWA-----PPGYOAFYCHGDCPP--PLADHLNSTNHAIVQTLVNSVN--S-SIPKACC--VPTLSAISMLYLDYDKVVK--NYQDMVVEGCGCR
DECA5DROME  HDDTCRRH--LYVDF--SDVGWMDIWA-----PLGYDAYFYCHGKCP--PLADHLNSTNHAIVQTLVNMNP--G-KVPKACC--VPTQLDSVAMLYLDQSTVVK--NYQDMVVEGCGCR
BMP3SHUMAN  EPRNCARRY--LKVDF--ADIGMSEWIIIS-----PKSEFADYICSGACQF--PMFKSLKPSNHATIQSIVRAVGVP--GIPEPC--VPEKMSLSILFFDENKNVVK--VYPNMVTEVCACR
VGLSKENLA   ASNICKRH--LYVEF--KDVGWMDIWA-----PPGYMANYCYGECPP--PLTEILNGSNHAILQTLVHSIE--PE-DIPLPCC--VPTKMSPLSMFLYDNNNDVVK--HYENMAVDEGCGCR
IHASBOVIN   AHADCHRA--LNISF--QELGMDRWIVH-----PPSQDIFLPVPGVPPTVQPLS--LV-PGAQPCCAALPGTMRPLHVRTTSDGGYSFKYEMVNP--LLTQHCACI
IHASPIG     VHADCHRA--LNISF--QELGMDRWIVH-----PPSFIFHYCHGGCGL--PTLPLNLSLVPVGPAPTPVQPL--LV-PGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVNP--LLTQHCACI
IHASHUMAN   AHANCHRA--LNISF--QELGMDRWIVH-----PPSFIFHYCHGGCGL--HIPPLNLSLVPVGPAPTPVQPL--LV-PGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVNP--LLTQHCACI
MISSBOVIN   ADGPCALRE--LSVDLRAERS-----VLI-----PETYOANNCQGCQWP--QSDRNPRYGNHVVLLKMOARG--AT-LARPPCC--VPTAYAGKLLISLSEERISAH--VPMNVATEGCGCR
MISSHUMAN   ADGPCALRE--LSVDLRAERS-----VLI-----PETYOANNCQGCQWP--QSDRNPRYGNHVVLLKMOARG--AA-LARPPCC--VPTAYAGKLLISLSEERISAH--VPMNVATEGCGCR
TGF1SCERAE  TEKNCVVRQ--LYIDFRKDLGWK--WIHE-----PKGYHANFCLGCPY-----IWSLDTQYSKVLALYNQHNPGA--SAAPCC--VPOALEPLIIVYVGRKPKVEQ--LSNMIVRSCKCS
TGF1SHUMAN  TEKNCVVRQ--LYIDFRKDLGWK--WIHE-----PKGYHANFCLGCPY-----IWSLDTQYSKVLALYNQHNPGA--SAAPCC--VPOALEPLIIVYVGRKPKVEQ--LSNMIVRSCKCS
TGF1SCHICK  TEKNCVVRQ--LYIDFRKDLGWK--WIHE-----PKGYHANFCLGCPY-----IWSLDTQYSKVLALYNQHNPGA--SAAPCC--VPOALEPLIIVYVGRKPKVEQ--LSNMIVRSCKCS
TGF1SPIG    TEKNCVVRQ--LYIDFRKDLGWK--WIHE-----PKGYHANFCLGCPY-----IWSLDTQYSKVLALYNQHNPGA--SAAPCC--VPOALEPLIIVYVGRKPKVEQ--LSNMIVRSCKCS
TGF1SMOUSE  TEKNCVVRQ--LYIDFRKDLGWK--WIHE-----PKGYHANFCLGCPY-----IWSLDTQYSKVLALYNQHNPGA--SAAPCC--VPOALEPLIIVYVGRKPKVEQ--LSNMIVRSCKCS
TGF2SHUMAN  VQDNCCLRP--LYIDFRKDLGWK--WIHE-----PKGYANFPCGACQY-----LWSSDTQHSRVLSTYNTINPEA--SASPCC--VSDQLEPLITILYIGKTPKIEQ--LSNMIVRSCKCS
TGF3SHUMAN  LEENCCVRP--LYIDFRKDLGWK--WIHE-----PKGYANFPCGACQY-----LWSSDTQHSRVLSTYNTINPEA--SASPCC--VSDQLEPLITILYIGKTPKIEQ--LSNMIVRSCKCS
TGF4SCHICK  DEKNCVVRP--LYIDFRKDLGWK--WIHE-----PKGYANFPCGACQY-----LWSSDTQHSRVLSTYNTINPEA--SASPCC--VSDQLEPLITILYIGKTPKIEQ--LSNMIVRSCKCS
IHBSHUMAN   KVNICKKK--FFVSF--KDIGNMDIWA-----PSGYHANYCEGECPSH--IAGTSGSSLSFHSVTINHYMRGHSPPFANLKS--VPTKLRPMSMLYDDGQNIKK--DIQNMIVEEGCGS
IHBSPIG     KVNICKKK--FFVSF--KDIGNMDIWA-----PSGYHANYCEGECPSH--IAGTSGSSLSFHSVTINHYMRGHSPPFANLKS--VPTKLRPMSMLYDDGQNIKK--DIQNMIVEEGCGS
IHBSBOVIN   RTNLCCRQ--FFIDF--RLIGNMDIWA-----PTGYGNYCEGSCPAYLAGVPGSSAFHTAVNQYRMGLNP--GTVNSCC--IPTKLSTMSMLYDDGQNIKK--DIQNMIVEEGCGS
IHBSBOVIN   RTNLCCRQ--FFIDF--RLIGNMDIWA-----PTGYGNYCEGSCPAYLAGVPGSSAFHTAVNQYRMGLNP--GTVNSCC--IPTKLSTMSMLYDDGQNIKK--DIQNMIVEEGCGS
IHBSBOVIN   KVNICKKK--FFVSF--KDIGNMDIWA-----PSGYHANYCEGECPSH--IAGTSGSSLSFHSVTINHYMRGHSPPFANLKS--VPTKLRPMSMLYDDGQNIKK--DIQNMIVEEGCGS
IHBSBOVIN   KVNICKKK--FFVSF--KDIGNMDIWA-----PSGYHANYCEGECPSH--IAGTSGSSLSFHSVTINHYMRGHSPPFANLKS--VPTKLRPMSMLYDDGQNIKK--DIQNMIVEEGCGS
TGF-β pattern  C-----L-IDL--D-GW--WI--          P--Y-A--C-G-C-----CC--VP-----M-----C-C
                  F VEF E V          F F          I          L
                  S L          G          L
GLRXSPIG     TFPFRKTKOELLSQLPFKQGLLEFVDITATSDTNEIQDYLOQLTGARTVPRVFIGKICGGGIDLESMDHKGRELLTRIQGIGALK
GLRXBOVIN    TFPFRKTKOELLSQLPFKQGLLEFVDITAGNISEIQDYLOQLTGARTVPRVFIGKICGGGIDLVNMMHGERGELLTRIKQMGALQ
GLRXSRABIT   TFPFRKTKOELLSQLPFKQGLLEFVDITATSDMSEIQDYLOQLTGARTVPRVFIGKICGGGIDLVNMMHGERGELLTRIKQMGALQ
                  C L QL K GL FV          P F G C G C
                  I          * * * * *
                  *          * * * * *

```

(b)

C....[ILF]...[LF]...G[WL]..[WF][IV]..(1,23)P...[YF].[AFG]..C.G.C

Fig. 1. Common pattern between the TGF- β family and glutaredoxins

(a) Alignment of the C-terminal domain of the TGF- β family members (top) with animal glutaredoxins (bottom, residue 21, the first shown in the figure). Residues at the bottom of the figure are the residues in the TGF- β consensus pattern for the first similar region in that domain, also conserved in glutaredoxins. Except for the big gap, almost all residues in the TGF- β consensus pattern appear in glutaredoxins. Residues with ** are residues absolutely conserved among TGF- β proteins and glutaredoxins. Residues with * are residues not more conserved within TGF- β proteins than between TGF- β proteins and glutaredoxins. Note that all absolute conserved residues among TGF- β 's in that region are also present in glutaredoxins. Boxes indicate the dithiol active sites in the glutaredoxin sequences. On the left, locus names from SWISS-PROT database, release 13, for the aligned sequences. (b) Common pattern to TGF- β 's are animal glutaredoxins expressed as a regular expression. Here a period indicates any amino acid, the capital letters in square brackets indicate conserved alternative amino acids, and the curly bracket the range of a variable spacer or gap.

met with negative results. In that respect, the similarity noted here between TGF- β superfamily polypeptides and glutaredoxins, particularly the presence of a potential dithiol active site in TGF- β , suggest a mechanism of action for ligand-induced receptor activation similar to the one proposed for gonadotropin hormones, in which dithiol-disulphide interchange reactions would be involved in the activation of the receptor. Although unexpected, such a relationship between the autocrine system and the glutaredoxin and thioredoxin systems has a precedent: the recent discovery that thioredoxin can act as an autocrine growth factor that would modulate interleukin 2 receptor induction through dithiol reduction (Tagaya *et al.*, 1989; Wakasugi *et al.*, 1990).

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