# Interaction of transcription factor Sp1 with the promoter of the gene for the multifunctional protein disulphide isomerase polypeptide

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Protein disulphide isomerase (PDI) is a unique polypeptide which resides in the lumen of the endoplasmic reticulum and also functions as the β-subunit of prolyl 4-hydroxylase, as a cellular thyroid hormone-binding protein, as the smaller subunit of the microsomal triacylglycerol transfer protein complex, as a dehydroascorbate reductase and as a protein that binds various peptides in a specific manner. We have recently demonstrated that the promoter of the PDI gene contains six CCAAT boxes and other elements which are needed for efficient transcription. We now demonstrate that purified human recombinant transcription factor Sp1 interacts with two perfect GGGCGG sequences and three other GC-rich elements of the PDI promoter. Sp1 also appears to participate in the regulation of PDI gene expression, since overexpression of Sp1 stimulated PDI promoter activity in HeLa cells and mutations introduced into each of these Sp1-binding sites separately reduced the promoter strength, although even the largest decrease was only about 50%. These results support our view that expression of the gene for this polypeptide with multiple functions is secured by several regulatory elements, some of which are functionally redundant.

## INTRODUCTION

Protein disulphide isomerase (PDI; EC 5.3.4.1) is an abundant protein of the lumen of the endoplasmic reticulum, with several distinct functions. As a homodimer, it catalyses thiol:disulphide interchange *in vitro* and is thought to be required for the correct folding and assembly of secretory proteins *in vivo* (Edman et al., 1985; see Anfinsen and Sheraga 1975; Freedman 1989; Freedman et al., 1989), but it also acts as the β-subunit of prolyl 4-hydroxylase, an $\alpha_2\beta_2$ enzyme tetramer catalysing the formation of the 4-hydroxyproline residues required for the stability of collagens and other proteins with collagen-like structures (Koivu et al., 1987; Pihlajaniemi et al., 1987; see Kivirikko et al., 1989, 1990, 1992). In addition, it has been shown to function as a cellular thyroid hormone-binding protein (Cheng et al., 1987; Yamauchi et al., 1987), as the smaller polypeptide of the microsomal triacylglycerol transfer protein dimer (Wetterau et al., 1990, 1991), as a dehydroascorbate reductase (Wells et al., 1990) and as an endoplasmic reticulum luminal polypeptide binding various peptides in a unique manner (Geetha-Habib et al., 1988; Noiva et al., 1991).

Although PDI is an abundant protein, the correlation between its activity in cells and the synthesis of disulphide-bonded proteins suggests that its expression is regulated in many situations (Roth and Koshland 1981; Myllylä et al., 1983; Edman et al., 1985; Helaakoski et al., 1990). The primary point of control of gene expression is the initiation of transcription, which is in general regulated by sequence-specific transcription factors which bind to *cis*-elements of promoters and enhancers. The promoter region of the human PDI gene contains several putative regulatory elements such as a TATA box, six CCAAT boxes and several GC-rich regions, which include GC boxes (GGGCGG sequences) (Tasanen et al., 1988). The PDI promoter thus resembles those of constitutively expressed housekeeping genes in containing several CCAAT boxes and GC-rich regions, but, in contrast to these genes, transcription of the PDI gene is initiated at a single location defined by the TATA box (Tasanen et al., 1988). A recent DNAase I footprinting analysis of the human PDI promoter revealed that the first 630 nt of the region upstream of the transcription initiation site contain eleven elements which are recognized by DNA-binding proteins present in HeLa cell and human fibrosarcoma HT-1080 cell nuclear extracts; both of these cell lines possess abundant quantities of PDI (Tasanen et al., 1992). These protein-binding regions include all six CCAAT elements, a GC box, an element resembling the AP-1-binding site, an element identical to one of the E-box consensus sequences of mammalian type C retroviruses acting as binding sites for SEF-2 proteins (Corneliussen et al., 1991), and two elements which did not display sequence similarity to any hitherto characterized transcriptional control elements. Elucidation of the transcriptional significance of these elements by point mutation analysis suggested that all six CCAAT boxes are required for efficient expression of the PDI gene, but that some of them are functionally redundant (Tasanen et al., 1992).

Transcription factor Sp1 binds to the GC box and related GC-rich regions found in a variety of cellular and viral promoters, especially in the promoters of constitutively expressed housekeeping genes (see Kadonaga et al., 1986, 1987, 1988; Courey and Tjian 1988; Courey et al., 1989; Saffer et al., 1990). Sp1-responsive genes usually contain multiple GC-box recognition sites, but a single binding site appears to be sufficient for a promoter to be stimulated by Sp1. Although Sp1 has been reported to exist in all mammalian cells, its levels were recently found to vary in some cell types during different stages of development, which suggests that it may be required not only for the transcription of constitutively expressed genes but also for the regulation of development and differentiation (Saffer et al., 1991). In genes containing multiple GC boxes, Sp1 bound to a distal enhancer has been shown to activate transcription synergistically with Sp1 bound to sites located in the vicinity of the

Abbreviations used: PDI, protein disulphide isomerase; GC box, GGGCGG or related DNA sequence; hGH, human growth hormone; CAT, chloramphenicol acetyltransferase; SV40, Simian virus 40; DMEM, Dulbecco's modified Eagle's medium.

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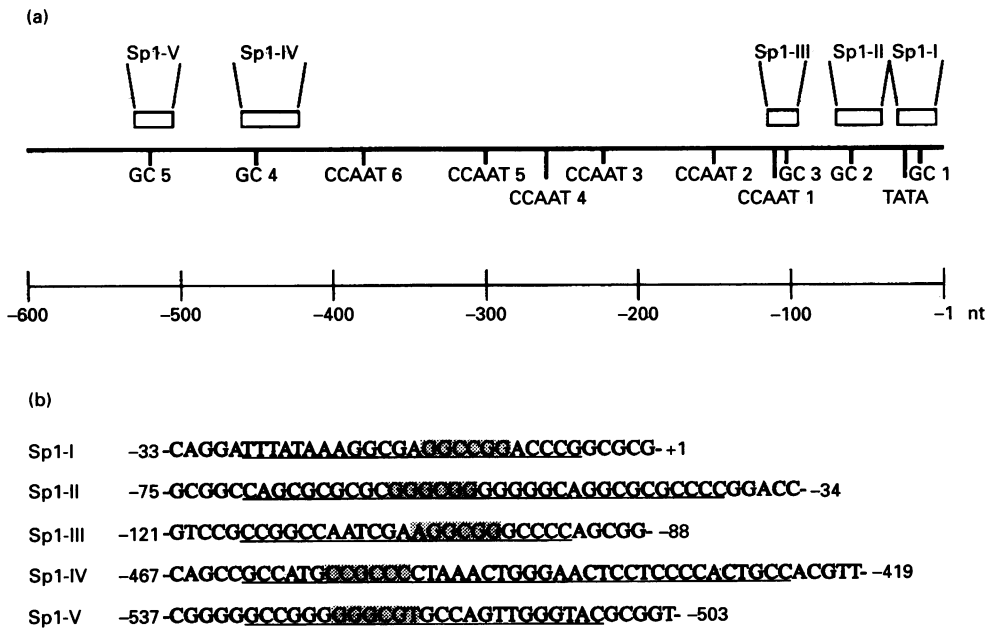


**Figure 1 Transcription factor Sp1-binding sites in the human PDI gene promoter**

(**a**) Schematic representation of transcription factor Sp1 interactions with the human PDI promoter. The five Sp1-binding sites are indicated by open boxes and numbered with Roman numerals beginning from the transcription initiation site. The TATA box, six CCAAT boxes (numbered 1–6, beginning with the one closest to the transcription initiation site) and five GC boxes are also shown. (**b**) Sequences of transcription factor Sp1-binding sites in the human PDI promoter. Five regions protected by DNAase I digestion are underlined and the footprinted regions are named Sp-I to Sp-V, beginning from the transcription initiation site. GC boxes are shaded and numbered relative to the transcription initiation site. Numbers at the ends of the lines show nucleotide positions.

transcription initiation site (Pascal and Tjian, 1991; Mastrangelo et al., 1991; Su et al., 1991), but the exact activation mechanism is not understood. DNAase I footprinting analysis with crude nuclear extracts may be insufficient to demonstrate binding of Sp1 to DNA, and therefore the interaction of Sp1 with several GC-rich stretches present in the promoter region of the human PDI gene was studied here by means of DNAase I footprinting assays using purified human recombinant Sp1. The data obtained in these experiments, and in subsequent functional analyses on the effects of point mutations introduced into the protected elements and Sp1 dose–effect co-transfection experiments, indicated that Sp1 may interact with five GC boxes of the promoter, all of which seem to be important for the expression of the human PDI gene.

## EXPERIMENTAL

### Plasmid constructs

A 952 nt *Aval*/*Aval* fragment covering nucleotides −827 to +125 had previously been isolated from the genomic clone SGB-22, which contains a 3.2 kb region of the human PDI gene including 1.8 kb of 5′ flanking sequences (Tasanen et al., 1992). For subcloning purposes, the 5′ protruding ends of the *Aval*/*Aval* fragment were filled with the Klenow enzyme, the ends digested with *Xba*I, and a *Bam*HI-compatible end generated at nucleotide +52 by digestion with *Sau*3A. This PDI promoter fragment was cloned between the *Xba*I/*Bam*HI sites of plasmid pφGH containing the human growth hormone (hGH) gene (Selden et al., 1986) to create a wild-type promoter construct termed PDI(−827/+52)hGH (Tasanen et al., 1992). To generate a single-stranded template for mutagenesis, the PDI(−827/+52) fragment was also cloned between the *Xba*I/*Bam*HI sites of the M13mp19 vector. Site-directed mutagenesis was performed using an oligonucleotide-directed *in vitro* mutagenesis system (Amersham). Oligonucleotide primers of 24 nt containing the desired point mutations were synthesized in an Applied Biosystems DNA synthesizer at the Department of Biochemistry, University of Oulu. The resulting mutant promoter fragments were cloned into plasmid pφGH as above. The DNA sequences of all the mutant clones were verified by dideoxy sequencing using the Sequenase enzyme (United States Biochemical).

### DNAase I footprinting

The probe for the DNAase I footprinting experiments was prepared by labelling the sense or antisense strand of the human PDI promoter fragment PDI(−563/+67), covering nucleotides −563 to +67 (Tasanen et al., 1992), using polynucleotide kinase and [$\gamma$-$^{32}$P]ATP (> 3000 Ci/mmol). The footprinting experiments were performed by means of an Sp1 footprinting system, using the reaction conditions recommended by the supplier (Promega), with the amount of Sp1 being either 1 or 5 footprinting units per reaction. This system makes use of purified human Sp1 produced in HeLa cells by a recombinant vaccinia virus. The digestion products were examined by electrophoresis on a 5% sequencing gel and the exact locations of the protected regions were determined by comparison with adjacent Maxam and Gilbert sequencing reactions of the probe.

### Transfections and gene expression assays

HeLa cells (CCL 2; American Type Culture Collection) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum. Cells for the transfection experiments were seeded at a density of $1 \times 10^5$/100 mm dish and maintained in DMEM containing 10% foetal calf serum. The

media were changed the next day and the cells were transfected 2–4 h later by the calcium phosphate precipitation method (Ausubel et al., 1987). All transfections were repeated at least five times using two or more independent plasmid preparations, and plasmid pSV2CAT, containing the simian virus 40 (SV40) early promoter driving the bacterial chloramphenicol acetyltransferase (CAT) gene (Gorman et al., 1982), was co-transfected in all experiments to provide an internal control for DNA uptake. In dose–effect Sp1 co-transfection experiments, HeLa cells were transfected with a wild-type promoter construct PDI(−827/+52)hGH, a mutated PDI construct containing a GC box 2 mutation (see Table 1), pSV2CAT and various amounts of a sense Sp1 expression vector, pSVSp1-F (Saffer et al., 1991), and the amount of DNA was adjusted using the frameshift expression vector pSVSp1-FX (Saffer et al., 1991). To measure the promoter activities of the various constructs, hGH levels in the media were determined 72 h after transfection with a two-site immunoradiometric assay (Pharmacia), and CAT activities from the cell lysates were measured as described (Ausubel et al., 1987).

## RESULTS

### Transcription factor Sp1-binding sites on the PDI promoter

The promoter region of the human PDI gene has several GC-rich regions (Tasanen et al., 1988, 1992), and 86 out of 103 nt (83%) between nucleotides −1 and −103 are GC, including one perfect GGGCGG sequence at −56 and numerous closely related elements. Another GC-rich region is located between nucleotides −518 and −544, where 22 out of 24 nt are GC, but this region has no perfect GGGCGG sequence. The other perfect GGGCGG sequence in the PDI promoter is located at −451, this element having previously been found to be recognized by nuclear proteins present in HeLa cell and HT-1080 cell extracts (Tasanen et al., 1992). The possible Sp1-binding sites among the GC-rich regions of the PDI promoter were analysed by DNAase I footprinting using two different concentrations (see the Experimental section) of purified human recombinant Sp1 and 5′-end-labelled sense and anti-sense chains of a fragment spanning the promoter from −563 to +67. The exact borders of the protected regions were determined by comparing them with the Maxam and Gilbert sequencing reactions of the untreated probe (results not shown).

Transcription factor Sp1 was found to become bound to five distinct regions in the PDI promoter, as detected by DNAase I footprinting, and these regions were termed Sp1-I, Sp1-II, Sp1-III, Sp1-IV and Sp1-V (Figures 1 and 2). Footprint segments Sp1-II and Sp1-IV both contain a GGGCGG sequence at positions −56 and −451, termed GC boxes 2 and 4 respectively, although the perfect GC box in segments Sp1-IV is found in the anti-sense strand. The other Sp1-binding regions contain sequences closely related to the GGGCGG element. Sp1-I, located downstream of the TATA box, between −5 and −28, contains a GGCCGG sequence (GC box 1), whereas the footprinted region Sp1-III contains an AGGCGG sequence (GC box 3). The most distal Sp1-binding site is located over 500 nt upstream of the transcription initiation site and contains GC box 5, which comprises a GGGCGT element. The above results were obtained with the higher amount of SP1 (5 footprinting units), while only footprint segment Sp1-IV was protected with the lower amount of SP1 (1 footprinting unit) (results not shown).

### Point mutation analysis of the five transcription factor Sp1-binding sites

The transcriptional properties of the five transcription factor Sp1-binding sites were determined by creating mutations in GC boxes 1–5 found in them by *in vitro* mutagenesis using the promoter fragment PDI(−827/+52) as a template. GC boxes 1, 2 and 5 were mutated to GTATCG, GC box 3 to ATATCG and GC box 4 to CTATCC (Table 1). The wild-type promoter fragment and the five mutated promoter fragments were cloned into the hGH reporter vector pϕGH (Selden et al., 1986) and introduced into HeLa cells by transient transfection. The transcriptional efficiency of each plasmid construct was determined by measuring the amount of hGH secreted into the culture medium, and the hGH values obtained were normalized with respect to the CAT activity of the co-transfected pSV2CAT (Gorman et al., 1982).

All five Sp1-binding sites were found to contribute to the transcriptional efficiency of the human PDI promoter, as mutation of each GC box separately decreased marker gene expression (Table 1). Mutation of one of the two perfect GGGCGG sequences, i.e. GC box 4, at nucleotide −451 produced the largest decrease in PDI promoter activity (50%). The effects of mutation of GC box 1, which is closest to the transcription initiation site, and of the most distal Sp1-binding site, GC box 5,

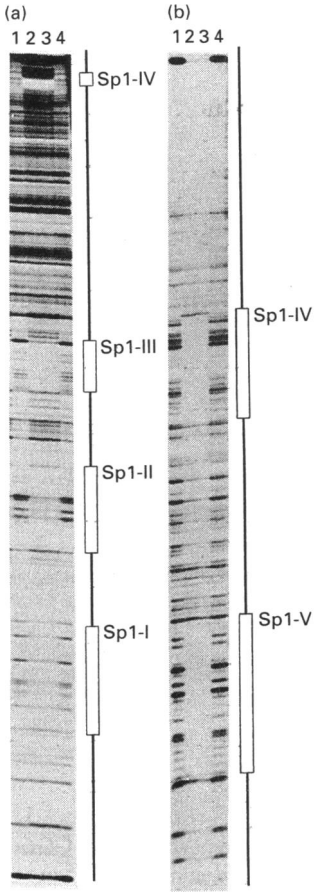


**Figure 2 DNAase I footprinting experiments with the human PDI gene promoter using recombinant transcription factor Sp1**

The five binding sites of transcription factor Sp1 are indicated by open boxes and are numbered Sp1-I to Sp1-V, beginning from the transcription initiation site. (**a**) A promoter fragment, PDI(−563/+67), with the anti-sense strand labelled was incubated with purified recombinant transcription factor Sp1 and treated with DNAase I. Lanes 1 and 4, DNAase I digestion pattern of the naked probe incubated without Sp1; lanes 2 and 3, pattern with Sp1. (**b**) The DNAase I digestion pattern of the same promoter fragment with the sense strand labelled shows the protected elements Sp1-IV and Sp1-V. Lanes 1 and 4, DNAase I digestion of naked DNA; lanes 2 and 3, digestion of DNA incubated with Sp1.

**Table 1 Effects of mutations of five GC boxes in the human PDI gene promoter on hGH expression in transiently transfected HeLa cells**

The values are presented as percentages of the activity obtained with the wild-type construct and are means ± S.E.M. for the number of analyses performed (*n*). The wild-type construct is the plasmid PDI(−827/+52) hGH, which consists of nucleotides −827 to +52 of the human PDI gene promoter sequences driving the hGH gene.

| Construct | Relative hGH expression (%) | *n* |
|---|---|---|
| Wild-type | 100 | |
| GC box 1 (GGCCGG) mutated to GTATCG | 62±10 | 12 |
| GC box 2 (GGGCGG) mutated to GTATCG | 68±11 | 9 |
| GC box 3 (AGGCGG) mutated to ATATCG | 78±4 | 7 |
| GC box 4 (CCGCCC) mutated to CTATCC | 55±11 | 7 |
| GC box 5 (GGGCGT) mutated to GTATCG | 61±15 | 5 |

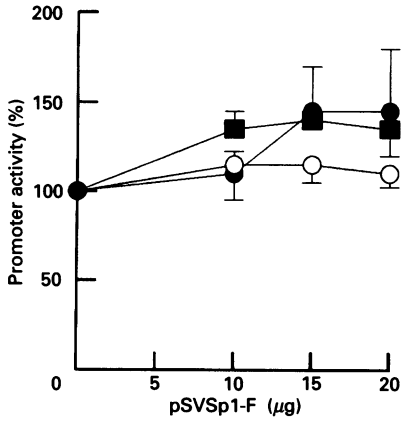


**Figure 3 Effects of Sp1 overexpression of PDI promoter and SV40 early promoter activities in HeLa cells**

A 15 μg portion of the wild-type PDI(−827/+52) hGH plasmid (●) or 15 μg of the plasmid containing the GC box 2 mutation (○) and 0.5 μg of the plasmid pSV2CAT (■) was co-transfected with increasing amounts of plasmid pSVSp1-F to HeLa cells. The total amount of DNA transfected was adjusted to 35.5 μg per dish using plasmid pSVSp1-FX. The amounts of hGH secreted and CAT activity were determined. The bars indicate S.E.M.

were almost as large, with the decrease being about 40%. Mutation of the other perfect GGGCGG element, GC box 2, had a lesser effect, reducing hGH expression by about 30%, while mutation of GC box 3 gave the smallest decrease, of about 20%.

### Effect of transcription factor Sp1 overexpression on PDI promoter activity

To study the regulation of the PDI promoter by transcription factor Sp1, an Sp1 expression vector, pSVSp1-F (Saffer et al., 1991), was co-transfected with the PDI promoter construct PDI(−827/+52)hGH. In order to show that the stimulation of the PDI promoter by Sp1 is specific, a PDI promoter construct containing the GC box 2 mutation (see Table 1) was also co-transfected with pSVSp1-F. The effect of increasing doses of pSVSp1-F was compared with a similar expression vector containing frame-shifted non-functional Sp1 cDNA. To include an internal control for the effect of Sp1 overexpression, plasmid pSV2CAT was also included in the co-transfection experiments. pSV2CAT contains the SV40 early promoter with six GC boxes.

Overexpression of Sp1 led to a dose-dependent increase in PDI promoter activity (Figure 3), indicating the functional significance of the GC boxes for transactivation by transcription factor Sp1. With increasing doses of the Sp1 expression vector, transactivation of the PDI promoter paralleled the effect on SV40 promoter activity (Figure 3). Further proof for regulation of the PDI promoter by Sp1 was obtained in an experiment showing that the construct with the mutated GC box 2 showed only a small response to co-transfection with the Sp1 expression vector (Figure 3).

## DISCUSSION

PDI is an abundant protein in the lumen of the endoplasmic reticulum in numerous species (Freedman, 1989; Freedman et al., 1989; Kivirikko et al., 1989, 1990, 1992) and is essential for cell viability in yeast (Farquhar et al., 1991; LaMantia et al., 1991; Scherens et al., 1991; Tachikawa et al., 1991). Our previous results indicated that efficient expression of the human PDI gene is ensured by six CCAAT boxes and other promoter elements, some of which are functionally redundant (Tasanen et al., 1992). The promoter region of this gene also contains several GC-rich stretches (Tasanen et al., 1988), but only one GC box was previously found to be protected in DNAase I footprinting experiments with nuclear proteins present in the crude HeLa cell and HT-1080 cell extracts (Tasanen et al., 1992). The present findings nevertheless suggest that purified human recombinant Sp1 binds five distinct promoter segments, including a region around −450 (the present Sp1-IV) previously detected with the crude nuclear extracts (Tasanen et al., 1992). This element contains the perfect consensus sequence for Sp1 binding and was found to be most efficiently protected in DNAase I footprinting experiments with recombinant Sp1. The inability to find the other four protected segments in experiments with crude nuclear extracts may be in part due to their lower binding affinity, or to general difficulties in identifying Sp1-binding sites with crude nuclear extracts. Competition between several transcription factors for adjacent promoter elements may explain the inability to find Sp1-III and -V in the previous experiment (Tasanen et al., 1992), as the GC box in Sp1-III is located very close to the first CCAAT box (Figures 1a and 1b) and Sp1-V overlaps with a previously identified protected segment (segment IX in Tasanen et al., 1992).

Co-transfection experiments with the Sp1 expression vector indicated that Sp1 is involved in the regulation of PDI promoter activity in HeLa cells. Point mutation analysis of the five Sp1-binding segments indicated that the largest decrease in PDI promoter activity (50%) was caused by mutation of Sp1-IV, which contains a GGGCGG sequence (in the anti-sense strand) and was the only Sp1-binding site identified in DNAase I footprinting experiments with crude nuclear extracts (Tasanen et al., 1992). All five Sp1-binding elements appeared to contribute to promoter strength, however. Efficient expression of the human PDI gene is thus secured not only by the six CCAAT boxes and other promoter elements which have not been characterized in detail (Tasanen et al., 1992), but also by the five Sp1-binding

sites, many of these promoter elements being functionally redundant. The regulation of the abundant expression of this gene in most situations (Freedman, 1989; Freedman et al., 1989; Kivirikko et al., 1989, 1990, 1992) and its low level of expression in some situations (e.g. Roth and Koshland, 1982; Myllylä et al., 1983; Helaakoski et al., 1990) therefore appear to involve highly complex mechanisms.

We are grateful to Mrs. Maija Seppänen for her expert technical assistance. Plasmids pSVSp1-F and pSVSp1-FX were a gift from Dr. Jeffrey Saffer, The Jackson Laboratory, Bar Harbor, ME, U.S.A. The work was supported by grants from the Medical Research Council of the Academy of Finland, the Emil Aaltonen Foundation and the Research and Science Foundation of Farmos.

## REFERENCES

Anfinsen, C. B. and Sheraga, H. A. (1975) Adv. Protein Chem. **29**, 205–300
Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman, J. G. and Struhl, K. (1987) Current Protocols in Molecular Biology, J. Wiley and Sons, New York, NY
Cheng, S.-Y., Gong, Q.-H., Parkison, C., Robinson, E. A., Apella, E., Merlino, G. T. and Pastan, I. (1987) J. Biol. Chem. **262**, 11221–11227
Corneliussen, B., Thornell, A., Hallberg, B. and Grundström, T. (1991) J. Virol. **65**, 6084–6093
Courey, A. J. and Tjian, R. (1988) Cell **55**, 887–898
Courey, A. J., Holtzman, D. A., Jackson, S. P. and Tjian, R. (1989) Cell **59**, 827–836
Edman, J. C., Ellis, L., Blacher, R. W., Roth, R. A. and Rutter, W. J. (1985) Nature (London) **317**, 267–270
Farquhar, R., Honey, N., Murrant, S. J., Bossier, P., Schultz, L., Montgomery, D., Ellis, R. W., Freedman, R. B. and Tuite, M. F. (1991) Gene **108**, 81–89
Freedman, R. B. (1989) Cell **57**, 1069–1072
Freedman, R. B., Bulleid, N. J., Hawkins, H. C. and Paver, J. L. (1989) Biochem. Soc. Symp. **55**, 167–192
Geetha-Habib, M., Noiva, R., Kaplan, H. A. and Lennarz, W. J. (1988) Cell **54**, 1053–1060
Gorman, C. M., Moffat, L. F. and Howard, B. H. (1982) Mol. Cell. Biol. **2**, 1044–1051
Helaakoski, T., Pajunen, L., Kivirikko, K. I. and Pihlajaniemi, T. (1990) J. Biol. Chem. **265**, 11413–11416
Kadonaga, J. T., Jones, K. A. and Tjian, R. (1986) Trends. Biochem. Sci. **11**, 20–23

Received 7 December 1992; accepted 21 December 1992

Kadonaga, J. T., Carner, K. R., Masiarz, F. R. and Tjian, R. (1987) Cell **51**, 1079–1090
Kadonaga, J. T., Courey, A. J., Ladika, J. and Tjian, R. (1988) Science **242**, 1566–1570
Kivirikko, K. I., Myllylä, R. and Pihlajaniemi, T. (1989) FASEB J. **3**, 1609–1617
Kivirikko, K. I., Helaakoski, T., Tasanen, K., Vuori, K., Myllylä, R., Parkkonen, T. and Pihlajaniemi, T. (1990) Ann. N. Y. Acad. Sci. **580**, 132–142
Kivirikko, K. I., Myllylä, R. and Pihlajaniemi, T. (1992) in Post-Translational Modifications of Proteins (Harding, J. J. and Crabbe, M. J. C., eds.), pp. 1–51, CRC Press, Boca Raton and London
Koivu, J., Myllylä, R., Helaakoski, t., Pihlajaniemi, T., Tasanen, K. and Kivirikko, K. I. (1987) J. Biol. Chem. **262**, 6447–6449
LaMantia, M., Miura, T., Tachikawa, H., Kaplan, H. A., Lennarz, W. J. and Mizunaga, T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 4453–4457
Mastrangelo, I. A., Courey, A. J., Wall, J. S., Jackson, S. P. and Hough, P. V. C. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5670–5674
Myllylä, R., Koivu, J., Pihlajaniemi, T. and Kivirikko, K. I. (1983) Eur. J. Biochem. **134**, 7–11
Noiva, R., Kaplan, H. A. and Lennarz, W. J. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 1986–1990
Pascal, E. and Tjian, R. (1991) Genes Dev. **5**, 1646–1656
Pihlajaniemi, T., Helaakoski, T., Tasanen, K., Myllylä, R., Huhtala, M.-L., Koivu, J. and Kivirikko, K. I. (1987) EMBO J. **6**, 643–649
Roth, R. A. and Koshland, M. E. (1981) Biochemistry **20**, 6594–6599
Saffer, J. D., Jackson, S. P. and Thurston, S. J. (1990) Genes Dev. **4**, 659–666
Saffer, J. D., Jackson, S. P. and Annarella, M. B. (1991) Mol. Cell. Biol. **11**, 2189–2199
Scherens, B., Dubois, E. and Messenguy, F. (1991) Yeast **7**, 185–193
Selden, K. F., Burke-Howie, K., Kowe, M. E., Goodman, H. M. and Moore, D. D. (1986) Mol. Cell. Biol. **6**, 3173–3179
Su, W., Jackson, S., Tjian, R. and Echols, H. (1991) Genes Dev. **5**, 820–826
Tachikawa, H., Miura, T., Katakura, Y. and Mizunaga, T. (1991) J. Biochem. (Tokyo) **110**, 306–311
Tasanen, K., Parkkonen, T., Chow, L. T., Kivirikko, K. I. and Pihlajaniemi, T. (1988) J. Biol. Chem. **263**, 16218–16224
Tasanen, K., Oikarinen, J., Kivirikko, K. I. and Pihlajaniemi, T. (1992) J. Biol. Chem. **267**, 11513–11519
Wells, W. W., Xu, D. P., Yang, Y. and Rocque, W. J. (1990) J. Biol. Chem. **265**, 15361–15364
Wetterau, J. R., Combs, K. A., Spinner, S. N. and Joiner, B. J. (1990) J. Biol. Chem. **265**, 9800–9807
Wetterau, J. R., Combs, K. A., McLean, L. R., Spinner, S. N. and Aggebeck, L. P. (1991) Biochemistry **30**, 9728–9735
Yamauchi, K., Yamamoto, T., Hagashi, H., Koya, S., Takikawa, H., Toyoshima, K. and Horiuchi, R. (1987) Biochem. Biophys. Res. Commun. **146**, 1485–1492