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A human homologue of the yeast *GST1* gene codes for a GTP-binding protein and is expressed in a proliferation-dependent manner in mammalian cells

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A human homologue (*GST1-Hs*) of the yeast *GST1* gene that encodes a new GTP-binding protein essential for the G_1-to-S phase transition of the cell cycle was cloned from the cDNA library of human KB cells. The *GST1-Hs* cDNA contained a 1497 bp open reading frame coding for a 499 amino acid protein with mol. wt 55 754 and with the amino acid sequence homologies of 52.3 and 37.8% to the *GST1* protein and polypeptide chain elongation factor EF1α respectively. The regions potentially responsible for GTP binding and GTP hydrolysis were conserved in the *GST1-Hs* protein as well. When expressed in yeast cell, the *GST1-Hs* gene could complement the ts phenotype of yeast *gst1* mutant. *GST1-Hs* and its mouse homologue were expressed in human fibroblasts and in various mouse cell types respectively, at relatively low levels in their quiescent states, and the level of those expressions increased rapidly, prior to the onset of DNA replication and the total RNA synthesis, when human or mouse fibroblasts were progressed out of the growth-arrested state by the addition of serum. A possible role of *GST1-Hs* in mammalian cell growth is discussed.

Key words: cell proliferation/GTP-binding protein/human homologue of yeast gene

Introduction

A variety of gene products have been found to play essential roles in the cell cycle of yeast as a result of application of extensive genetic approaches to this model organism. A search for mammalian proteins homologous to these yeast gene products provides a useful means for identification of the proteins involved in regulation of the mammalian cell growth, since genetic approaches are not readily applicable directly to mammalian cells. For instance, Lee and Nurse (1987) have succeeded in cloning the human homologue of the *cdc2* gene of fission yeast *Schizosaccharomyces pombe* or the *CDC28* gene of budding yeast *Saccharomyces cerevisiae*, based on the homologue's ability to complement the *cdc2* mutants that are unable to proceed past the G_1 or G_2 control points.

The *GST1* gene that can complement a temperature-sensitive *gst1* mutant of *S.cerevisiae* was recently isolated from the yeast genomic library (Kikuchi *et al.*, 1988). The *gst1* cell was a *cdc*-like mutant whose execution point seemed to be distal to the mating factor-sensitive step. DNA synthesis was substantially arrested in this mutant at non-permissive temperature, suggesting that the *GST1* gene is essential for the G_1-to-S phase transition in the yeast cell cycle. The gene product appeared to be a GTP-binding protein of mol. wt 76 565 having 38% identity in protein sequence with polypeptide chain elongation factor EF1α.

There are two major families of GTP-binding proteins i.e. the *ras* and related oncogene products (Barbacid, 1987) and the membrane signal transducer G proteins (Gilman, 1987), in higher eukaryotes. Both must play important roles in the regulation of the cell growth. Reduction of spontaneous GTPase activity of *ras* proteins, together with their concurrent insusceptibility to cytosolic GAP (GTPase activating protein) (McCormick, 1989), after oncogenic mutation of cells, affords strong support to the idea that the ability of the GTP-binding proteins to interact with, or hydrolyze GTP at a moderate rate is essential for normal cell growth.

More direct evidence is currently available for the involvement of signal transducer G proteins in cell proliferation. They are homologous proteins with a common $\alpha\beta\gamma$ heterotrimeric structure. The α-subunits are GTP-binding proteins serving as the selective substrates of mono (ADP-ribosyl)ation catalyzed by cholera toxin and/or pertussis toxin. Any signal arising from membrane receptors is efficiently blocked at the subsequent G protein step of the signaling pathway, when the whole pool of the receptor-coupled G proteins has been ADP-ribosylated after exposure of the cells to pertussis toxin or islet-activating protein (IAP) for several hours (Ui, 1984, 1986). The DNA synthesis or the G_0/G_1-to-S phase transition as triggered by the addition of serum or certain growth factors to cloned or primary cultured mammalian cells was inhibited by prior exposure of the cells to IAP in a manner dependent on the toxin-induced ADP-ribosylation of membrane G proteins under these condtiions (see Ui, 1989 for review). Thus, signals arising from certain growth factor receptors must be mediated by IAP-substrate G proteins leading to eventual proliferation in these cell types.

Genes coding for proteins homologous to mammalian *ras* proteins (*RAS1* and *RAS2*) (Powers *et al.*, 1984) or to G protein α-subunits (*GPA1* and *GPA2*) (Nakafuku *et al.*, 1987, 1988; Dietzel and Kujan, 1987) have been isolated from *S.cerevisiae* and found to behave as one of the yeast cell cycle control genes. It would be expected, therefore, that the homologue of the yeast *GST1* gene is likewise expressed in human cells. Here, we describe the isolation and characterization of a human cDNA clone that is highly

homologous to the yeast *GST1* gene. The gene may play a significant role in the mammalian cell growth, since its expression in human and mouse cells was proliferation dependent.

Results

Isolation and nucleotide sequence analysis of human cDNA that is homologous to S.cerevisiae GST1 gene

Poly(A)$^+$ RNA or genomic DNA prepared from human KB cells was subjected to Northern or Southern blotting analysis using yeast *GST1* as a probe under low stringency conditions. Several bands, including one of 2.7 kb and the 9 kb *Eco*RI fragment, were detected in Northern and genonic Southern blotting respectively (data not shown), suggesting that a *GST1* gene homologue occurs in human cells.

About 6×10^5 plaques of a λgt10 human KB cell cDNA library were therefore prepared and screened by the plaque hybridization technique using the yeast *GST1* gene as a probe. Thirty-three positive clones were isolated under low stringency conditions. DNA was prepared from eight selected clones, subcloned into pUC19 and analyzed by restriction endonuclease mapping. Since they all showed similar restriction maps, one of the clones, pGH5, which contained the longest insert (~2.6 kb) was subjected to DNA sequencing analysis.

The restriction map of pGH5 and sequencing strategy are shown in Figure 1. Analysis of the complete nucleotide sequence (2612 bp) of the cloned cDNA revealed an open reading frame of 1497 nucleotides, starting at the first available ATG codon (nucleotide positions 1–3) and ending at the TAA termination codon (1495–1497) (Figure 2). The open reading frame codes for a polypeptide consisting of 499 amino acids with a mol. wt of 55 754 daltons. The nucleotide sequence downstream to the stop codon contained a sequence ATTTA (nucleotide positions 1851–1855) which is potentially involved in mRNA selective degradation (Shaw and Kamen, 1986), and an additional AATAAA consensus polyadenylation signal (Birnstiel *et al.*, 1985) (nucleotide positions 1922–1927) was located 13 nucleotides upstream from the poly(A)$^+$ tail. This human homologue of yeast *GST1* will be henceforth referred to as *GST1-Hs*.

Predicted amino acid sequence of GST1-Hs as compared with the yeast gene

The predicted amino acid sequence of *GST1-Hs* contained a glutamic acid-rich region, 56 amino acids long in the N terminus, which was followed by a region characteristic of GTP-binding proteins. The second region was homologous to human EF1α. The extent of homology was 38.5% in the nucleotide sequence and 37.8% in the amino acid sequence throughout the entire sequence. Figure 3 shows the alignment of the amino acid sequence of the *GST1-Hs* protein with those of the yeast *GST1* protein and human EF1α. Disregarding the sequence of a stretch of 183 extra amino acids that is present in the N terminus of the yeast *GST1* protein, amino acid (or nucleotide) identities were 52.3% (or 58.6%) between *GST1-Hs* and yeast *GST1*. A potential target site of cAMP-dependent protein kinase (Cohen, 1985) in yeast *GST1* (Kikuchi *et al.*, 1988) was conserved in *GST1-Hs*.

GTP-binding proteins are known to share sequence similarities in some regions (G1–G5) that must be responsible for guanine nucleotide binding and hydrolysis (Halliday, 1984). Figure 4 compares amino acid sequences of these regions between *GST1-Hs* and other GTP-binding proteins. The G1 region is involved in phosphoryl binding and is important for GTP-hydrolysis; GTPase activity was markedly reduced when glycine-12 present in this region of the mammalian *c-ras* protein was replaced by valine. It is noteworthy that the amino acid corresponding to glycine-12 in the *ras* protein is replaced by valine in *GST1-Hs* as well as in EF1α, IF2α and EF2.

The G2 region, which contains a consensus sequence Asp-X-X-Gly, is considered to form a salt bridge with magnesium ion, while the G3, G4 and G5 regions are involved in the binding of guanine nucleotides (Jurnak, 1985). These regions are also conserved in *GST1-Hs*.

Complementation of yeast gst1 mutants by human GST1-Hs

The *gst1*^{ts} mutants were transformed with plasmids containing *GST1* or *GST1-Hs* and allowed to grow at a restrictive temperature (Figure 5). Not only the cells transformed with pYK810 (carrying yeast *GST1*) but also the transformants with p56G (carrying *GST1-Hs*) grew at significant rates, though the rate was lower in the latter case than in the former case (Figure 5A and C). In contrast, the transformants with the vector pTRP56 (carrying neither *GST1* nor *GST1-Hs*) did not grow under the same conditions (Figure 5B). The cells transformed with the plasmid containing *GST1-Hs* but lacking the yeast *GAL1* promoter region grew also at a significant rate; the human gene could be expressed in yeast even without the yeast promoter. Thus, the human gene, *GST1-Hs*, shared similarities in function as well as in structure with the yeast gene, *GST1*.

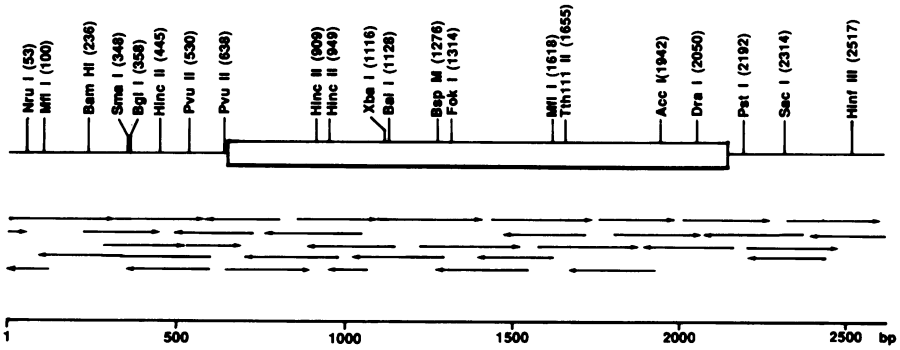

Fig. 1. Restriction endonuclease map and sequencing strategy for pGH5 (*GST1-Hs*). The scale under the restriction map indicates the nucleotide positions in base pairs. The amino acids coding region is represented by an open bar. Arrows indicate the direction and extent of sequence determination using the dideoxynucleotide chain-termination method.

Occurrence and expression of the GST1-Hs gene in human cells and its mouse homologue in mouse tissues and cells

DNA was prepared from human KB cells, digested with *Eco*RI and subjected to the Southern blot analysis using ^{32}P-labeled cDNA of *GST1-Hs* as a probe (Figure 6B). Two DNA fragments of 9.5 and 2.0 kb displayed the hybridization signal.

Poly(A)$^+$ RNA was prepared from exponentially growing cells and analyzed by Northern blot hybridization using ^{32}P-radiolabeled cDNA of *GST1-Hs* as a probe. As shown in Figure 6A a predominant mRNA band of ~2.7 kb, together with a minor band of 3.5 kb, was present in human KB cells. There exists one more band in KB cells whose mobility corresponds to that of 28S rRNA. The intensity of this band flucutates in each experiment; therefore,

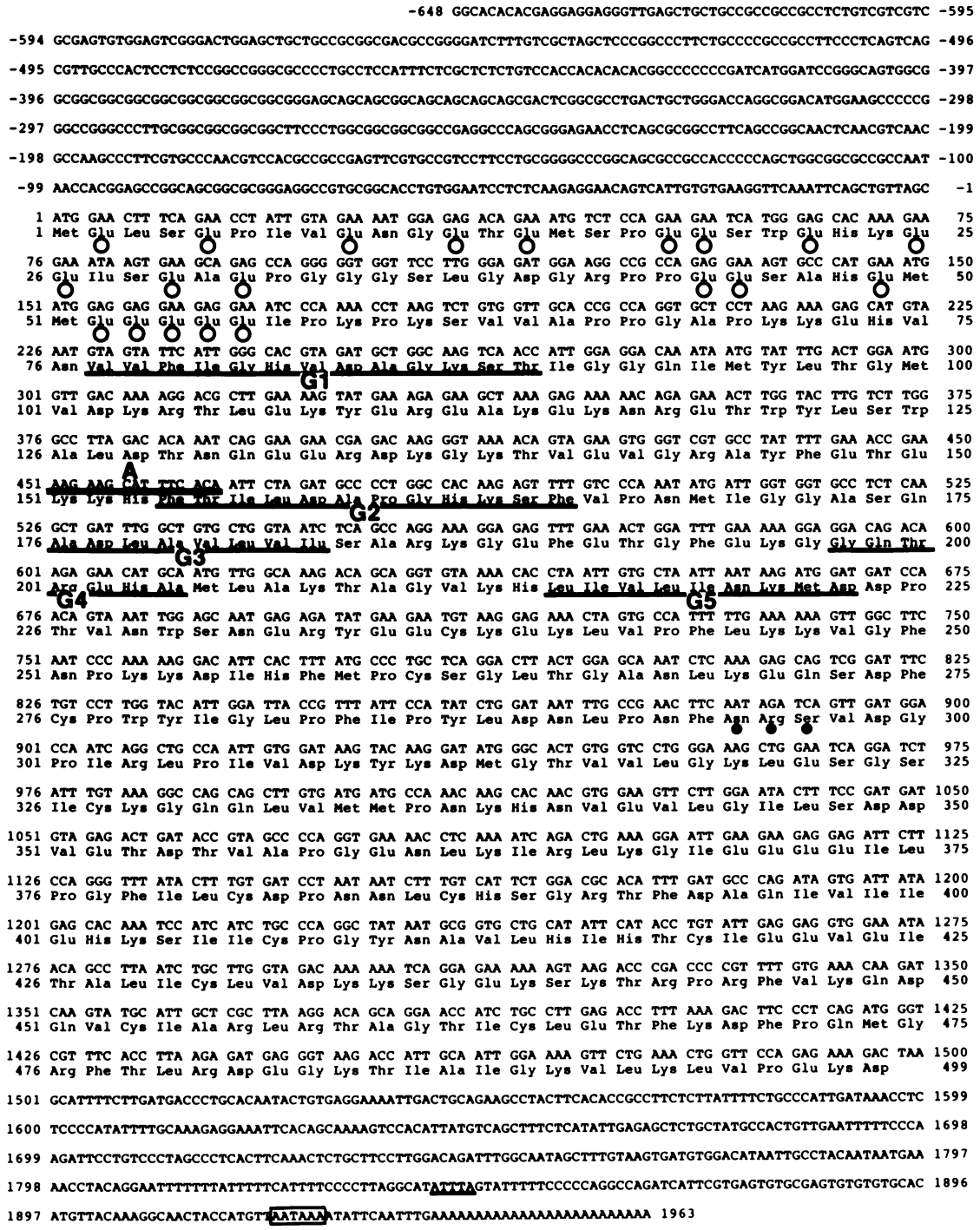

Fig. 2. Nucleotide and predicted amino acids sequences of the cDNA insert of pGH5 (*GST1-Hs*). The nucleotide and the deduced amino acid residues are numbered from the first ATG codon and from the initiation methionine respectively. 'Glu' marked with open circles show the presence of a glutamic-acid rich region near the N terminus. Conservative domains G1–G5 (involved in GTP-binding; Kohno *et al.*, 1986) are indicated by solid lines and the putative recognition site of cAMP-dependent protein kinase is doubly underlined. The AATAAA box close to the polyadenylated 3′ end of the mRNA is boxed. The ATTTA sequence motif upstream of the AATAAA box is underlined. Potential glycosylation sites are indicated by filled circles.

it could be a contaminating 28S rRNA. The 2.7 kb message was also detected in mouse FM3A cells. Since the 2.7 kb message was the only detectable band in RNA from mouse FM3A cells, and intensity and mobility of the band were comparable to those of the major band in human KB cell RNA, we regarded the transcript as the mouse homologue of *GST1-Hs*. In contrast, no hybridizing band was detected in RNA preparations from fly cells (NIH SaPe4).

The mouse homologue of *GST1-Hs* gene was expressed in all the mouse tissues studied; a 2.7 kb transcript hybridizing to *GST1-Hs* was seen in liver, kidney, spleen, brain, lung and heart of adult mice, though the hybridization signal was much less intense in liver than in other tissues (Figure 7) (see Discussion).

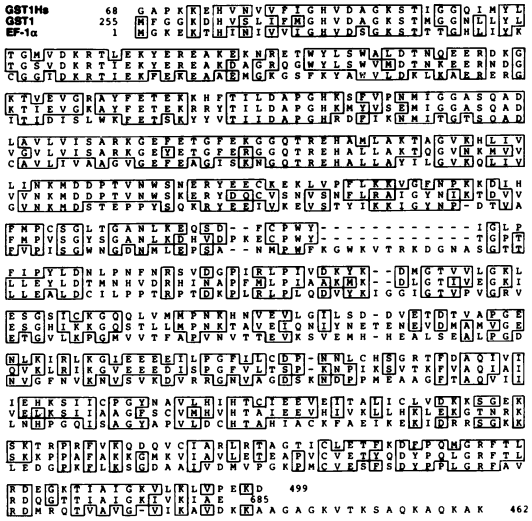

Fig. 3. Homologies in amino acid sequences in human *GST1-Hs*, *S.cerevisiae GST1* and human EF1α (Brands *et al.*, 1986). The alignment was optimized by computer analysis. Identical amino acid residues are boxed. The numbers refer to the amino acid residues, with the initiating Met = 1.

Proliferation-dependent expression of the mammalian homologues of GST1 gene in human and mouse fibroblasts

Since *GST1* had been identified as an essential gene for the G_1-to-S phase transition in the yeast cell cycle (Kikuchi *et al.*, 1988), we studied a possible role of *GST1-Hs* and its mouse homologue in the regulation of mammalian cell growth. Serum was added to quiescent IMR90 human fibroblasts to induce the progression of the G_0 cells through G_1 to the S phase. The total RNA obtained from these cells at various culture times was then analyzed by Northern blot hybridization using *GST1-Hs* cDNA as a probe.

The *GST1-Hs* mRNA tended to increase at 2 h and actually increased dramatically at 4 h after serum stimulation (Figure 8A-2, top panel). This enhanced expression of the *GST1-Hs* gene was an early event and preceded the onset of total RNA synthesis and DNA replication measured in the same cells (Figure 8A-1). The *GST1-Hs* mRNA reached its maximal level at 4−8 h and decreased gradually thereafter despite a still steady increase in the total RNA up to 24 h. As has been shown for KB cells in Figure 6, two mRNA bands with estimated sizes of 2.7 and 3.5 kb were detected in this human cell line as well. At present, we do not know anything about the relationship between these two bands. Their intensities fluctuate in parallel, indicating that they might arise from an alternative splicing of the primary transcript. Similar results were obtained with mouse Swiss 3T3 cells (Figure 8B-2), top); there was a marked increase in the 2.7 kb transcript of the mouse homologue of *GST1-Hs* DNA at 5 h after serum stimulation of the cells, which was followed by a gradual decrease in the gene expression during the period of rapid increase in DNA and total RNA synthesis up to 24 h.

These proliferation-dependent gene expressions were specific to *GST1-Hs* in the sense that genes coding for other GTP-binding proteins were expressed in distinctly different manners as shown in the lower panels in Figure 8(A-2) and (B-2). For instance, EF1α with a DNA sequence

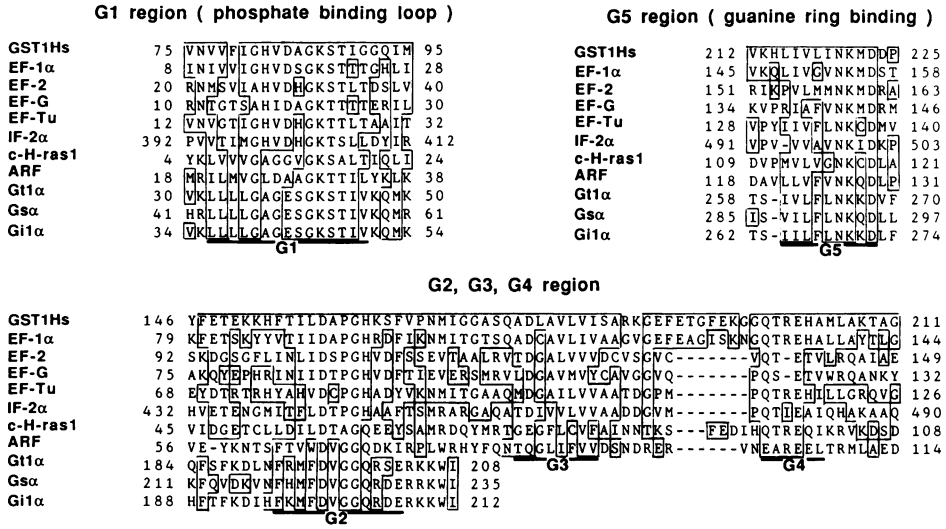

Fig. 4. Comparison of five *GST1-Hs* peptide regions with corresponding sequences conserved in GTP-binding protein families. Amino acid sequences for the conserved regions G1−G5 of GTP-binding proteins including human EF1α (Brands *et al.*, 1986), hamster EF2 (Kohno *et al.*, 1986), bacterial EF-Tu (Arai *et al.*, 1980), EF-G (Zengel *et al.*, 1984), bacterial IF2α (Sacerkot *et al.*, 1984), human *c-H-ras* (Capon *et al.*, 1983), bovine ARF (Price *et al.*, 1988), human G protein $G_i1\alpha$ (Itoh *et al.*, 1988), Gsα (Kozasa *et al.*, 1988) and bovine $G_t1\alpha$ (Tanabe *et al.*, 1985) are aligned with corresponding regions of *GST1-Hs* protein. Regions of exact homology or conservative Dayhoff substitutions between the *GST1-Hs* protein and other proteins are boxed. The following Dayhoff conservative categories (Dayhoff, 1978) were used: C; A, G, P, S, T; H, R, K; I, L, M, V; N, D, E, Q; F, Y, W.

homologous to *GST1-Hs* (Figure 3) was highly expressed even in the G_0 phase before serum stimulation, and this high level of expression was maintained over the 24 h period following the stimulation with a trend to decrease only slightly toward the end of this period in either human or mouse fibroblasts (the second panel in Figure 8A-2 and B-2).

Similarly, continuous gene expression was observed during the cell cycle of human fibroblasts for $G_i2\alpha$, the α-subunit of one of the IAP-sensitive G proteins. In the case of mouse Swiss 3T3 cells, however, the expression of the $G_i2\alpha$ mRNA showed a proliferation-dependent fluctuation, although the manner of the fluctuation was different from that for the *GST1-Hs* gene expression and levels of expression were, as a whole, several-fold lower than those for the same mRNA in human cells. The level of the $G_i2\alpha$ mRNA was the highest at the G_0-phase before serum stimulation, fell strikingly at 5 and 10 h, i.e. as the cell cycle proceeded to the G_1-phase, increased again at 15 and 20 h when DNA synthesis peaked in the S-phase, and finally decreased slightly at the end (25 h) of the DNA synthesis in 3T3 cells. Conversely, the gene coding for $G_i3\alpha$, the α-subunit of another IAP-sensitive G protein, was expressed at a lower level before serum stimulation, and the expression increased rapidly and transiently at the early G_1-phase in 3T3 cells (the bottom panel of Figure 8B-2). It would be thus likely that the expression of *GST1-Hs*, unlike that of EF1α but together with some of the transducer G protein α-subunits, is closely related to mammalian cell proliferation or cell cycle progression.

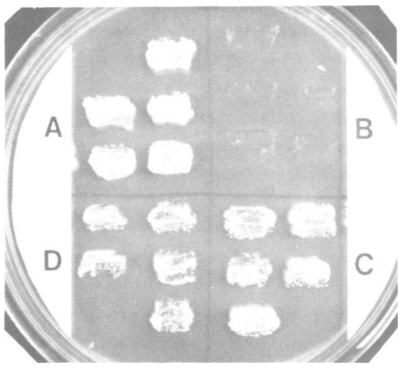

Fig. 5. Complementation of the yeast *gst1* mutant by human *GST1-Hs* as well as by yeast *GST1* gene. The *S.cerevisiae* temperature-sensitive strain (YK21-02) was transformed with the plasmid of pYK810 (*A*), pTRP56 (**B**), p56G (**C**) or p56G BamΔ (**D**). Five *Trp*$^+$ or *Ura*$^+$ transformants in each case were streaked on YPD-plates and incubated for 60 h at the restrictive temperature of 37°C. See Materials and methods for explanation of plasmids and other details.

Discussion

We have isolated a human cDNA clone (*GST1-Hs*) coding for a new type of GTP-binding protein with striking homology to the yeast *GST1* gene. The *GST1-Hs* protein predicted from the DNA sequence data was characterized by the presence of a GTP-binding domain and GTPase activity center which was highly homologous to the first half of EF1α. The yeast GST1 gene, as compared with *GST1-Hs*,

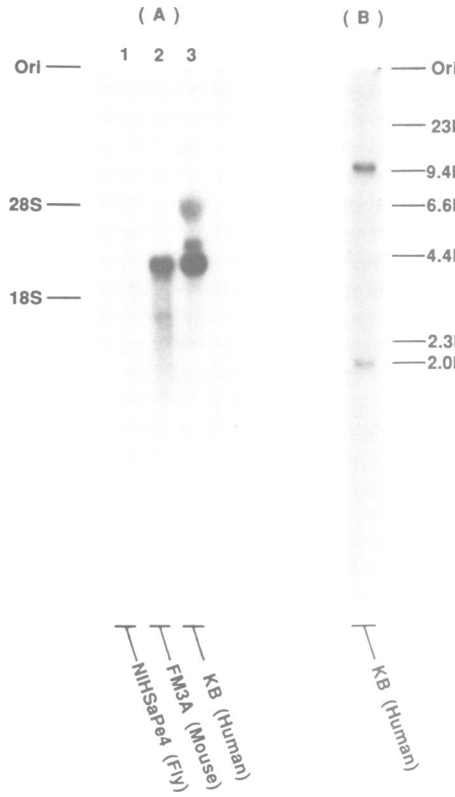

Fig. 6. (**A**) Blot hybridization analysis of poly(A)$^+$ RNA from various cells. Poly(A)$^+$ RNA (2 μg/each lane) was electrophoresed in a 1.2% formaldehyde agarose gel. RNA was transferred to a nylon membrane filter aned hybridized with the ^{32}P-labeled 1.6 kb *Pvu*II−*Pst*I fragment of pGH5 under high stringency conditions. Positions of 28S (4718 nucleotides) and 18S (1874 nucleotides) RNA are indicated. **Lane 1**; fly NIH SaPE4 (cells; **lane 2**, mouse FM3A cells.; **lane 3**, human KB cells. (**B**) Southern blot analysis of human genomic DNA digested with *Eco*RI. Genomic DNA (10 μg) isolated from human KB cells was digested with a 10-fold excess of *Eco*RI and electrophoresed in a 0.8% agarose gel. DNA was denatured, transferred to a nylon membrane filter and hybridized with the ^{32}P-labeled 1.6 kb *Pvu*II−*Pst*I fragment of the pGH5 under high stringency conditions. Positions of fragments of *Hin*dIII-digested λDNA are indicated.

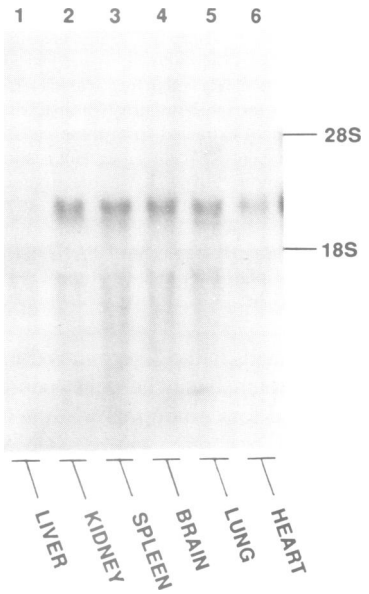

Fig. 7. Blot hybridization analysis of the mouse homologue of *GST1-Hs* mRNA in various mouse tissues. Total tissue RNA (20 μg) was fractionated by electrophoresis, transferred to a nylon membrane filter and hybridized with ^{32}P-labeled 1.6 kb *Pvu*II−*Pst*I fragment of pGH5 under high stringency conditions. The source of RNA is indicated under each lane.

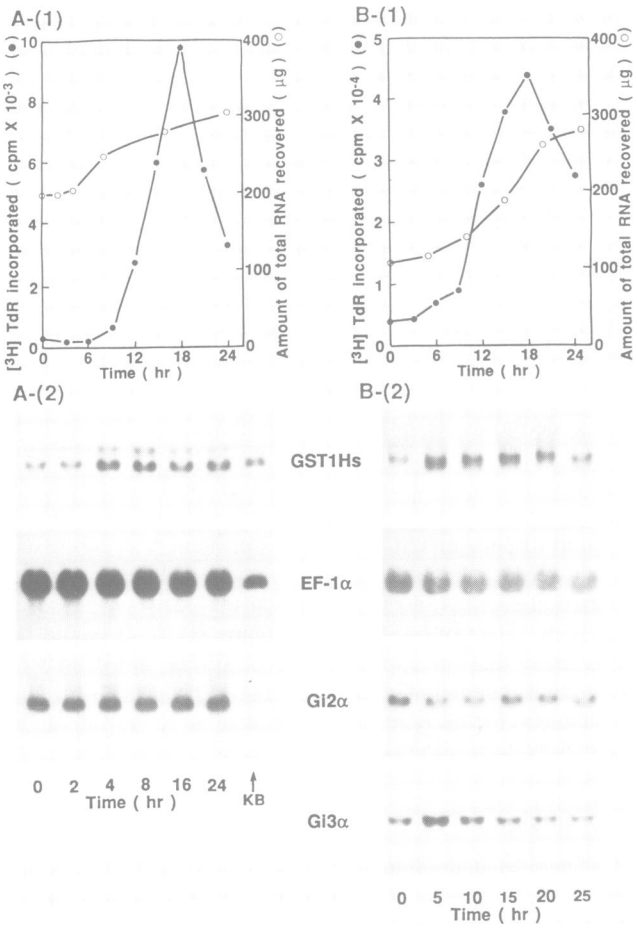

Fig. 8. Periodical changes in proliferation-dependent gene expression of *GST1-Hs*, its mouse homologue and other GTP-binding protein α-subunits, as compared with simultaneous changes in DNA synthesis and total RNA level, after serum stimulation of human IMR90 (**A**) or mouse Swiss 3T3 (**B**) cells. DNA synthesis as measured by the incorporation of [^{3}H]thymidine into the acid-insoluble fraction and total RNA measured by the absorbance at 260 nm are plotted as function of time (hours) after the addition of serum to quiesent cells in upper panels (**A-1** and **B-2**). These values shown on vertical axis are based on 2.8 × 10^7 (**A**) or 1 × 10^7 (**B**) cells. In lower panels (**A-2** and **B-2**) are shown autoradiograms for the Northern blot analysis of total cytoplasmic RNA (2 μg/lane) extracted from these cells The blot for poly(A)$^+$ RNA from quiescent KB cells (2 μg/lane) is also shown for comparison (as indicated by an arrow 'KB'). Electrophoresis, blotting and hybridization were achieved as described in Figure 6(A). Probes used for hybridization were the 1.6 kb *Pvu*II−*Pst*I fragment of pGH5 for *GST1-Hs*, the 2.3 kb *Bam*HI fragment of PAN7 for EF1α, the 1.7-kb *Eco*RI fragment of pGi13 for $G_i2\alpha$ and the 1.1 kb *Eco*RI fragment of pGX14 for $G_i3\alpha$.

had 183 extra amino acids in the N terminus. Similar deletion of an amino acid stretch from the yeast peptide has been occasionally observed for certain mammalian GTP-binding proteins. For instance, *ras* proteins in yeast (*RAS1* and *RAS2*) contain 98 and 111 excess amino acids respectively in their C termini (Powers *et al.*, 1984), and the yeast G proteins (*GP1α* and *GP2α*) possess 110 and 83 extra amino acids at their N-terminal regions, as compared with their mammalian counterparts (Nakafuku *et al.*, 1987, 1988; Dietzel and Kujan, 1987). The omission of a stretch of amino acid residues from the yeast counterpart thus never unique to *GST1-Hs* but a common feature that may enable otherwise structurally analogous proteins to play essentially the same physiological role in evolutionally different species, namely mammals and yeast.

RAS proteins are involved in the progression of the glucose-dependent yeast cell cycle as a result of their role as signal transcucer between the nutrient receptor and adenylate cyclase effector system (Toda *et al.*, 1985). There is evidence that the product of proto-oncogene *ras* plays a similarly significant role in normal proliferation of mammalian cells, although no decisive information is yet available as to identification of membrane receptors or cytosolic effectors directly mediated by *ras* proteins in these cells. *GP1α*, the yeast counterpart of mammalian IAP-substrate G protein α-subunits, is also assumed to be a transducer that is coupled to the mating factor receptor (Miyajima *et al.*, 1987) whose primary structure is homologous to mammalian G protein-coupled receptors especially in seven membrane-spanning regions (Burkholder and Hartwell, 1985; Nakayama *et al.*, 1985; Hagen *et al.*, 1986). In view of these functional similarities between yeast and mammalian G protein counterparts, the putative protein encoded by *GST1-Hs* is likely to share analogous properties in mammalian cells with the *GST1* protein which is essential for the G_1-to-S transition in the cell cycle of yeast (Kikuchi *et al.*, 1988).

The following data presented in this paper would be consistent with the idea that the *GST1-Hs* protein is somehow involved in regulation of mammalian cell growth. First, the multicopy vector carrying *GST1-Hs* mimicked the yeast *GST1*-containing plasmid by complementing the yeast *ts* mutant, *gst1* whose cell cycle was otherwise arrested at a stage prior to DNA synthesis at non-permissive temperature. Similar functions supporting yeast cell growth are thus suggested for both GTP-binding proteins encoded by these genes. Second, the expression of the *GST1-Hs* gene was proliferation dependent in both human or mouse cells. The expression started promptly after the addition of serum, prior to the onset of total RNA synthesis and DNA replication. This expression pattern was distinctly different from the pattern of expression of EF1α that is homologous in primary structure to the *GST1-Hs* protein but is involved in protein synthesis rather than DNA replication.

Third, *GST1-Hs* was expressed ubiquitously in all tissues tested at relatively low levels, in accordance with the similarly low-level expression in cultured cells before serum stimulation. As for the extremely low-level expression of the mouse *GST1-Hs* homologue in liver, we do not know the reason for this. We should point out, however, that rat primary hepatocytes showed an increase in the expression of *GST1-Hs* homologue before onset of DNA replication, indicating that liver cells have a potential for expressing *GST1-Hs* homologue (unpublished observation). Fourth, the DNA sequence in the 5′ non-coding region of *GST1-Hs* was highly homologous to that of the corresponding regions of some oncogenes, such as *ras* (McGrath *et al.*, 1983) and *sis* (Collins *et al.*, 1985; Rao *et al.*, 1986), that encode proteins seemingly responsible for cell growth regulation. These genes might be expressed in an analogous fashion to play related roles in cells. An exact role of the *GST1-Hs* gene in mammalian cell functions is under current investigation in our laboratory and will be the subject of forthcoming papers.

Materials and methods

Isolation and RNA

Total cellular RNA was prepared from cells or tissues using the guanidinium−cesium chloride method (Chirgwin *et al.*, 1979). Poly(A)$^+$

RNA was isolated therefrom by oligo(dT)–cellulose column chromatography (Maniatis *et al.*, 1982).

Construction of the λgt10 cDNA library

Poly(A)$^+$ RNA isolated from KB cells was converted into double-stranded cDNA by the RNase H method of Gubler and Hoffman (1983). Resultant double-stranded cDNA was blunt-ended by T4 DNA polymerase, treated with *Eco*RI methylase, ligated to synthetic *Eco*RI linkers and finally digested with *Eco*RI. The linker-ligated cDNA thus prepared was passed through a Sepharose CL-4B column to remove linker fragments and then inserted into phage λgt10 and packaged *in vitro* using Gigapack Gold (Vector Cloning System, San Diego, CA). Thus prepared bacteriophage particles were grown on *Escherichia coli* C600 Hfl. About 10^6 recombinants were obtained from 110 ng of double-stranded cDNA.

Screening of the cDNA library

The hybridization probe used to screen the λgt10 cDNA library was the 1.35 kb *Kpn*I fragment of pYK812 (Kikuchi *et al.*, 1988) which carries almost the entire coding region of the *GST1* gene. About 6×10^5 recombinant plaques were screened by the plaque-hyridization method (Benton and Davis, 1977). Hybridization was performed overnight at 42°C in a solution containing 20% formamide, 5 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate), 1 × Denhardt's solution (0.002% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidone) and 20 μg/ml of heat-denatured salmon sperm DNA. Filters were washed twice at room temperature with 2 × SSC buffer (1 × SSC buffer = 1 × SSC, 10 mM sodium phosphate, 0.025% SDS), then twice at 68°C with 1 × SSC buffer for 10 min per wash, dried at room temperature and finally subjected to autoradiography. The λgt10 phage DNA was isolated from positive clones by the standard procedure (Maniatis *et al.*, 1982).

Subcloning and DNA sequence analysis

The cDNA insert of the DNA of the positive clone was again inserted into pUC19 to produce the subclone pGH5, which was subjected to nucleotide sequence analysis by the dideoxy chain-termination method using denatured plasmid templates (Hattori and Sakaki, 1986).

Cell culture of IMR-90 and Swiss 3T3 fibroblasts

IMR-90 human embryo fibroblasts (Nicols *et al.*, 1977) and mouse embryo fibroblasts (Swiss 3T3, obtained from the Japanese Cancer Research Resources Bank) were routinely maintained in monolayer cultures in tissue culture flasks containing Dulbecco's modified Eagle's medium (DMEM) fortified with 10% fetal bovine serum (FBS). These cells were transferred to plastic Petri dishes, some of which were provided with a glass coverslip at the bottom for the purpose of DNA synthesis determination (see below), to grow further to semiconfluence. The cells were then maintained in the culture medium containing FBS at a concentration as low as 0.5% for 4 days. During this serum starvation procedure, 24 h [^{3}H]thymidine labeling did not occur in most (>95%) of nuclei. DNA synthesis was then induced in these G_0/G_1-arrested cells by changing the medium to the fresh one containing 10% FBS. The cells were harvested at the desired times for RNA isolation. To detemrine the rate of DNA synthesis, cells grown on glass coverslips were incubated with [^{3}H]thymidine (1 μCi/ml; New England Nuclear, 25 Ci/mmol) for 60 min, and the radioactivities in the acid-insoluble fraction fixed on the coverslip were determined. The IMR-90 cell line used here had a limited lifespan of 60 population doubling level (60 PDL) under our culture conditions. We used cells at 30–36 PDL in the experiments shown in Figure 8.

RNA blot hybridization analysis

Total cellular RNA or poly(A)$^+$ RNA was denatured by heating at 65°C for 5 min in 2.2 M formaldehyde, 50% (v/v) formamide and size fractionated by 1.2% agarose gel containing 2.2 M formaldehyde. The RNA was transferred directly to nylon membrane filters. Hybridizations were carried out overnight at 42°C in a solution containing 50% formamide, 5 × SSC, 3 × Denhardt's solution, 50 mM sodium phosphate (pH 6.8) and 100 μg/ml of heat-denatured salmon sperm DNA. The filters were washed twice at room temperature in 2 × SSC buffer, twice at 68°C in 1 × SSC buffer for 10 min per wash and subjected to autoradiography. The plasmid carrying cDNA sequences for EF1α (PAN7), $G_i2\alpha$ (pGT13) and $G_i3\alpha$ (pGX14) were kind gifts from Dr Y.Kaziro and were used as the hybridization probes.

Construction of plasmids to transform yeast cells

A plasmid pYK810 containing yeast *Gst1* on the single copy vector, YCp50 containing *URA3*, was described previously (Kikuchi *et al.*, 1988). A plasmid p56G, a multicopy vector carrying *GST1-Hs*, was constructed by ligating the 2.6 kb *Nru*I–*Kpn*I fragment of the above-mentioned pGH5 with the 5 kb *Bam*HI–*Kpn*I fragment of pTRP56 (Miyajima *et al.*, 1984) and annealing with the 1 kb *Bam*HI–*Pst*I fragment of pG1 (Miyajima *et al.*, 1984) in which the *Pst*I site was blunt-ended. The plasmid p56G BamΔ was constructed from p56G by removing the *Bam*HI fragment which carried the yeast *GAL1* promoter region. Yeast transformation was performed by the alkali ion method (Ito *et al.*, 1983). *Ura*$^+$ and *Trp*$^+$ transformants were selected at 26°C in the case of pYK810 and other plasmids respectively.

Southern blot analysis

Human genomic DNA was isolated from KB cells digested to completion with *Eco*RI, fractionated by electrophoresis and transferred to a nylon membrane filter by the method of Southern (1975). Blots were hybridized and washed as described above for RNA.

Yeast strain and media

The strain of *S.cerevisiae* used in this study was YK21-02, α *gst1-1 ura3 trp1 his3* Gal$^-$ (Kikuchi *et al.*, 1988). Media for yeast experiments were described by Sherman *et al.* (1986).

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