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Regulated high efficiency expression of human interferon-alpha in *Saccharomyces cerevisiae*

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The 5′ control region of the yeast phosphoglycerate kinase gene *(PGK)* was fused to the coding sequence of a human interferon-alpha. This *PGK*-interferon fusion was then introduced into yeast on a high copy number 2μ-based plasmid vector. Strains containing this plasmid produced a *PGK*-interferon-alpha fusion protein as 1–2% of cell protein and the expression of interferon activity was regulated by the availability of a fermentable carbon source. The system is capable of making as much as 15 mg of human interferon-alpha per litre of batch culture.

Key words: yeast/interferon/expression vectors

Introduction

The production of useful and interesting polypeptides by the exploitation of recombinant DNA techniques has been largely centred around *Escherichia coli*. However, in some situations *E. coli* may prove to be unsuitable as a host/vector system. For example, *E. coli* contains a number of pyrogenic factors that must be eliminated from any potentially useful pharmaceutical product, and higher eukaryotic proteins are not processed and modified accurately by prokaryotic cells. These and other considerations have led to increased interest in alternative host/vector systems; in particular, the use of eukaryotic systems for the production of eukaryotic products is appealing. Amongst the eukaryotic organisms suitable for exploitation, perhaps the easiest to manage is the yeast, *Saccharomyces cerevisiae*. It has a highly developed genetic system and, recently, plasmids that can be used as vectors in yeast have been developed (Kingsman *et al.*, 1979; Struhl *et al.*, 1979).

When Beggs *et al.* (1980) introduced the rabbit β-globin gene into yeast on a 2μ-based recombinant molecule, transcription started at a position down-stream from the normal initiation site and terminated prematurely in the second intron. The first intron was not excised from this aberrant transcript. Also, the herpes simplex virus (HSV) thymidine kinase gene is not expressed in yeast unless it is placed under the control of a yeast promoter (Kiss *et al.*, 1982). These results suggest that transcription initiation and termination signals in yeast are specific and that yeast cannot process mammalian gene transcripts containing introns. This is interesting because yeast genes contain many of the canonical sequences in their 3′ and 5′ regions that are common to higher eukaryotic genes (Dobson *et al.*, 1982) and, at least in the case of the yeast actin gene, the coding sequence is interrupted by a single intron with exon-intron boundaries similar to those of mammalian and avian genes (Gallwitz and Sures, 1980; Breathnach *et al.*, 1978). To express a mammalian gene in yeast it appears that a coding sequence, lacking introns, must be placed under the control of a yeast promoter. Recently Hitzeman *et al.* (1981) fused a human interferon-alpha coding sequence to the yeast *ADH1* "promoter" region and obtained expression of the human gene in yeast. However, levels of expression were relatively low, apparently even with high copy number 2μ plasmid vectors.

We have recently analysed the 5′ control regions of a number of yeast genes (Dobson *et al.*, 1982) and, in addition to there being considerable conservation of signals likely to be involved in the specificity of transcription initiation, there is also significant conservation of structure in the ATG translation initiation environment. The importance of these translation initiation signals has been emphasised by Kozak (1981). In yeast the most striking conserved structures are an A at −3 and a pyrimidine, usually T, at +6 (Dobson *et al.*, 1982). Neither of these conditions were met in previous reports in which transcription fusions were used (Hitzeman *et al.*, 1981). Therefore, to maximise both transcriptional and translational efficiency we chose to fuse a human interferon-alpha coding sequence to a few codons of an abundant yeast protein, phosphoglycerate kinase *(PGK)* so that transcription and translation signals would be "wild-type". The 5′ control regions of the glycolytic enzyme genes of yeast are particularly attractive for incorporation into yeast expression vectors as each gene encodes 1–5% of total mRNA and protein and they are readily regulated by glucose. This gives the potential of placing high level heterologous gene expression under the simple control of the carbon source.

We describe here the transcriptional and translational fusion of the yeast *PGK* 5′ region to a human interferon-alpha coding sequence. The yeast plasmids containing this fusion also contain a 2μ-plasmid replication origin and the yeast *LEU2* gene which permit stable replication and high fidelity segregation of a high copy number of plasmids. The result is synthesis of human interferon-alpha at levels much higher than previously reported and under the simple control of glucose. This system can produce at least 15 mg of interferon/l of batch culture and can be used to direct the high level expression of a wide range of coding sequences (A.J. Kingsman *et al.*, unpublished data).

Results

PGK-interferon fusion plasmid construction

The source of the human interferon-alpha coding sequence was plasmid N5H8 (Slocombe *et al.*, 1982). This plasmid is a pAT153 derivative containing an interferon-alpha-2 cDNA inserted at the *Bam*HI site using *Bam*HI synthetic linkers (Figure 1a). There are two *Pvu*II sites in N5H8 both within the interferon-coding sequence. One is at a position corresponding to signal sequence amino acid S15 and the other between the codons corresponding to amino acids 92 and 93 in the mature protein. N5H8 was partially cleaved with *Pvu*II and full length linear molecules were purified by electroelution from an agarose gel. These were then mixed with a 50-fold molar excess of a synthetic double-stranded oligonucleotide with the sequence 5′-GGATCC.ATG.GG-3′ and ligated. This mix was used to transform *E. coli* strain

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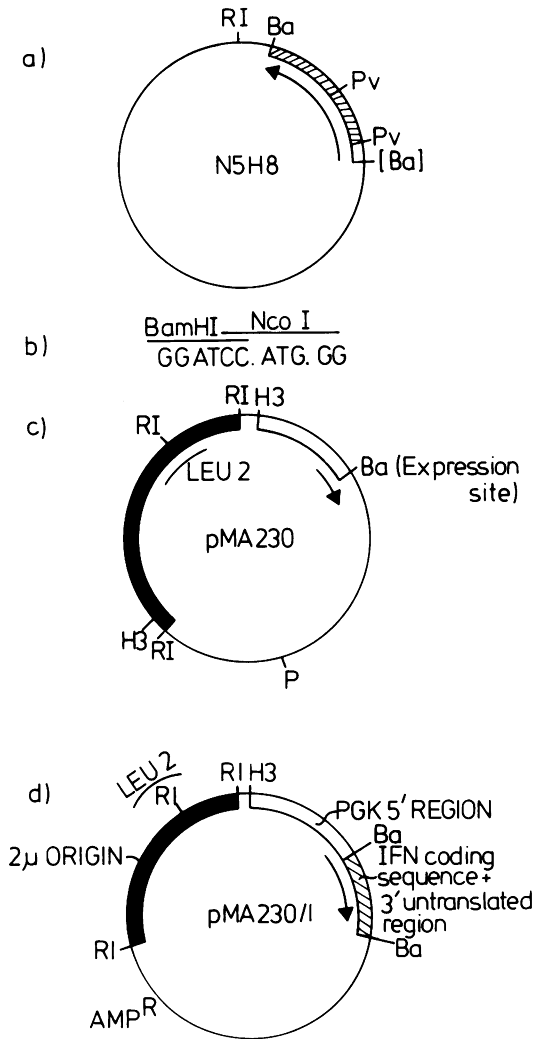

Fig. 1. (**a**) Plasmid N5H8. Hatched area is the region of the interferon-alpha coding sequence used in the construction of pMA230-1. Arrow marks orientation of coding sequence. (**b**) Synthetic oligonucleotide. (**c**) Plasmid pMA230. Thin line = pBR322 sequences; thick line = yeast DNA region containing 2μ origin of replication and *LEU2* gene; open box = 5′ region of *PGK*. Arrow marks direction of transcription. (**d**) Plasmid pMA230-1. Thin and thick lines and open box as in (c); hatched area as in (a). Arrow marks direction of transcription. RI = *Eco*RI; Ba = *Bam*HI; Pv = *Pvu*II; H3 = *Hin*dIII; P = *Pst*I; (Ba) = defective *Bam*HI site.

AKEC28 to ampicillin resistance. Plasmids from individual transformants were screened for the presence of a *Bam*HI site at the position of the site closest to the 5′ end of the interferon insert in N5H8. Plasmids of this type therefore contain a convenient *Bam*HI fragment containing the entire interferon-alpha coding sequence with most of the signal sequence codons removed (Figure 2b). The orientation of the synthetic oligonucleotide with respect to the coding sequence was checked by determining whether an *Nco*I site was linked to the interferon-alpha *Bam*HI fragment when it was inserted in other vectors (Figure 1b). A plasmid, pMA25, has the oligonucleotide inserted at the 5′-proximal *Pvu*II site and in the orientation *Bam*HI-*Nco*I-'interferon-coding sequence'. The *Bam*HI fragment containing the interferon-coding sequence in pMA25 therefore can be used in both transcriptional and translational fusions as the oligonucleotide contains an ATG at which translation initiation may occur.

The expression vector, plasmid pMA230, is shown in Figure 1c. It comprises a 2μ plasmid origin of replication and the yeast *LEU2* gene on a double *Eco*RI fragment inserted into the *Eco*RI site of pBR322. It also contains ~1500 nucleotides of *PGK* 5′-flanking region and 11 codons of the *PGK*-coding sequence contained on a *Hin*dIII-*Bam*HI fragment. Plasmid pMA230 is a member of a series of molecules designated the pMA22a deletion series described by Dobson *et al.* (1982). Figure 2a shows part of the *PGK* 5′-flanking region on pMA230 and the position of a *Bam*HI linker, CCGGATCC, 11 codons into the coding sequence. This *Bam*HI linker forms the unique *Bam*HI expression site in pMA230.

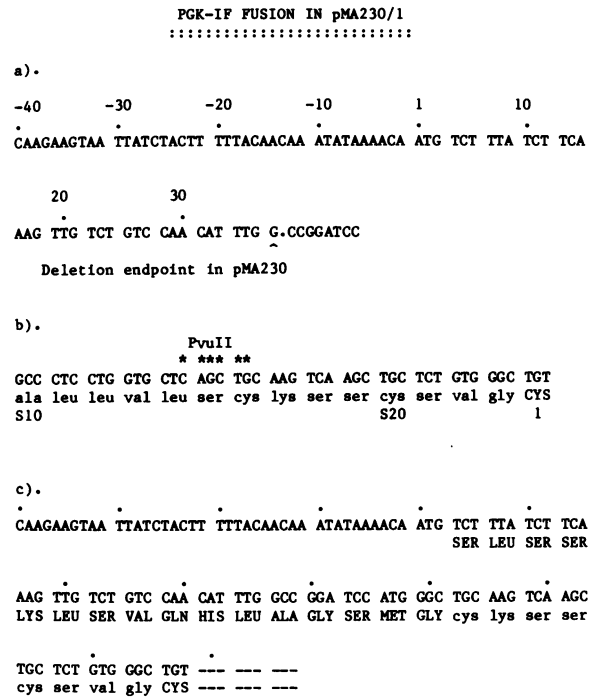

Fig. 2. (**a**) Nucleotide sequence of *PGK* gene preceding the *Bam*HI expression site in pMA230. The *Bam*HI linker is shown after the full stop. (**b**) Nucleotide sequence of plasmid N5H8 in the vicinity of the 5′ proximal *Pvu*II site. (**c**) Nucleotide sequence of plasmid pMA230-1 in the vicinity of the *PGK*-interferon junction. Amino acids in lower case are derived from the signal sequence or the *Bam*HI linker.

The *Bam*HI fragment carrying the interferon-alpha coding sequence from pMA25 was inserted into the *Bam*HI site of pMA230 to fuse the 11 codons of *PGK* to the interferon-alpha sequence. The resulting plasmid, pMA230-1, is shown in Figure 1d and the precise structure of the fusion is shown in Figure 2c.

Expression of human interferon-alpha in yeast

Interferon yields from batch cultures of yeast strain, MD40-4c transformed with either plasmid pMA230, the expression vector alone, or plasmid pMA230-1, containing the interferon-alpha coding sequence, were determined initially by the virus RNA reduction assay in EBTr cells (Table I). Using a value of 2×10^8 units/mg for the specific activity of interferon-alpha on bovine cells (Hitzeman *et al.*, 1981) and an approximate mol. wt. of 20 K for the *PGK*-interferon fusion protein, the data indicate a yield of 1.2×10^7 molecules of interferon per viable yeast cell containing pMA230-1. The interferon activity in extracts from yeast containing pMA230-1 gave a dose-response curve identical to that of reference interferon-alpha (data not shown) and was confirmed as inter-

Table I. Expression of interferon-alpha in yeast

Plasmid	Total cells	Total protein (mg)	Interferon titre[a] RU/ml[b]	Interferon titre[c] in presence of specific antibody	Molecules[d] of interferon /cell
pMA230	3 x 10^9	9	5.6 x 10^2	3.2 x 10^2	–
pMA230-1	2 x 10^9	10	1.6 x 10^8	6.3 x 10^2	1.2 x 10^7

[a]Interferon was assayed by SFV RNA reduction in EBTr cells.
[b]Volume of extract was 1 ml. RU = reference unit. An international leukocyte interferon standard (5 x 10^3 RU/ml) was included in all assays and all titres were adjusted relative to the standard. In this experiment actual titres were 10-fold higher than shown in the table.
[c]Cells were treated with a 1:2000 dilution of an anti-leukocyte interferon antibody before adding dilutions of the test interferon.
[d]Calculations based on a specific activity of 2 x 10^8 units/mg and a mol. wt. of 20 K.

feron-alpha since its activity was reduced by >100 000-fold by specific anti-interferon-alpha antibody. In addition, 100% of the activity was retained on NK2-Sepharose, a monoclonal antibody affinity column specific for interferon-alpha-2 (Secher and Burke, 1980). The control yeast extracts from strains containing pMA230 always displayed a low level of apparent activity (<10^3 units/ml) which did not show a typical interferon dose-response curve and was not affected by specific anti-interferon-alpha antibody. The data in Table I suggest that 2 x 10^9 cells containing pMA230-1 yield 2.4 x 10^{16} molecules of interferon-alpha which, assuming a specific activity of 2 x 10^8 units/mg, is ~1 mg of interferon, that is 10% of the total protein in the culture. However, analysis of the protein profile of interferon-containing yeast extracts by SDS-polyacrylamide gel electrophoresis (PAGE) indicates that this is an over-estimate of yields (Figure 3). A prominent new protein band is clearly present in extracts of MD40-4c containing pMA230-1 (lane b) and is not detectable in pMA230 containing cell extracts (lane a). This *PGK*-interferon fusion protein is clearly one of the major proteins but it is probably 1–2% of the total protein rather than 10%. That this new band is in fact the *PGK*-interferon fusion protein is confirmed by its identical mobility to the major band eluted from the NK2-Sepharose column (Figure 3, lanes b and c) and the mol. wt. of 20 K estimated from the gel is that expected for the fusion protein. The other bands in the NK2-Sepharose eluate are non-specifically bound major yeast proteins which are also seen in control extracts which lack interferon activity.

To resolve the difference between the relative yields of interferon indicated by SDS-PAGE and levels calculated by the virus RNA reduction assay on bovine cells, the assays were repeated using bovine (EBTr), human (HEp-2), and mouse (L929) cells and compared with human reference interferon-alpha (69/19) (Table II). The relative species specificity (bovine>human>mouse) of the interferon produced in yeast is characteristic of interferon-alphas (Stewart, 1979). However, it is clear that interferon-alpha-2, as a single interferon species made in yeast, has a higher relative titre on bovine cells than on human cells when compared to the reference interferon-alpha. This has also been observed for single interferon-alpha species synthesised in *E. coli* (Slocombe *et al.*, 1982; Weck *et al.*, 1981). Because of the higher sensitivity of bovine cells to a pure interferon-alpha species, it is likely that a specific activity of 2 x 10^8 units/mg only holds for assays on human cells and consequently our interferon yields,

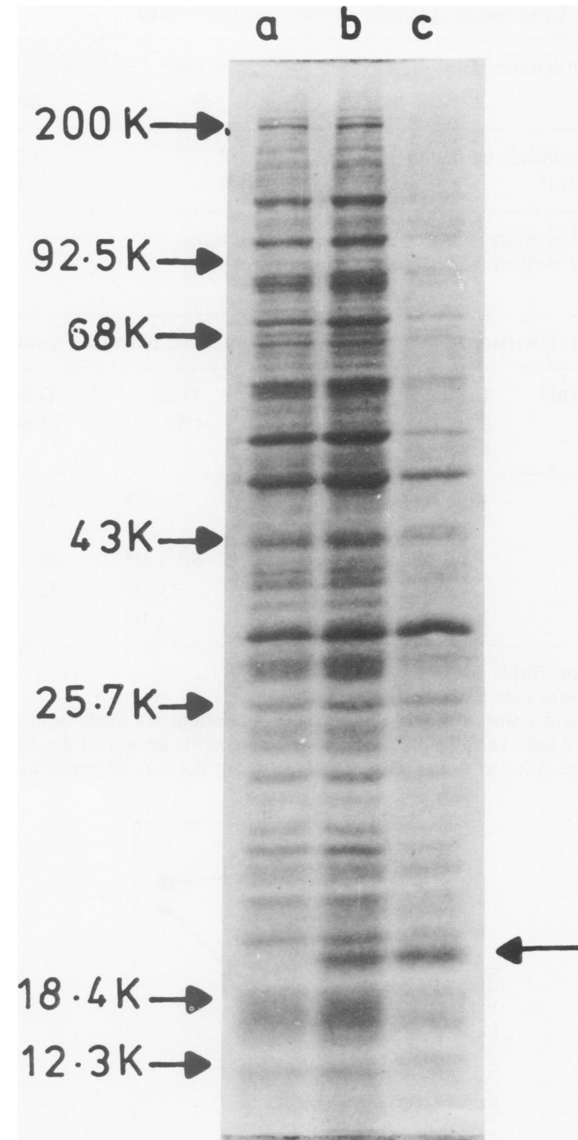

Fig. 3. Coomassie stained SDS-PAGE protein profiles. **(a)** Total protein from MD40-4c containing pMA230. **(b)** Total protein from MD40-4c containing pMA230-1. **(c)** Protein from MD40-4c containing pMA230-1 after partial purification on an NK2 column. The positions of mol. wt. markers are shown. An arrow marks the position of the *PGK*-interferon fusion protein.

except for those in Table I for direct comparison with previous data (Hitzeman *et al.*, 1981), are based on specific activity values for the fusion protein of 2 x 10^8 units/mg on human cells and 10^9 units/mg on bovine cells. Using this adjusted specific activity, protein yields calculated from the assay data are 1–2% of total protein which agrees with the SDS-PAGE analysis.

The data from several separate experiments are combined in Table III. Yields in terms of molecules/cell are very reproducible, irrespective of culture density or whether growth is in media selective for the plasmid-borne *LEU2* marker, and the average yield is 2.3 x 10^6 molecules/cell. As yeast cultures grown in rich medium attain cell densities of 2 x 10^8 cells/ml, the theoretical yield of interferon from 1 litre of batch culture using this system is 15 mg.

Interferon expression is regulated by glucose

The expression of the unlinked glycolytic enzyme genes of

Table II. Cross species specificity of yeast interferon-alpha.

Source of interferon	Interferon titre[a]			
	Human cells	Bovine cells	Mouse cells	Bovine/human ratio
Reference interferon (69/19)	2.8×10^3	3.2×10^4	10^2	11.4
Yeast/230-1[b]	2.2×10^5	9×10^6	2.5×10^2	41

[a]Assayed by RNA reduction assay; uncorrected titres.
[b]Partially purified by NK2 column chromatography.

Table III. Interferon levels in yeast containing plasmid pMA230-1 grown under different conditions.

Experiment[b]	Culture density (cells/ml)	Medium	Total cells	Total protein (mg)	Total[d] interferon (RU)	Total[c] interferon molecules	Interferon molecules /cell	Interferon as percent total protein
1[a]	3.6×10^6	−Leucine	2×10^9	10	1.6×10^8	4.8×10^{15}	2.4×10^6	1.6
2	2×10^6	−Leucine	10^9	7	10^7	1.5×10^{15}	1.5×10^6	0.7
3	10^7	−Leucine	6.5×10^9	24	8×10^7	1.2×10^{16}	1.8×10^6	1.6
4	7×10^7	+Leucine	3×10^9	12	6.4×10^7	9.6×10^{15}	3.2×10^6	2.6
5	3.8×10^7	+Leucine	2×10^9	–	1.2×10^7	2×10^{15}	1.0×10^6	–

[a]Data from Table I.
[b]All cultures were shaken at 30°C.
[c]Experiment 1 was assayed on EBTr cells, calculations are made from a specific activity of 10^9 units/mg/3×10^{16} molecules. Experiments 2–5 were assayed on HEp-2 cells, calculations are made from a specific activity of 2×10^8 units/mg/3×10^{16} molecules.
[d]Titres are adjusted to the reference standard but not for cell sensitivity.

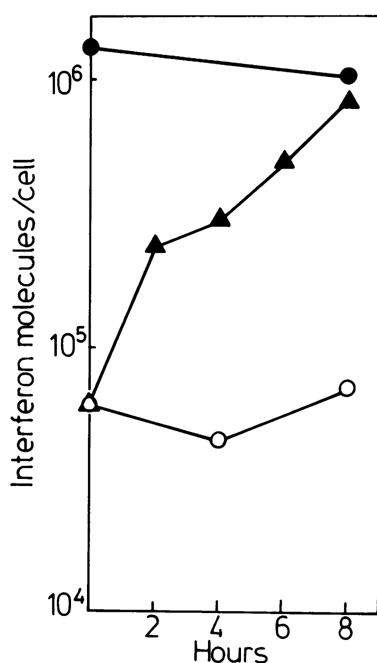

Fig. 4. Regulation of interferon production by glucose. Time 0 h is the time at which inocula were introduced into fresh media. ● = glucose/glucose; ○ = acetate/acetate; ▲ = acetate/glucose. See text for explanation.

yeast is coordinately regulated by the carbon source. When cells are grown on a fermentable carbon source, such as glucose, enzyme levels are 100-fold higher than when they are grown on a non-fermentable source (Hommes, 1966; Maitra and Lobo, 1971) and there is good evidence that this regulation occurs at the level of transcription (Holland and Holland, 1978). Therefore, it was of interest to determine whether the structures necessary for the recognition of this regulatory system are present on the 1500-nucleotide *PGK* fragment in pMA230-1 and if so whether human interferon-alpha expression could be regulated by glucose.

Yeast strain MD40-4c containing pMA230-1 was grown in rich medium with acetate as carbon source for 12 generations to a density of 2×10^6 cells/ml. These cells were used as inocula for two flasks of fresh medium, one containing glucose as carbon source and the other acetate. A second batch of cells grown on glucose was used to inoculate a fresh glucose culture. Therefore, there were three inoculum/culture conditions: acetate/acetate; acetate/glucose; glucose/glucose. Aliquots of these cultures were taken at various intervals, extracts were prepared and interferon levels were assayed by c.p.e. reduction in HEp-2 cells. The data in Figure 4 show that the glucose/glucose culture contains relatively high interferon levels while the acetate/acetate culture has low levels over the course of the experiment. The acetate/glucose culture exhibits increasing levels of interferon after the cells are transferred to glucose medium (time 0, Figure 4). This induction of interferon occurs over a period of ~8 h and the levels of interferon produced by cells grown on glucose are 20- to 30-fold higher than in cells grown on acetate.

While these results strongly suggest that carbon source control of interferon levels is being mediated by the 5′ control region of the *PGK* gene, it is important to establish that there is no difference in plasmid stability in cells grown on acetate or glucose. Therefore, total DNA was prepared from aliquots of yeast cells taken at various points during the experiment described in Figure 4. The DNA was digested with *Eco*RI and fragments were separated on a 1% agarose gel. The fractionation bands were then blotted onto nitrocellulose and hybridised with [^{32}P]YRp7. Plasmid YRp7 is pBR322 containing a unique 1.45-kb *Eco*RI fragment from the yeast genome (Struhl *et al.*, 1979). The pBR322 component of this probe served to quantitate levels of pMA230-1 in the yeast DNA preparations while the sequences of the 1.45-kb fragment were used to control for amounts of DNA, transfer efficien-

CODON USAGE - B:N5H8LeIF.SEQ

	Codon	(%)	No.		Codon	(%)	No.		Codon	(%)	No.
LEU:	TTA	(5)	1	SER:	AGT	(0)	2	ARG:	AGA	(89)	5
:::	TTG	(91)	4	:::	AGC	(0)	6	:::	AGG	(0)	4
	CTA	(2)	1		TCA	(2)	3		CGA	(0)	0
	CTG	(0)	10		TCG	(0)	0		CGG	(0)	0
	CTT	(2)	1		TCT	(46)	5		CGT	(10)	0
	CTC	(0)	8		TCC	(49)	2		CGC	(0)	0
ALA:	GCA	(0)	2	GLY:	GGA	(0)	1	VAL:	GTA	(0)	0
:::	GCG	(0)	0	:::	GGG	(0)	2	:::	GTG	(0)	7
	GCT	(78)	4		GGT	(99)	1		GTT	(50)	1
	GCC	(22)	3		GGC	(1)	2		GTC	(50)	2
PRO:	CCA	(89)	0	THR:	ACA	(0)	3	ILE:	ATA	(0)	1
:::	CCG	(0)	0	:::	ACG	(0)	0				
	CCT	(11)	3		ACT	(48)	3		ATT	(50)	1
	CCC	(0)	2		ACC	(52)	4		ATC	(50)	6
PHE:	TTT	(8)	4	CYS:	TGT	(100)	3	HIS:	CAT	(8)	2
:::	TTC	(92)	6	:::	TGC	(0)	3	:::	CAC	(92)	1
GLN:	CAA	(100)	4	ASN:	AAT	(3)	2	ASP:	GAT	(16)	2
:::	CAG	(0)	8	:::	AAC	(97)	2	:::	GAC	(84)	6
GLU:	GAA	(97)	6	TYR:	TAT	(2)	1	LYS:	AAA	(14)	5
:::	GAG	(3)	8	:::	TAC	(98)	4	:::	AAG	(86)	7
MET:	ATG	(100)	5	TRP:	TGG	(100)	2	STOP:	TAA		0
:::				:::				::::	TAG		0
									TGA		1

Fig. 5. Comparison of interferon-alpha-2 and abundant yeast protein codon usage. Figures in brackets show the frequency of use of codons in abundant yeast proteins as a percentage. Figures not in brackets show the absolute number of times a codon is used in interferon-alpha coding sequence from N5H8.

cies, and hybridisation efficiencies. In addition to this Southern blot analysis, the proportion of Leu^+ cells in the aliquots was measured by comparing colony counts on media with and without leucine. In all cases Southern hybridisation profiles were identical and >99% of cells were Leu^+ (data not shown) showing that growth on acetate or glucose has no effect on plasmid copy number or stability.

Discussion

We have shown that the synthesis of high levels of human interferon-alpha can be directed by yeast expression signals and that the amounts of interferon produced can be controlled by the availability of fermentable carbon source. This system can be modified to direct the regulated expression of potentially any eukaryotic or prokaryotic coding sequence; for example we have obtained efficient expression of the *E. coli* β-galactosidase gene and the HSV thymidine kinase gene in this system.

Comparison of the levels of interferon produced in this system with those obtained in *E. coli* shows that there is little difference between the systems on the basis of quantities of interferon produced per litre of batch culture (e.g. Goeddel *et al.*, 1980; Slocombe *et al.*, 1982). Comparison with the levels produced previously in yeast (Hitzeman *et al.*, 1981) are complex. It is clear from the discrepancies between the levels of interferon produced in the *PGK*-based system calculated from assays on bovine cells and estimated from SDS-PAGE that a specific activity of 2×10^8 units/mg is too low when applied to bovine cell assays (Figure 3, Tables I, II, and III). The higher susceptibility of bovine, as compared to human cells to interferon-alpha is well established (Stewart, 1979) and is accommodated in our assays by standardisation to an international reference interferon-alpha preparation. However, it also appears that individual interferon-alpha species may have different relative specific activities on bovine and human cells (Weck *et al.*, 1981; Slocombe *et al.*, 1982). This means that quantitation of interferon levels on the basis of converting activity in units to an estimate of mass *via* a specific activity (Hitzeman *et al.*, 1981) is prone to error. In the *PGK* system, sufficient interferon is produced to permit reasonable quantitation in SDS-PAGE (Figure 3). We are therefore confident that the estimation of the amounts of interferon produced in the *PGK*-based system are correct.

While the levels of interferon produced in this system are high they are perhaps not as high as they might be for a system that exploits a 5′ region that directs the expression of at least 1% of cell mRNA and protein (Holland and Holland, 1978) borne on a multicopy 2μ-based plasmid. The reason for this may be manifold but two deficiencies in this system are obvious. Firstly, the 3′ end of the interferon coding sequence has not been fused to a yeast 3′ region. This may result in increased lability of the interferon transcript in yeast. Zaret and Sherman (1982) have recently shown that a 38-bp deletion in the 3′ non-translated region of the *CYC1* gene in yeast decreases specific mRNA levels by 90−95% suggesting that specific 3′ signals are required for efficient gene expression. Secondly, the codon usage for the human interferon-alpha coding sequence used in this study differs in some respects from that of abundant yeast proteins. Figure 5 shows a comparison of the codon usage for abundant yeast proteins and the coding sequence for interferon-alpha from plasmid N5H8. There are several minor differences but the major discrepancy is in the leucine codons. In yeast by far the most favoured codon is TTG whereas in the interferon-alpha sequence the preferred codon is CTG.

Clearly this system has great potential for the production of important polypeptides in yeast. However, it also provides a very sensitive system for the analysis of the 5′ control region of the yeast *PGK* gene. The observation that interferon levels can be increased by growing cells in the presence of glucose means that all the regulatory signals that mediate this control are on the 1.5-kb *Hind*III-*Bam*HI *PGK* fragment in pMA230. The system is therefore amenable to a detailed *in vitro* mutagenesis study in which gene expression can be monitored with great precision by using the sensitivity of the interferon bioassays.

Materials and methods

Bacterial and yeast strains and media

E. coli strain AKEC28 = C600 *thrC leuB6 thyA trpC1117 hsdRk hsdMk*. *S. cerevisiae* strain MD40-4c = *ura2 trp1 leu2-3 leu2-112 his3-11 his3-15*. *E. coli* were grown in Luria Broth (Miller, 1972). Yeast media were prepared according to Hawthorne and Mortimer (1960).

DNA isolation

Plasmid DNA was isolated from *E. coli* by a procedure described in Chinault and Carbon (1979). Total yeast DNA was isolated according to Cryer *et al.* (1975).

Yeast transformation

The method described by Hinnen *et al.* (1978) was used.

Enzymes and restriction fragment purification

Restriction endonucleases were purchased from Bethesda Research Laboratories (BRL). Fragments were purified from agarose by the method of Tabak and Flavell (1978).

Southern transfers, hybridisation, and in vitro labelling

DNA fragments fractionated on agarose gels were transferred to nitrocellulose sheets by a modification of Southern's procedure (Southern, 1975) as previously described (Chinault and Carbon, 1979). DNA was labelled using [^{32}P]TTP (Amersham) by nick-translation (Rigby *et al.*, 1977). Hybridisation was carried out in 0.3 M NaCl, 0.03 M sodium citrate, 0.02% polyvinyl pyrrolidine, 0.02% bovine serum albumin (BSA), 0.02% ficoll at 65°C for 48 h.

Ligation reactions

Blunt end ligations were carried out at 20°C for 6 h in 20 mM Tris-HCl, 7.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM ATP, 1 mM dithiothreitol, 1 mM spermidine with 400 units of T4 DNA ligase (New England Biolabs.).

Yeast extracts, protein estimation, and gels

Yeast cells were grown in synthetic complete medium lacking leucine at 30°C. Cultures were harvested at $2 \times 10^6 - 2 \times 10^7$ cells/ml and spheroplasted with zymolyase (Kuo and Yamamoto, 1975). Spheroplasts were washed in 1 M sorbitol before lysing in 7 M guanidine hydrochloride, 1 mM phenylmethylsulphonyl fluoride (Hitzeman *et al.*, 1981). Cell debris was removed by centrifugation and lysates stored at -70°C; spheroplasts from 500-ml cultures were lysed in $1 - 3$ ml. No effect of culture density up to 7×10^7 cells/ml or dialysis of extracts on subsequent interferon yields was observed. Total protein concentrations of yeast extracts were estimated by standard procedures (Lowry, 1951). Extracts were analysed by SDS-PAGE (Kingsman *et al.*, 1980). Samples ($\sim 30\ \mu g$) were precipitated from acetone before loading (Tuite *et al.*, 1980). The mol. wt. of the interferon produced in yeast was estimated from the relative mobility of myosin (200 000), phosphorylase b (92 500), BSA (68 000), ovalbumin (43 000), α-chymotrypsin (25 700), β-lactoglobulin (18 400), and cytochrome c (12 300).

Interferon assays and immunochromatography

Interferon was assayed either on Hep-2 (human) in a c.p.e. reduction assay (Nagata *et al.*, 1980) using encephalomyocarditis virus as the challenge virus or on HFF (human), L929 (mouse), or bovine (EBTr) cells (a gift from J.Vilcek, New York University Medical Centre, NY) using a virus RNA reduction assay with Semliki Forest virus as the challenge (Atherton and Burke, 1975). Cells were grown in Glasgow Modified Eagles medium with 10% new born calf serum. Interferon in crude yeast extracts was purified by immunochromatography on NK2-Sepharose as described by Secher and Burke (1980). Reference interferon was the Medical Research Council human interferon-alpha (69/19). The anti-leukocyte interferon antibody was a gift from A.Meager, National Institute for Biological Standards and Controls, London.

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