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Saccharomyces cerevisiae Secretes and Correctly Processes Human Interferon Hybrid Proteins Containing Yeast Invertase Signal Peptides

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Synthetic oligonucleotides coding for the yeast invertase secretion signal peptide were fused to the gene for the mature form of human interferon (huIFN-α2). Two plasmids (E3 and F2) were constructed. E3 contained the invertase signal codons in a reading frame with the mature huIFN-α2 gene. F2 had a deletion of the codon for alanine at amino acid residue −5 in the invertase signal and an addition of a methionine codon located between the coding sequences for the invertase signal and mature huIFN-α2. Both hybrid genes were located adjacent to the promoter from the 3-phosphoglycerate kinase gene on the multicopy yeast expression plasmid, YEp1PT. Yeast transformants containing these plasmids produced somewhat more IFN than did the same expression plasmid containing the IFN gene with its human secretion signal sequence. HuIFN-α2, purified from the medium of yeast cells containing E3, was found to be processed at the correct site. The huIFN-α2 made by plasmid F2 was found to be completely processed at the junction between the invertase signal (a variant) and the methionine of methionine-huIFN-α2. These results strongly suggested that the invertase signal (or its variant) attached to huIFN was efficiently recognized by the presumed signal recognition particle and was cleaved by the signal peptidase in the yeast cells. These results also suggested that amino acid changes on the right side of the cleavage site did not necessarily prevent cleavage or secretion.

The mechanisms for protein secretion in the lower eucaryote *Saccharomyces cerevisiae* are similar to those found in animal cells. Novick and Schekman (21, 24) have isolated various temperature-sensitive yeast mutants which have defects in this secretion process. By using these mutants, they have elucidated a secretory pathway in yeast cells, which involves the transfer of secretory proteins from the site of their synthesis at the rough endoplasmic reticulum to the site of their discharge at the plasma membrane. Perlman and Halvorson (22) have shown by in vitro translation that yeast secretory proteins such as invertase and acid phosphatase are made as larger precursors bearing their respective amino terminal signal sequences. The function of the signal sequence in yeast cells is presumably the same as in mammalian cells, i.e., to interact with the signal recognition particle allowing translocation of secretory proteins across the membrane of the rough endoplasmic reticulum (30). Subsequently, the signal sequence is removed by the signal peptidase, a membrane-associated endopeptidase, to generate the mature protein.

Although yeast and mammalian cells have many similarities in the secretion of their proteins, yeast cells cannot necessarily process and secrete mammalian proteins as precisely as they do their own secretory proteins. For example, when human growth hormone (hGH) is expressed in yeast cells, only 10% of hGH is processed and secreted, whereas the vast majority of hGH remains in an intracellular location as a preprotein (11). Furthermore, expression of the human alpha interferon (huIFN-α2) gene has yielded three forms of huIFN-α2 that are cell associated as well as two forms that are free in the medium (13). Two of these forms are the mature protein, and the rest contain various portions of the signal sequence preceding the mature protein (see Fig. 5). Therefore, it appears that the signal sequences of these two human secretory proteins are to some extent improperly recognized by the presumed signal recognition particle (31) and signal peptidase of yeast cells.

To investigate whether the signal sequence of a yeast secretory protein was more efficient for secretion and processing of huIFN-α2 than was its natural human signal sequence, we constructed plasmids in which the gene encoding huIFN-α2 was fused to the yeast invertase signal coding sequence. These hybrid genes contained no mature invertase sequence and were expressed under the control of the 3-phosphoglycerate kinase (PGK) promoter. Yeast cells transformed with these plasmids efficiently synthesized and secreted the mature form of huIFN-α2. In this paper we describe the synthesis, processing, and secretion by yeast cells of hybrid proteins containing the yeast invertase signal fused to the mature form of huIFN-α2.

(A preliminary account of this work has been presented [C. N. Chang, M. Matteucci, L. J. Perry, C. Y. Chen, and R. A. Hitzeman, Abstracts of the 1983 Meeting on the Molecular Biology of Yeast, Cold Spring Harbor, N.Y.].)

MATERIALS AND METHODS

Materials. All DNA restriction enzymes, *Escherichia coli* DNA polymerase large fragment (Klenow polymerase I), T4 DNA ligase, and avian myeloblastosis virus reverse transcriptase, were products of either New England BioLabs, Inc. (Beverly, Mass.) or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and were used as recommended by the manufacturers. The deoxynucleotide triphosphates

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dATP, dGTP, dCTP, and dTTP were obtained from P-L Biochemicals, Inc. (Milwaukee, Wis.).

Bacterial and yeast strains. *E. coli* K-12 294 (*endA thi hsr hsm*$^+$; ATCC 31446) (1) and yeast strain 20B-12 (α *trp1 pep4-3*; ATCC 20626) (14) were used throughout this study.

Growth media. *E. coli* was grown in LB medium (20) which contained 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract (Difco), and 0.5% NaCl. Antibiotic (20 μg of ampicillin or tetracycline per ml) was added for growth of the plasmid-containing bacteria.

Yeast minimal medium plates contained 0.67% yeast nitrogen base (Difco) without amino acids, 2% glucose, and 3% Bacto-Agar. Minimal medium with the addition of 1 M sorbitol was used for yeast transformation. The minimal medium was supplemented with 10 mg of adenine, 10 mg of uracil, and 5 g of Casamino Acids (Difco) per liter to form YNB + CAA medium. YEPD medium contained 1% yeast extract (Difco), 2% Bacto-Peptone, and 2% glucose.

Yeast cells were grown in a 10-liter fermentor at 30°C in 2.6% yeast nitrogen base and 1% glucose to an A_{550} between 3 and 4. At this cell density, the initial 1% glucose was completely exhausted; cells were then slowly fed with glucose until A_{550} reached 50 to 100.

Transformations. *E. coli* 294 was transformed according to the published procedure (18). Yeast strain 20B-12 was transformed by the method of Hinnen et al. (10).

Preparation and analysis of DNA. Plasmid DNAs from individual *E. coli* transformants were prepared by the method of Birnboim and Doly (3). This method was also used for large-scale preparation of plasmid DNAs. They were further fractionated by Bio-Gel A-50M (100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) column chromatography to remove tRNA.

Polyacrylamide and agarose gel electrophoresis for analysis of plasmid DNAs were performed as described by Maniatis et al. (19) and by Sharp et al. (25), respectively. DNA restriction fragments were isolated by electroelution from the gels, followed by phenol and chloroform extraction and ethanol precipitation.

Northern blotting of mRNA. Total yeast RNA was prepared from the log phase of yeast cell culture (A_{660} = 1), which was denatured and electrophoresed on a 1.0% agarose gel containing MOPC buffer (20 mM MOPC, 5 mM sodium acetate, and 1 mM EDTA at pH 7.0) as described previously (12). The gel was then transferred to nitrocellulose paper and hybridized with either the *Bgl*II fragment of the huIFN-α2 gene (9) or the *Eco*RI-*Bgl*II fragment of the yeast PGK gene (12). These probes had previously been labeled with [α-^{32}P]ATP by using small fragments of calf thymus DNA as primer (12).

Design of oligonucleotides coding for the invertase signal peptide. Two 40mer oligonucleotides were designed as shown in Fig. 1, based on the known nucleotide sequence coding for the yeast invertase signal region (29). An additional ATG just preceding the ATG initiation site of translation was retained as in the original nucleotide sequence of yeast invertase. A convenient *Eco*RI restriction site was placed in front of the ATGATG with two additional nucleotides (CC) at the 5′ end. These two oligonucleotides have 12 base pairs (bp) complementary to each other at their 3′ ends. After these oligonucleotides were repaired with the reverse transcriptase, the 3′ end of the newly formed double-stranded DNA became a blunt end which was subsequently ligated to the blunt end of a mature methionine-huIFN-α2 (MetIFN-α2) gene maintaining the proper translation reading frame. The 5′ end of the invertase signal sequence was digested with *Eco*RI, facilitating its ligation to the *Eco*RI-digested end of an appropriate promoter fragment.

Preparation of yeast extract and IFN assays. For IFN assays, 10 ml of the yeast cell culture was centrifuged at 10,000 rpm for 10 min in a Sorvall RC3B (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant (media) was diluted in phosphate-buffered saline-bovine serum albumin buffer (20 mM NaH_2PO_4 [pH 7.4], 150 mM NaCl, 0.5% bovine serum albumin) immediately before the assay. The cells (about 10 A_{550} U) were suspended in 0.4 ml of 7 M guanidine hydrochloride in an Eppendorf (1.5-ml) tube containing about 0.4 ml of glass beads (0.45 to 0.5 mm; B. Braun Melsurgen AG), vortexed twice for 2 min at the highest speed, and centrifuged in an Eppendorf microcentrifuge for 0.5 min. The cell lysate was then diluted in phosphate-buffered saline-bovine serum albumin buffer as above for bioassays.

Yeast extracts and media were assayed for IFN by comparison with an IFN standard by using the cytopathic effect inhibition assay (27). Cells used in the assay were bovine kidney cells. IFN activity was expressed as units relative to the National Institutes of Health alpha IFN standard G203-901-527.

Purification of the huIFN-α2 from yeast media. Yeast 20B-12 transformants containing plasmids obtained from clones F2 and E3 were separately grown in a 10-liter fermentor, and after removal of yeast cells, the two IFNs were purified from the media. Since both huIFN-α2 proteins were purified in the same manner, only one purification is described below.

Medium (8 liters) was concentrated and diafiltered against 6 liters of ammonium acetate (pH 5.0) in a 2.5-liter Amicon stirred cell (Amicon 2000) with a YM-5 ultrafiltration membrane to a final volume of 1.7 liters. The concentrate was then diafiltered against 6 to 8 liters of 25 mM Tris hydrochloride–10 mM EDTA (pH 8.0) to a final volume of approximately 50 ml. The retentate was diluted to 530 ml, and 430 ml was passed over a 1.0-ml immunoaffinity column containing a monoclonal antibody to huIFN-α2 covalently bound to Affigel 10 at a flow rate of less than 10 ml/h. The column was washed with 25 mM Tris hydrochloride–10 mM EDTA (pH 8.0). The IFN activity was eluted from the column with 0.2 M acetic acid. Fractions (1 ml each) were collected and assayed (see Fig. 3). Fraction 20 (4.0×10^7 U/ml) was further purified by high-pressure liquid chromatography (HPLC) on a Synchropak RP-P column. The column was eluted at a flow rate of 1 ml/min with a linear gradient of 0 to 100% acetonitrile in 0.1% TFA (pH 2.5) (see Fig. 4, bottom). Fractions (1 ml each) were collected and assayed. A 5-μg sample of purified MetIFN-α2 produced in *E. coli* was also chromatographed as a control (see Fig. 4, top). The IFN activity eluted was from the column as a peak centered about fraction number 43 (9.0×10^6 U/ml). Fractions 41 to 43 were pooled and sequenced as follows.

Amino-terminal sequencing of purified huIFN-α2. HuIFN-α2 isolated from the yeast medium was subjected to consecutive Edman degradations in the Beckman 890B sequencer (8) by using the Quadrol Program. Polybrene [poly (*N*,*N*,*N*′,*N*′,-tetramethyl-*N*-trimethylenehexamethylene-diammonium diacetate) (2 mg)] was added to the sequencer cup as a protein carrier (28). The resulting 2-anilino-5-thiazolinone was added to 25% trifluoroacetic acid and heated to 70°C for 10 min to convert it into the 3-phenyl-2-thiohydantoin (PTH derivative). The PTH-amino acid residue was then dissolved in 50% acetonitrile and analyzed by a reverse-phase HPLC. Each PTH-amino acid was then

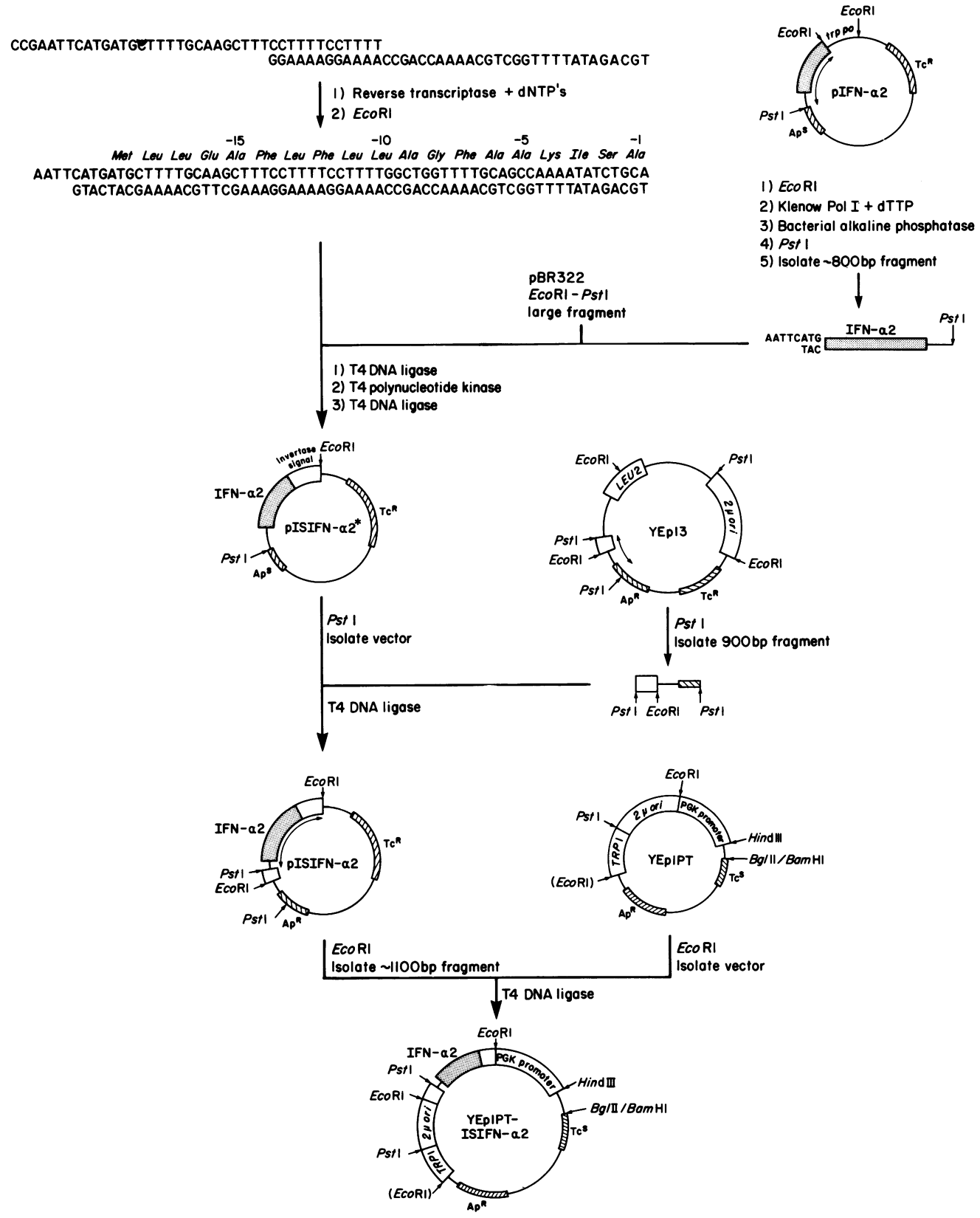

FIG. 1. Construction of a yeast secretion plasmid containing a gene coding for the hybrid invertase signal and mature form of huIFN-α2. The transcription of the hybrid gene is under the control of the yeast PGK promoter.

TABLE 1. Production of huIFN-α2 in yeast cells

IFN type[a]	Volumetric activity[b] (10^6 U/liter)				Total sp act (10^6 U/g of cells [dry/wt])	Relative product comparison[c]
	Cell associated[d]	Supernatant	Total	% in supernatant		
Preinvertase-IFN-α2 (E3)	1,155.0	88.0	1,243.0	7.0	29.0	2.4
Preinvertase-IFN-α2 (F2)	530.0	225.0	755.0	30.0	22.0	1.8
PreIFN-α2	410.0	26.0	436.0	6.0	12.0	1.0

[a] Values for E3 and F2 are the averages of two 10-liter fermentor runs; values for preIFN-α2 are the averages of five 10-liter fermentor runs.
[b] Yeast transformants were grown in 10-liter Biolafitte fermentors, and samples were taken every 6 h and processed for assays of huIFN-α2 activity as described in the Materials and Methods section. Values are peak values for a given fermentation.
[c] Compared with preIFN-α2.
[d] HuIFN-α2 may be present in yeast cell cytoplasm, within the secretory pathway, in the periplasm, or in a combination of these locations (see text).

identified by comparison to the retention times of a mixture of standard PTH-amino acids.

RESULTS

Construction of the hybrid genes. The invertase signal coding sequence was constructed from two synthetic oligonucleotides (40mers) which complemented each other for 12 bases as described in the Materials and Methods section (Fig. 1). Reverse transcriptase was used to synthesize DNA complementary to the single-stranded regions of these two oligonucleotides, resulting in a double-stranded DNA with a blunt 3′ end and an *Eco*RI-digested 5′ end after using this restriction enzyme.

The plasmid pIFN-α2 is an *E. coli* expression plasmid which contains the structural gene for mature MetIFN-α2 (9). This MetIFN-α2 gene resides within an *Eco*RI-*Pst*I fragment and does not have its natural signal coding sequence. The *Eco*RI site is located just preceding the ATG initiation codon of MetIFN-α2 (9). In order to generate the correct reading frame for MetIFN-α2 after its ligation with the invertase signal coding sequence, the nucleotide G at the 3′ strand of the *Eco*RI site had to be eliminated. This was accomplished by treating the *Eco*RI fragment of the entire plasmid pIFN-α2 with Klenow polymerase I in the presence of 25 μM dTTP. The presence of dTTP in the reaction prevents further removal of 3′ nucleotide from the coding frame of MetIFN-α2 by causing polymerization and 3′ exonuclease activities to be in equilibrium at an adenine nucleotide in the template (Fig. 1). Restriction of this Klenow-treated *Eco*RI fragment with the enzyme *Pst*I yielded an ~810-bp fragment containing the mature MetIFN-α2 gene.

Three-way ligation of the invertase signal coding sequence, the mature MetIFN-α2 gene described above, and an *Eco*RI-*Pst*I vector fragment of plasmid pBR322 (5) results in a hybrid plasmid designated as pISIFN-α2*, which should possess the correct coding frame from the invertase signal codons through the gene for mature MetIFN-α2. *E. coli* was then transformed with this ligation mix, and plasmid DNAs from individual transformants were prepared by a simple screening procedure (3). Restriction analysis of plasmid DNAs showed 6 among 30 transformants bearing the ~870-bp *Eco*RI-*Pst*I fragment, whereas the *Eco*RI-*Pst*I fragment of the mature MetIFN-α2 gene was ~810 bp (without the signal coding sequence). Dideoxy DNA sequencing (23) of these six clones showed two clones (F2 and E3) with the desired hybrid gene in the plasmid. In plasmid F2, the junction between the invertase signal codons and the MetIFN-α2 gene was as expected, whereas a trinucleotide GCC corresponding to an amino acid residue alanine (at position −5) of the invertase signal was deleted (Fig. 1; see Fig. 5). This deletion probably resulted from an error in the synthesis of oligonucleotides coding for the invertase signal region.

Plasmid E3 coded for a fusion gene encoding an invertase signal attached to mature huIFN-α2. There was no ATG prior to the mature huIFN-α2 gene of this clone. The reason for deletion of the ATG (codes for methionine) is unknown. Presumably, the limited 3′ exonuclease digestion with the Klenow fragment of DNA polymerase I did not cease at T, but rather proceeded for three more nucleotides, deleting the codon TAC (Fig. 1). However, this construction is most like the huIFN-α2 with its own natural signal (see Fig. 5).

For convenient construction of a yeast expression plasmid, we converted the *Pst*I site of plasmid pISIFN-α2* (F2 or E3) into an *Eco*RI site by using an adaptor fragment (1 kilobase) isolated from the yeast episomal plasmid YEp13 (7). This adaptor fragment contains an internal *Eco*RI site and has *Pst*I sites on both ends; the 247 bp from *Pst*I to *Eco*RI is derived from yeast 2μm DNA, and 754 bp from *Eco*RI to *Pst*I is from pBR322. Thus, the proper insertion of this adaptor fragment into the *Pst*I site of the plasmid results in the restoration of ampicillin resistance in bacteria containing this plasmid.

To construct the final plasmid for expression in yeast cells, we isolated the *Eco*RI fragment from plasmid pISIFN-α2 which contains the invertase signal coding sequence fused to the huIFN-α2 gene and subsequently inserted it into the yeast episomal plasmid YEp1PT (13). The resulting plasmids were designated YEp1PT-ISIFN-α2 (F2 or E3). These expression plasmids also contained a portion of pBR322 with the ampicillin resistance gene and the *E. coli* origin of replication for selection and stable growth in *E. coli*. They also contained the yeast *TRP1* gene allowing for selection in *trp1*$^-$ yeast cells and a 2μm plasmid origin of replication allowing for autonomous replication in yeast cells. Transcription of the hybrid genes was under the control of the yeast PGK promoter.

Synthesis and secretion of huIFN-α2. A plasmid containing the huIFN-α2 gene with its own natural secretion signal has been previously constructed by using the yeast episomal vector YEp1PT (13). In the present study this plasmid was used as a control to compare the levels of production and release into the medium of huIFN-α2 with that of the plasmid YEp1PT-ISIFN-α2 (F2 or E3). The activity of IFN associated with both cells and media was measured (Table 1). Production of huIFN-α2 by yeast cells was somewhat

higher with the yeast invertase signal (or its signal variant in the case of F2) than with the natural human signal. This difference in production was somewhat higher in shake flasks, with the invertase signal constructions consistently producing three- to fourfold more IFN than did the IFN construction with its mammalian secretion signal.

The amount of huIFN-α2 released from the cells varied among fermentation runs. However, we consistently observed that transformants containing F2 produced higher levels of huIFN-α2 in the medium. The higher secretion of huIFN-α2 in F2 could have been due to either a deletion of an alanine residue at position −5, making a more efficient signal for secretion of protein, or an addition of a methionine residue at +1, making the secreted huIFN-α2 more stable in the medium (or during the processes of secretion). For F2, about 10 to 20% of the huIFN-α2 that was cell associated was in the periplasm (data not shown); however, the majority (more than 50%) of the huIFN-α2 remained intracellular. We have not determined whether this intracellular huIFN-α2 is present in the secretory apparatus, in the cytosol, or as a combination of both.

Analysis of huIFN-α2 mRNA. These results indicate that the plasmid with the invertase signal sequence was somewhat more efficient than that with the natural human signal sequence for production of huIFN-α2 by yeast cells. The difference in the synthesis of huIFN-α2 could have occurred

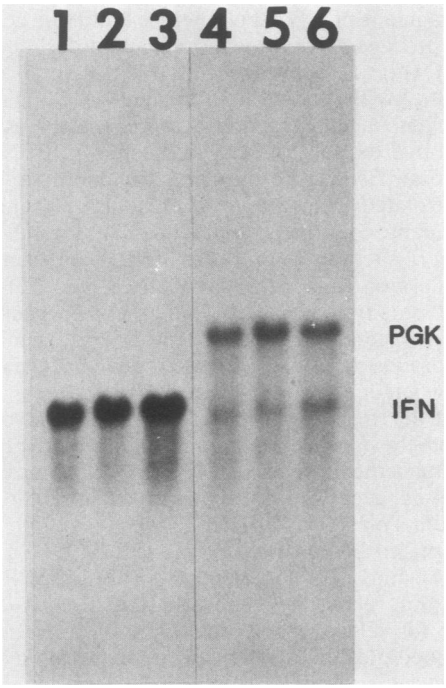

FIG. 2. Analysis of huIFN-α2 mRNA. Total RNA isolation, gel electrophoresis, transfer of RNA to nitrocellulose paper, hybridization with DNA probes, and autoradiography were performed as described in the Materials and Methods section. RNAs were derived from yeast transformants containing plasmids YEp1PT-ISIFN-α2 (F2) (lane 1), YEp1PT-ISIFN-α2 (E3) (lane 2), and YEp1PT-preIFN-α2 (lane 3) and were probed with ^{32}P-labeled DNA of the huIFN-α2 gene. Lanes 4 through 6 are the same as lanes 1 through 3, except that they were washed with boiling water and subsequently probed with ^{32}P-labeled DNA of the PGK gene. One A_{260} of total RNA was loaded in each lane. Note that the ^{32}P-labeled probe of the huIFN-α2 gene in lanes 4 through 6 was not completely washed off.

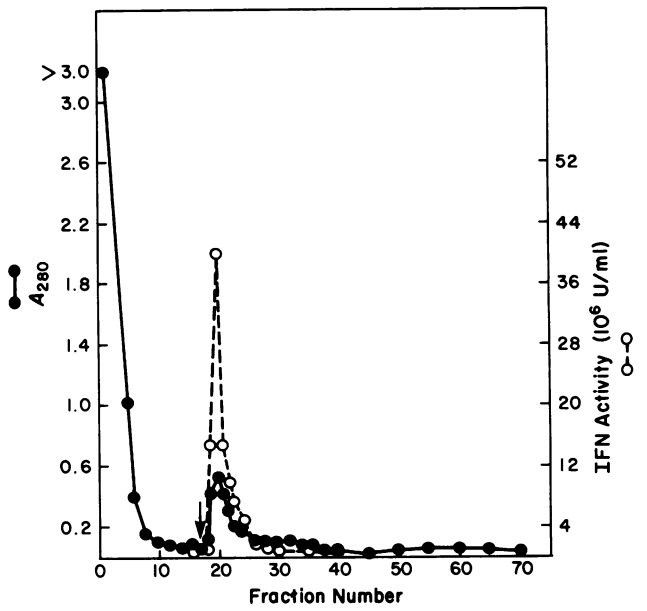

FIG. 3. Immunoaffinity column chromatography of huIFN-α2 from media of the yeast strain 20B-12 transformed with the plasmid YEp1PT-ISIFN-α2 (F2). Arrow, start of elution with 0.2 M acetic acid.

at the level of either mRNA or protein. To examine these possibilities, we first investigated the steady-state levels of mRNA from yeast cells bearing each of three different constructions of the huIFN-α2 gene as determined by using shake flasks. The results (Fig. 2) indicated that there was little variation in the level of mRNA among the three transformed yeast cells (Fig. 2, lanes 1, 2, and 3), suggesting that both the amount of transcription and the stability of IFN mRNA for these three constructions were quite similar (within ±50%). Thus, the difference in the amounts of huIFN-α2 produced probably was at the level of translation, secretion, or protein stability.

To confirm this experiment, we standardized the amount of mRNA loaded on the gel by measuring the amount of PGK mRNA in each sample. Since the haploid yeast cells that we transformed contained a signal copy of the PGK gene, total RNAs from all three yeast transformants should have and did contain the same amount of PGK mRNA (Fig. 2, lanes 4, 5, and 6) by densitometer scans of autoradiograms (data not shown).

The nature of processing of huIFN-α2. The gene of huIFN-α2 with its natural signal has been expressed in yeast cells, and the IFN produced has been characterized by determination of the amino-terminal sequences (13). Only a portion of the IFN produced was correctly processed, suggesting that yeast signal peptidase(s) cannot process the signal of huIFN-α2 with complete fidelity. To investigate whether huIFN-α2 expressed by the F2-containing plasmid was properly processed, huIFN-α2 from the yeast medium was purified by a combination of monoclonal antibody affinity chromatography (Fig. 3) and HPLC (Fig. 4). Gel electrophoresis in sodium dodecyl sulfate of purified huIFN-α2 showed a single Coomassie blue-staining band (data not shown). Analysis of the amino-terminal sequence of purified huIFN-α2 (F2) showed Met-Cys-Asp-Leu-Pro as the amino terminus, indicating that cleavage had occurred before the methionine residue of huIFN-α2 (Fig. 5).

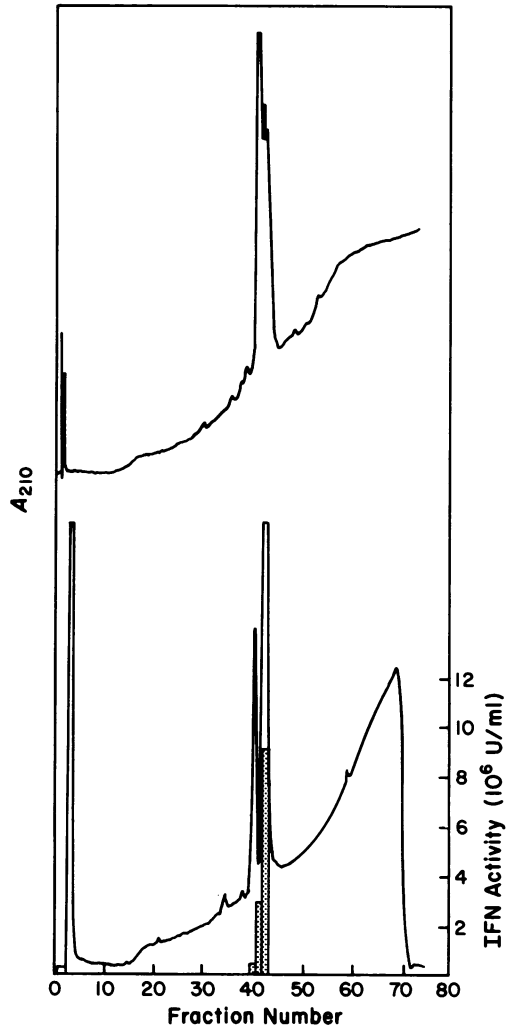

FIG. 4. HPLC of huIFN-α2 (F2) from fraction 20 of the immunoaffinity column shown in Fig. 3. MetIFN-α2 purified from *E. coli* was run as a control and is shown above.

HuIFN-α2 from the medium of yeast cells transformed with the E3-containing plasmid was also purified by the same methods as were used for huIFN-α2 produced by the F2-containing plasmid. The amino-terminal sequence of purified huIFN-α (E3) was identical to the known amino terminus of natural huIFN-α2 (Fig. 5). Thus, in both yeast transformants, the processing of huIFN-α2 precursor to remove the invertase signal (or its slight variant, in the case of the F2 plasmid) appeared to be complete for the IFN found in the medium.

DISCUSSION

We demonstrated that a homologous (yeast) signal derived from the yeast invertase gene was effective in the biosynthesis and secretion of huIFN-α2. Unlike its natural heterologous (human) signal sequence, which is not removed with complete fidelity by yeast cells, all of the invertase signal was removed from the IFN found in the medium after the secretion process. Plasmids used for the study of expression and secretion of huIFN-α2 were identical except for their signal coding sequences. The yeast cells transformed with these individual plasmids had the same steady-state level of mRNA coding for huIFN-α2 suggesting that differences in the amount of protein produced was at the level of translation or posttranslation.

To determine whether the use of the yeast invertase signal to secrete the mature forms of heterologous proteins was a general phenomenon, we fused the invertase signal codons directly to the genes of the mature forms of human insulin-like growth factor I and human serum albumin. Both proteins were expressed, properly processed, and secreted by yeast cells (unpublished results). It is worthwhile to note that in both cases the proteins have no prosequence (albumin prosequence removal; 16). Nevertheless, yeast cells secrete and process these two proteins individually to their mature forms.

Human insulinlike growth factor I and human serum albumin had completely different amino-terminal amino acids at their mature amino termini. The amino-terminal sequence of mature human insulinlike growth factor I began with Gly-Pro-Glu-Thr-Leu, whereas that of mature human serum albumin began with Asp-Ala-His-Lys-Ser. These results, together with the results shown in Table 1, suggest that the yeast signal peptidase could tolerate the change in the mature sequence and also could recognize and cleave the hybrid junctions between the invertase signal and other heterologous proteins. These results are consistent with the notion that the amino-terminal signal sequence of a secretory protein possesses information sufficient for translocation of protein across its targeted membrane (17). Perhaps the cleavage information is also defined by this region and is

	-23 -22 -21 -20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1	1 2 3 4 5
1. Preinvertase	Met Leu Leu Glu Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys Ile Ser Ala ↓	Ser Met Thr Asn Glu
2. Invertase signal - huIFN-α2 (E3)	Met Leu Leu Glu Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys Ile Ser Ala ↓	Cys Asp Leu Pro Gln
3. Invertase signal - huIFN-α2 (F2)	Met Leu Leu Glu Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Lys Ile Ser Ala ↓	Met Cys Asp Leu Pro
4. Human signal - huIFN-α2	Met Ala Ser Pro Phe Ala Leu Leu Met Ala Leu Val Val Leu Ser ↓	Cys Lys Ser Ser Cys ↓ Ser Val Gly ♀ ↓ Cys Asp Leu Pro Gln

FIG. 5. The amino-terminal sequences of huIFN-α2 from clones F2 and E3. Preinvertase and preIFN-α2 were taken from Taussig and Carlson (30) and Hitzeman et al. (13), respectively. Fifteen cycles of Edman degradations were carried out for huIFN-α2 of F2 and E3, respectively. PTH amino acids were determined as described in the Materials and Methods section. In this figure, only five amino acids from the amino termini of mature proteins are shown. Downward arrows, places where cleavage occurred in media material. ♀, correct processing site.

independent of the other side of the junction for this particular secretory system. Further experiments may elucidate these possibilities.

Recently there have been several reports of secretion of heterologous protein products by using the α-factor gene of yeast cells (4, 6, 26). These systems result in proper processing of heterologous proteins during the secretion process. However, the processing is at the level of a pro-sequence since both the signal sequence and the natural pro-sequence (89 amino acids) of α factor are retained in these constructions. Processing probably occurs in the Golgi, where an endopeptidase (*KEX2* gene product) cleaves after dibasic amino acids at the junction between the α factor pro-sequence and the heterologous gene product (15). Recent results of others with *E. coli* suggest that some hybrid secretion signal-heterologous gene fusions may not function properly with respect to secretion because of the loss of essential information present in the mature portion of the homologous genes that has been removed (2). Such information may be retained in these α-factor systems because of the presence of a pro-sequence, but would not be present in the invertase signal-huIFN-α2 hybrids discussed here. Since such hybrids do function, it is possible that sequences in the signal and the mature IFN sequence may act together in a hybrid fashion; or alternatively for invertase, all information for secretion may be contained in the secretion signal sequence itself. Since other heterologous proteins (see above) are properly secreted and processed in such invertase signal hybrids, the latter possibility seems more likely; however, further studies will be necessary to better characterize the nature of these hybrid systems.

ACKNOWLEDGMENTS

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ADDENDUM

After we submitted this manuscript for publication, Smith et al. (27) reported that yeast can secrete a hybrid protein consisting of the signal sequence plus 12 amino acids from the amino terminus of yeast invertase fused to prochymosin. Since the amino-terminal sequence of this secreted invertase-prochymosin fusion protein was not reported, it is not known what signal processing occurred.

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