Efficient translation of synthetic and natural mRNAs in an mRNA-dependent cell-free system from the dimorphic fungus *Candida albicans*

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An mRNA-dependent cell-free translation system has been developed from the human pathogenic fungus *Candida albicans* using either S30 or S100 lysates prepared from glass-bead-disrupted whole cells. Translation of the synthetic template poly(U) in this system is highly efficient at temperatures up to 37 °C and is ATP-dependent. Studies using a range of elongation-specific inhibitors suggest that the mechanism of translational elongation in *C. albicans* is similar to that of another yeast, *Saccharomyces cerevisiae*. A micrococcal-nuclease-treated *C. albicans* S100 lysate was able to translate exogenously-supplied homologous mRNAs, and a range of heterologous natural mRNAs, using an initiation mechanism that is inhibited by the antibiotic edeine and the 5' cap analogue 7-methylguanosine 5'-monophosphate (m⁷GMP). As with cell-free lysates prepared from *S. cerevisiae*, the *C. albicans* lysate is unable to initiate translation upon natural mRNAs at temperatures above 20 °C.

Introduction

The pathogenic fungus *Candida albicans* has been the subject of increasing interest, generated in part by its potential to cause disease in human patients with immune deficiencies (e.g. AIDS), and by its ability to undergo a dimorphic transition from a unicellular yeast-like organism to a multicellular hyphal form (Odds, 1988). Presently little is known about the cellular mechanisms responsible for either pathogenicity or dimorphism, largely because the appropriate genetic and biochemical tools have yet to be developed to an adequate degree to facilitate the necessary detailed studies of gene structure and function. Methods for overcoming the experimental handicap of studying the genetics of an organism with no known sexual cycle (*C. albicans* is a member of the Deuteromycetes or Fungi Imperfecti) are now becoming available (Kurtz *et al.*, 1988; Magee *et al.*, 1988), but little effort has yet gone into providing the necessary *in vitro* biochemical assays for the key steps in gene expression, namely transcription and translation.

The development of a cell-free translation system that

is capable of translating either exogenously supplied synthetic or natural mRNA templates has led to a detailed understanding of the mechanism and control of translation in eukaryotes, particularly in mammalian cells, although much of the work in this context has been confined to an 'atypical' cell, the rabbit reticulocyte (Clemens, 1985). Such cell-free systems have also been valuable in defining the mode of action of inhibitors and in assessing the coding capabilities of genes isolated by molecular cloning and transcribed *in vitro* using either the SP6 or T7 RNA polymerases (Little & Jackson, 1987). To provide the ability to carry out such studies in *C. albicans*, and to complement the increasingly sophisticated molecular genetic experiments with this fungus, requires the development of such a cell-free system.

To date, mRNA-dependent cell-free systems capable of initiating translation upon exogenously supplied natural mRNAs have only been reported for a limited number of fungi, with the most extensively studied being that derived from the budding yeast *Saccharomyces cerevisiae* (Tuite & Plesset, 1986). *S. cerevisiae* cell-free lysates, produced by disruption of either whole cells (Hofbauer *et al.*, 1982) or spheroplasts (Gasior *et al.*, 1979), are only able to translate exogenous mRNAs at temperatures below 23 °C, a property apparently unique to this organism and initially thought to be due to a temperature-activated nuclease (Herrera *et al.*, 1979).

Abbreviations: BMV, brome mosaic virus; TMV, tobacco mosaic virus; TLCK, tosyl-lysine chloromethyl ketone; TPCK, tosyl-phenylalanine chloromethyl ketone.

Recent evidence has shown, however, that this inhibition of translation at high temperatures is reversible (Mandel & Trachsel, 1989).

In this paper we report the development of an active cell-free system from *C. albicans* that translates efficiently both the synthetic template polyuridylic acid [poly(U)], and a range of exogenously supplied homologous and heterologous mRNAs. In addition, we demonstrate that the mechanisms of initiation and elongation in *C. albicans* are very similar to those of *S. cerevisiae*.

Methods

Strains. *Candida albicans* strain 2005E (ATCC 10261) was obtained from Glaxo Group Research, Greenford. *Saccharomyces cerevisiae* strain ABYS1, a protease-deficient strain, was obtained from Dr D. H. Wolf (Freiburg, FRG).

Materials. Biochemicals were obtained from Boehringer Mannheim (BCL), unless otherwise stated. Leupeptin was purchased from the Peptide Institute, Japan. Micrococcal nuclease was obtained from Worthington Biochemicals. Sephadex G-25-150 and m⁷GMP were obtained from Sigma. Edeine was a kind gift from Dr B. S. Cox (Oxford, UK) and glass beads (0.4 mm diameter) were obtained from BDH. [³H]Phenylalanine (53 Ci mmol⁻¹; 1.96 TBq mmol⁻¹) and [³⁵S]-methionine (1000 Ci mmol⁻¹; 37 TBq mmol⁻¹) were purchased from Amersham, UK.

Preparation of cell-free lysates. *C. albicans* was grown in YEPD (1%, w/v, yeast extract, 1%, w/v, bacto-peptone, 2%, w/v, glucose) at 37 °C to early exponential phase ($1-3 \times 10^7$ cells ml⁻¹). Cells were harvested at 2500 r.p.m. for 15 min at 4 °C, washed with cold sterile water and resuspended in cold lysis buffer A (20 mM-Hepes/KOH, pH 7.4, 100 mM-potassium acetate, 2 mM-magnesium acetate, 2 mM-dithiothreitol) at 1 ml per 2 g wet weight of cells. Buffer A was supplemented with the following protease inhibitors; phenylmethylsulphonyl fluoride (PMSF), 0.5 mM; benzamidine.HCl, 0.5 mM; tosyl-lysine chloromethyl ketone (TLCK), 0.1 mM; tosyl-phenylalanine chloromethyl ketone (TPCK), 0.1 mM; and leupeptin, 0.1 µg ml⁻¹.

The cell slurry was transferred to a sterile 50 ml Oakridge centrifuge tube and chilled on ice. Acid-washed and baked glass beads were added to just below the meniscus. The tube was tightly capped and shaken vigorously by hand in an 'up-and-down' motion for 30 s, then placed on ice for a further 30 s (Hussain & Liebowitz, 1986). This was repeated for eight cycles of shaking and cooling, and the degree of lysis checked microscopically. If necessary, shaking was continued to achieve the desired level (greater than 90%) of cell lysis. The glass beads were left to settle out in the tubes held on ice and the supernatant was transferred with a sterile pipette to a sterile baked 30 ml Corex tube. The beads were then washed with a further 1 ml of lysis buffer A and the supernatants combined.

For the poly(U)-dependent cell-free translation system, the lysate was centrifuged at 30000 g for 15 min at 4 °C. For the natural mRNA-dependent cell-free translation system, the lysate was initially centrifuged at 13000 g for 10 min at 4 °C, and the supernatant from this step centrifuged at 100000 g for 30 min at 4 °C to produce a post-polysomal fraction, as described previously (Tuite & Plesset, 1986).

The S100 or S30 supernatants were applied to a Sephadex G-25-150 column (1.6 × 30 cm) equilibrated in buffer B (buffer A plus 20%, v/v, glycerol) containing the protease inhibitors detailed above. The column was then eluted with buffer B and 2 ml fractions collected. The A₂₆₀ of the eluting fractions was measured and the fractions with peak

absorbances were put individually into sterile microcentrifuge tubes, frozen on dry ice and stored at -80 °C, where they maintained their protein synthetic activity for several months.

Preparation of mRNA templates. The synthetic template poly(U) was purchased from Boehringer Mannheim. Crude mRNA samples were prepared from *S. cerevisiae* and *C. albicans* essentially as described by Tuite *et al.* (1980) using the polysomes pelleted during the preparation of the S100 post-polysomal lysates (see above). Poly(A⁺) RNA was not prepared because previous studies have demonstrated that such highly purified mRNA samples translate relatively inefficiently in *S. cerevisiae* cell-free lysates (Gasior *et al.*, 1979). TMV RNA was purchased from Amersham. BMV RNA was prepared from purified BMV particles (generously provided by Dr B. S. Cox, University of Oxford) essentially as previously described (Santos *et al.*, 1990).

Cell-free translation assays. Assays (25 µl) were performed essentially as described by Tuite & Plesset (1986) with the following modification: treatment with micrococcal nuclease was performed in the presence of 1 mM-CaCl₂ and this incubation was stopped by the addition of 2 mM-EGTA. The final concentrations in this 25 µl assay were as follows unless otherwise stated in the text: 20 mM-Hepes/KOH pH 7.4, 2 mM-dithiothreitol, 180 mM-potassium acetate, 1.5 mM-magnesium acetate, 430 µM-ATP, 90 µM-GTP, 22 mM-creatine phosphate, 70 µg creatine phosphokinase ml⁻¹, 40 mM total amino acids, 200 µg mRNA ml⁻¹, 0.5-1.0 µCi [³H]phenylalanine or 2-5 µCi [³⁵S]methionine.

The incorporation of radiolabelled amino acids into protein in the cell-free assays was determined by spotting 20 µl volumes onto a 2.5 cm Whatman 3MM filter disc, which was then dropped into ice-cold 5% (w/v) TCA. After at least 10 min in TCA, the filters were washed successively with: 200 ml 5% TCA at room temperature; 200 ml boiling 5% TCA for 5 min, and finally 200 ml 5% TCA at room temperature. The filters were then rapidly swirled in 100 ml each of ethanol, ethanol/acetone (1:1, v/v) and acetone, after which they were separated and dried at 60 °C for 30 min. The dried filters were placed in a scintillant (LKB Optiphase T with 10%, v/v, H₂O) and their radioactivity measured in a Beckman LS7800 scintillation counter. All assays were carried out in duplicate and the means calculated.

Analysis of cell-free translation products. Following incubation of the cell-free translation system for 60 min at 20 °C with [³⁵S]methionine, a 5 µl sample was used to measure radioactivity incorporated into protein. The remainder was treated with ribonuclease A (100 µg ml⁻¹) at 20 °C for a further 5 min. Subsequent SDS-PAGE analysis and autoradiography were as previously described (Tuite *et al.*, 1980).

Preparation of aminoacylated [³H]tRNA^{Phe}. *C. albicans* was grown to a cell density of 1×10^8 cells ml⁻¹, harvested by centrifugation, washed once in cold double-distilled water and frozen at -70 °C for 30 min. The cells were then gently thawed and resuspended in 15 ml buffer J (20 mM-Hepes/KOH pH 7.5, 100 mM-potassium acetate, 5 mM-magnesium acetate, 1 mM-dithiothreitol) and lysed by five passages through a French pressure cell (Amicon). The resulting lysate was centrifuged at 30000 g for 2 h at 4 °C and the supernatant from this was further centrifuged at 100000 g for 2 h at 4 °C. The resulting S100 supernatant was then dialysed for 16 h against 2×1 l buffer K (50 mM-Tris/HCl pH 7.6, 10 mM-magnesium chloride, 150 mM-potassium chloride). The dialysed extract was then applied to a DEAE-cellulose column (3 × 10 cm) equilibrated with buffer K and the column washed extensively with this buffer. Fractions containing aminoacyl-tRNA synthetase activity were identified as described below, then pooled and dialysed against buffer L (20 mM-Tris/HCl pH 7.6, 20 mM-potassium chloride, 1 mM-dithiothreitol, 2 mM-EDTA, 10%, v/v, glycerol) for 2 h at 4 °C. The dialysed pooled fractions were applied to a phosphocellulose column (1.5 × 7 cm) equilibrated with buffer L. The column was eluted with buffer L containing 100 mM-potassium chloride, then with

buffer L containing 250 mM-potassium chloride. Aminoacyl-tRNA synthetase activity eluted in the 100 mM-salt wash. The peak fractions were pooled and used as a tRNA-depleted aminoacyl-tRNA synthetase preparation for the aminoacylation of tRNA^{Phe}.

A 2.5 ml reaction mixture containing 100 µCi [³H]phenylalanine, 1 mg *E. coli* tRNA^{Phe} (BCL), 4 mM-ATP, 0.1 mM-CTP, 12 mM-magnesium chloride, 20 mM-HEPES/KOH pH 7.5, 1 mM-dithiothreitol and 500 µl of the crude tRNA-depleted aminoacyl-tRNA synthetase preparation was incubated at 25 °C for 30 min then cooled on ice for 10 min. The reaction mixture was then loaded onto a 1 ml DEAE-cellulose column and washed extensively with buffer K (150 mM-potassium chloride), then the [³H]tRNA^{Phe} was eluted with buffer K (500 mM-potassium chloride). The peak fractions of charged tRNA^{Phe} were pooled, precipitated at -20 °C for 16 h with 2.5 vols ethanol (95%, v/v) and dried.

Results

Translation of synthetic templates

Extensive studies with *S. cerevisiae* cell-free lysates (Sissons, 1978) have shown that to translate the synthetic template poly(U) into poly-phenylalanine only requires a functional elongation system comprising cytoplasmic ribosomes, elongation factors, ATP and GTP, providing the reaction is carried out at very high, non-physiological Mg²⁺ concentrations to 'force' initiation on the synthetic template. An S30 lysate prepared by glass bead disruption of exponentially growing cells of *C. albicans* (see Methods for details) was found to translate poly(U) with very high efficiency in a template- and Mg²⁺-dependent manner. Incorporation of [³H]phenylalanine was linear for 20 min at 20 °C, with a complete cessation of protein synthesis by 30 min. Efficient translation of poly(U) only occurred in the *C. albicans* lysate at Mg²⁺ concentrations between 10 and 25 mM, with the optimum being about 15 mM (results not shown).

Table 1 summarizes the basic parameters for efficient translation of poly(U) in the *C. albicans* S30 lysate and shows that they are very similar to those previously obtained for an equivalent *S. cerevisiae* S30 lysate (Tuite & Plesset, 1986; M. F. Tuite, unpublished data).

Translational elongation mechanism in *C. albicans* and *S. cerevisiae*

A wide range of antibiotics are available that have well-documented effects on specific steps in the translational elongation cycle of eukaryotes (Vazques & Jiminez, 1980). Table 2 shows the I₅₀ values of a selection of antibiotics in both *C. albicans* and *S. cerevisiae* S30 lysates. These antibiotics affect aminoacyl-tRNA binding to the ribosome, peptide bond formation, or translocation of the ribosome. With the exception of cycloheximide, the inhibitors examined appeared to be

Table 1. Comparison of the optimal parameters for the translation of the synthetic template poly(U) in *C. albicans* and *S. cerevisiae* S30 lysates

Parameter	Optimal conditions	
	<i>C. albicans</i> lysate	<i>S. cerevisiae</i> lysate*
Time of incubation	20–30 min	20–30 min
Mg ²⁺ concn	15 mM	12 mM
Potassium acetate concn	180 mM	150 mM
Temperature	30–37 °C	30–37 °C
Protein concn	1.2–1.6 mg ml ⁻¹	ND
Poly(U) concn	500 µg ml ⁻¹	400 µg ml ⁻¹

ND, not determined.

* Data from Tuite & Plesset (1986) and M. F. Tuite (unpublished).

Table 2. Inhibition of translational elongation in cell-free systems from *S. cerevisiae* and *C. albicans* by antibiotics

For each antibiotic seven different concentrations were used to determine the I₅₀ value, i.e. the concentration, in µg ml⁻¹, required to give 50% inhibition of [³H]phenylalanine incorporation in a poly(U)-programmed cell-free system.

Site of action*	Antibiotic	I ₅₀ (µg ml ⁻¹)	
		<i>S. cerevisiae</i> lysate	<i>C. albicans</i> lysate
1	Tetracycline	110	75
	Fusidic acid	85	200
2	Puromycin	19	4.5
3	Cycloheximide	0.2	200
	Emetine	65	65
	Diphtheria toxin	1	1

* 1, Aminoacyl-tRNA binding to the ribosome; 2, peptide bond formation; 3, ribosomal translocation.

equally effective against both the *C. albicans* and *S. cerevisiae* translational elongation systems, suggesting that the partial elongation steps are very similar in the two fungi. The resistance to cycloheximide observed in the *C. albicans* lysate strongly suggests that *C. albicans* shows natural resistance to cycloheximide, a not uncommon property of many species of fungi including some strains of *S. cerevisiae* (Rao & Grollman, 1967). Such resistance is probably due to a natural variation in the structure of a ribosomal protein of the 60S subunit (Fried & Warner, 1982).

The poly(U)-dependent cell-free system of *S. cerevisiae* has also been shown to exhibit a very specific requirement for ATP to allow the functioning of translation elongation factors independent of aminoacylation

Fig. 1. Effect of increasing ATP concentrations on the translation of the synthetic template poly(U), in an S30 cell-free lysate of *C. albicans*, in the absence (●) or presence (▲) of 0.3 mM-GTP added exogenously.

(Skogerson, 1979; Uritani & Miyazaki, 1988). Fig. 1 shows that the *C. albicans* poly(U)-dependent system also has a strict requirement for ATP. To demonstrate this, the *C. albicans* cell-free system was supplemented with purified tRNA^{Phe} aminoacylated *in vitro* with [³H]phenylalanine, in place of [³H]phenylalanine alone, to make the system independent of the ATP requirement for aminoacylation. The ATP requirement for elongation was saturated at 100 μM and could not be substituted for by increased concentrations of GTP (data not shown). In contrast, the requirement for GTP was less strict and significant levels of translation could be obtained in the absence of exogenously supplied GTP, providing ATP was present in concentrations $\geq 10^{-5}$ M. These results suggest that, like *S. cerevisiae*, *C. albicans* has an ATP-dependent translation elongation factor (or factors) absolutely required for poly(U) translation *in vitro*.

Translation of exogenous natural mRNAs

For a cell-free translation system to be of wide use it must be able to translate a wide range of natural mRNAs both efficiently and authentically in a concentration-dependent manner. This requires, in turn, that the cell-free system can be made free of endogenous mRNAs by treating the cell-free lysate with micrococcal nuclease (Pelham & Jackson, 1976). Preliminary experiments with an S100 lysate of *C. albicans* (see Methods) indicated that endogenous mRNAs could be effectively removed by treating the lysates with 25 μg micrococcal nuclease ml⁻¹ for 15 min. To establish whether such an mRNA-depleted system could now translate exogenously sup-

plied natural mRNA, total RNA prepared from *C. albicans* polysomes was used to programme the system at 20 °C. Over a range of Mg²⁺ concentrations the addition of the *C. albicans* polysomal RNA stimulated incorporation of [³H]phenylalanine into protein by between 10- and 15-fold, with the maximal efficiency being observed at 1.5 mM-Mg²⁺ (data not shown). This value is in good agreement with the Mg²⁺ optimum observed with the *S. cerevisiae* mRNA-dependent cell-free system (Tuite *et al.*, 1980). The optimal potassium acetate concentration for translation of the *C. albicans* polysomal RNA was found to be 160 mM (data not shown), again in good agreement with the K⁺ optimum of the *S. cerevisiae* cell-free system (Tuite *et al.*, 1980).

To examine the nature of the translation products being synthesized in the *C. albicans* lysate, proteins were labelled with [³⁵S]methionine *in vitro* and analysed by one-dimensional SDS-PAGE. As shown in Fig. 2, there was a high level of endogenous protein synthesis in the *C. albicans* lysate which could be entirely eliminated by treating the lysate with micrococcal nuclease (compare lanes 1 and 2). Furthermore this micrococcal-nuclease-treated lysate was able to translate exogenously supplied complex mixtures of both heterologous (Fig. 2, lane 3) and homologous (Fig. 2, lane 6) mRNAs. That this translation was being mediated by natural initiation mechanisms was indicated by the strong inhibition of translation of exogenously supplied homologous and heterologous mRNAs by two initiation-specific inhibitors, edeine and the 5' mRNA cap analogue m⁷GMP (Fig. 2, lanes 4, 5 and 7, 8), as found previously for the analogous *S. cerevisiae* cell-free system (Tuite *et al.*, 1980; Tuite and Plesset, 1986).

In an attempt to improve the *in vitro* synthesis of high-molecular-mass proteins we supplemented the *C. albicans* cell-free lysate with either a cocktail of protease inhibitors (see Methods) or human placental ribonuclease inhibitor or a combination of both. As shown in Fig. 3, only the combination of protease and ribonuclease inhibitors significantly improved the level of incorporation and synthesis of high-molecular-mass proteins programmed by exogenously supplied *C. albicans* mRNA, with proteins in excess of 100 kDa being detected. The addition of total tRNA from either *C. albicans* or rat liver had no further stimulatory effect (data not shown).

The results presented in Fig. 4 confirm the potential of the system to synthesize polypeptides with molecular masses in excess of 100 kDa. In this case the system was programmed with TMV RNA (Fig. 4, lane 3). The ability of the *C. albicans* lysate to translate a second plant viral RNA genome is shown in Fig. 4, lane 2; unfractionated BMV RNA directed the synthesis of a single major polypeptide, the 19 kDa coat protein,

Fig. 2. The products of translation of homologous (*C. albicans*) and heterologous (*S. cerevisiae*) mRNAs in a *C. albicans* S100 lysate. Lane 1, endogenous protein synthesis prior to treatment with micrococcal nuclease; lane 2, endogenous protein synthesis after treatment with micrococcal nuclease ($25 \mu\text{g ml}^{-1}$); lane 3, as for lane 2 except programmed with polysomal RNA from *S. cerevisiae* ($200 \mu\text{g ml}^{-1}$); lane 4, as for lane 3 except $10 \mu\text{g edeine ml}^{-1}$ was also present; lane 5, as for lane 3 except $5 \text{ mM-}m^7\text{GMP}$ was also present; lane 6, as for lane 2 except programmed with polysomal RNA from *C. albicans* ($200 \mu\text{g ml}^{-1}$); lane 7, as for lane 6 except $10 \mu\text{g edeine ml}^{-1}$ was also present; lane 8, as for lane 6 except $5 \text{ mM-}m^7\text{GMP}$ was also present. *In vitro* translation products were labelled with [^{35}S]methionine and analysed as described in Methods. The positions of molecular mass standards are indicated. NB: The band of 47 kDa observed in all lanes is an artefact arising from the use of methionine as the label.

although the *in vitro*-synthesized product migrated slightly more slowly in the gel than authentic BMV coat protein co-electrophoresed on the same gel. We have recently shown that this aberrant migration is due to efficient 'read-through' of the BMV coat protein cistron UAG termination codon by an endogenous tRNA (Santos *et al.*, 1990). We have not, however, observed significant read-through of translational terminators in *C. albicans* or other natural mRNAs, suggesting that the BMV RNA4 UAG terminator may be leaky with respect to termination (Skuzeski *et al.*, 1990).

One of the unusual characteristics of the *S. cerevisiae* mRNA-dependent cell-free lysate is its sensitivity to temperature, with little or no translation of natural mRNAs being detectable above 20°C (Gasior *et al.*, 1979). In a similar way, translation was not detectable in *C. albicans* cell-free lysates incubated at either 30°C or 37°C (results not shown), temperatures at which the majority of eukaryotic cell-free translation systems efficiently translate exogenous mRNAs (Clemens, 1985). Failure to translate at temperatures above 25°C was observed with exogenously supplied natural mRNAs, and with endogenous-RNA-directed protein synthesis (data not shown). That this temperature sensitivity was not due to a block in translation elongation was confirmed by using the synthetic template poly(U) (Table 3); the elongation efficiency of both the *C.*

Table 3. Translation elongation is not temperature-sensitive in either the *C. albicans* or *S. cerevisiae* cell-free systems, programmed with the synthetic template poly(U)

Incorporation of [^3H]phenylalanine was measured in a 20 min assay in the absence or presence of poly(U). The data represent means of two independent assays run in parallel.

Temp. of incubation ($^\circ\text{C}$)	$10^{-3} \times$ Incorporation of [^3H]phenylalanine (d.p.m.)			
	<i>C. albicans</i> lysate		<i>S. cerevisiae</i> lysate	
	Minus poly(U)	Plus poly(U)	Minus poly(U)	Plus poly(U)
20	6.2	224.8	8.7	413.9
30	5.2	356.5	7.8	431.0
37	5.6	342.9	9.5	436.5

albicans and *S. cerevisiae* S100s was not impaired at either 30°C or 37°C , and the *C. albicans* lysate translated poly(U) optimally at 30°C .

Discussion

In many ways the characteristics we describe for the *C. albicans* system are similar to those previously described for an analogous system developed for the budding yeast *S. cerevisiae* (Gasior *et al.*, 1979; Herrera *et al.*, 1979;

Fig. 3. Effect of addition of protease and ribonuclease inhibitors on the synthesis of translation products programmed by *C. albicans* mRNA in a micrococcal-nuclease-treated cell-free lysate. Lane 1, no mRNA; Lanes 2–5, as for lane 1 except programmed with polysomal RNA from *C. albicans* ($200 \mu\text{g ml}^{-1}$). The following additions were made: lane 3, 0.5 mM-PMSF, 0.5 mM-benzamidine.HCl, 0.1 mM-TLCK, 0.1 mM-TPCK, 0.1 $\mu\text{g leupeptin ml}^{-1}$ (protease inhibitors); lane 4, 1 unit human placental ribonuclease inhibitor; lane 5, a combination of the components added to lanes 3 and 4. *In vitro* translation products were labelled with [^{35}S]methionine and analysed as described in Methods. The positions of molecular mass standards are indicated.

Fig. 4. Translation of plant viral RNAs in a *C. albicans* S100 cell-free lysate treated with micrococcal nuclease. Lane 1, no RNA added; lane 2, 40 $\mu\text{g BMV RNA ml}^{-1}$ added; lane 3, 40 $\mu\text{g TMV RNA ml}^{-1}$ added. *In vitro* translation products were labelled with [^{35}S]methionine and analysed as described in Methods. The positions of molecular mass standards are indicated.

Tuite & Plesset, 1986). In particular, the absolute requirement for exogenously supplied ATP for translation elongation, and the failure of the system to initiate *in vitro* at temperatures much above 20°C , appear to be two characteristics unique to yeast cell-free systems (Gasior *et al.*, 1979; Skogerson, 1979). In most other respects – ionic requirements, sensitivity to antibiotic inhibitors, ability to translate a wide range of natural mRNAs, etc. – the two fungal systems behave very similarly to those developed from higher eukaryotic cells (Clemens, 1985).

The nucleotide requirements (i.e. ATP and GTP) of the crude *C. albicans* elongation system are intriguing. All eukaryotic cells examined to date require two translation elongation factors, each with associated GTPase activities – EF-1 α , which mediates tRNA binding to the ribosome, and EF-2, which mediates translocation (reviewed by Moldave, 1985); the yeast *S. cerevisiae* is no exception to this (Skogerson, 1979). However, *S. cerevisiae* has an absolute requirement for a third soluble elongation factor, EF-3 (Skogerson & Wakatama, 1976). EF-3 is required for the translation of both synthetic and natural mRNAs *in vitro* (Hutchinson *et al.*, 1984; Herrera *et al.*, 1984) and has an 80S-ribosome-dependent GTPase and ATPase activity displayed by the same active site (Uritani & Miyazaki, 1988). The dramatic stimulation of polyphenylalanine synthesis on yeast ribosomes by ATP, initially described by Skogerson (1979), has now been ascribed to the essential requirement for the ATPase activity of EF-3 during the elongation cycle (Uritani & Miyazaki, 1988). Our results (Fig. 1) strongly suggest that *C. albicans* may also possess an ATP-dependent elongation factor analogous to the *S. cerevisiae* EF-3. We have shown that *C. albicans* does indeed have a protein of approximate molecular mass 130 kDa, that strongly cross-reacts with a polyclonal antibody raised against the *S. cerevisiae* EF-3 translation factor, which has a molecular mass of 125 kDa (D. Colthurst and others, unpublished). The apparent non-requirement for exogenous GTP at high ATP concentrations in the crude *C. albicans* lysate indicates either a high endogenous level of GTP in the S30 lysate or that excess ATP can be used to generate GTP from endogenous GDP.

One unusual aspect of translation in *C. albicans* uncovered by our *in vitro* translation studies is the failure to recognize the termination codon UAG as such, but rather as a sense codon (Santos *et al.*, 1990).

Our studies with an *in vitro* translation system developed from *C. albicans* have therefore already provided evidence for there being a number of aspects of the translational machinery of this human pathogen which differ from those of higher eukaryotes and may thus represent potential targets for chemotherapeutic agents.

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