An edeine resistant mRNA-dependent protein synthesis system from a *Saccharomyces cerevisiae* mutant

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A cell-free protein synthesizing system from a mutant of *Saccharomyces cerevisiae* translated exogenous mRNA in the presence of 2 μM edeine, while a similar system from wild-type strain was completely inhibited by the drug. The mutant ribosomes showed an affinity for [^{125}I]edeine comparable to the wild-type ribosomes, thereby suggesting that these macromolecules alone were not responsible for the edeine-resistant capacity of the mutant.

Cell-free system Saccharomyces cerevisiae *Edeine-resistant mutant* *Initiation protein synthesis*

1. INTRODUCTION

The yeast *Saccharomyces cerevisiae*, with its low genetic complexity and innumerable mutant availability, makes a convenient model for the study of mechanisms controlling gene expression in eukaryotes. The development of a yeast cell-free protein synthesizing system which translates exogenous mRNA faithfully and efficiently [1–3] has provided the means for studying the mechanism and regulation of protein synthesis in this eukaryotic cell. In addition, cell-free systems from yeast mutants resistant to inhibitors of protein synthesis can be used as a tool for these analyses.

Antibiotics inhibiting specific steps in translation have increased knowledge of this process. In particular, the basic oligopeptide, edeine has been shown to block initiation in lysates from prokaryotic and eukaryotic organisms with no observable effect on polypeptide elongation [4–8]. While it has been suggested that edeine may prevent the union of the ribosomal subunits [6,8,9], it has also been reported to destabilize the GTP-dependent interaction of Met-tRNAi with 40 S subunits [10]. Others have indicated that the drug may impede the AUG recognition event [11].

From the above observations, it is possible to surmise that yeast mutant lysates resistant to edeine are systems which may permit analyses of various steps in peptide chain initiation. Here, we have identified a mutant of *S. cerevisiae* with a cell-free system capable of synthesizing protein in the presence of edeine. It is also shown that the mutant ribosomes alone are not responsible for this resistance.

2. EXPERIMENTAL

2.1. *Preparation of cell-free protein synthesizing systems (S-100')*

Strains of *S. cerevisiae* designated A364A (wild-type), E1, E3, E5, E6, E10, E28 and E54 (mutants of A364A resistant to edeine in vivo) were grown as in [12] and the S-100' extract was prepared according to [1].

2.2. *Assay for protein synthesis*

The assay was performed according to [2].

2.3. *Preparation of yeast mRNA*

Yeast mRNA was prepared following the procedure in [13].

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2.4. *[^{125}I]-labeling of edeine*

The antibiotic was iodinated as in [10].

2.5. *Binding of [^{125}I]edeine to ribosomes*

The final concentrations of all reaction components in a total volume of 150 μl were: 0.5–0.6 mg S-100′ extract from the mutant or wild-type strain, 20 mM Hepes–KOH buffer (pH 7.4), 210 mM potassium acetate, 3 mM magnesium acetate, 3 mM dithiothreitol, 0.5 mM ATP, 0.1 mM GTP, 20 mM creatine phosphate, 10 μg creatine phosphokinase, 10% glycerol, and 250 pmol [^{125}I]edeine (80 cpm/pmol). Incubation was then carried out for 4 min at 20°C followed by cooling to 0°C and then these samples were layered onto 12 ml of 10–38% linear gradients of sucrose as in [3].

3. RESULTS AND DISCUSSION

The effect of increasing concentrations of edeine on the translation of exogenous yeast mRNA in the wild-type lysate is shown in fig.1. A concentration of 2.0 μM completely inhibited the protein synthesizing activity of the wild-type cell-free system. Lysates of 8 *S. cerevisiae* mutants resistant to edeine in vivo were tested for their ability to synthesize protein in the presence of 2 μM edeine. The data presented in table 1 demonstrate that the in vitro protein synthesizing system from the mutant E6 was the only one capable of translating exogenous mRNA in the presence of the drug while maintaining a 70% residual activity with respect to the control; a higher concentration of 3 μM was required to produce 95% inhibition. The other mutants, as sensitive to edeine as the wild-type strain, may have changed membranes which, in turn, impeded the entry of the antibiotic into the cell.

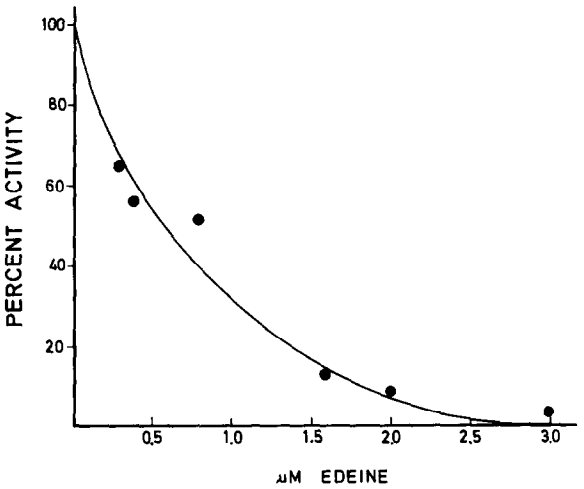

Fig.1. Effect of edeine on exogenous messenger translation in the whole wild-type lysate system. Incubations were carried out as described in the text. Edeine (Calbiochem Behring, La Jolla, CA) was added prior to the messenger. The results are expressed as percent activity obtained in the presence of edeine as compared to controls in the absence of the antibiotic. 100% corresponded to 532128 cpm. Data have been corrected for endogenous mRNA radioactivity incorporation by subtracting it (22320 cpm) from the [^{3}H]leucine incorporated in the presence of exogenous mRNA.

It has been shown that [^{125}I]edeine is able to interact with 40 S subunits and 80 S ribosomes in reticulocyte lysates [10]. To determine this interaction of the drug with both ribosome populations, the binding of [^{125}I]edeine was tested in the wild-type and E6 extracts. Fig.2 shows that [^{125}I]edeine was bound in both lysates to the 40 S and 80 S ribosomes in very similar proportions.

The result indicates that the mutant ribosomes have an affinity for the drug comparable to that of

Table 1

Effect of 2 μM edeine on exogenous messenger translation in whole lysate systems

Strain	% activity
A364A	3
E1	5
E3	2
E5	0
E6	70
E10	2
E12	0
E28	3
E54	0

Incubations were carried out as described in the text. Results are expressed as percent activity obtained in the presence of edeine as compared to controls in the absence of the antibiotic. Controls were similar in all cases, resulting in a mean value of 540500 cpm. Data have been corrected for endogenous mRNA radioactivity incorporation by substracting it from the [^{3}H]leucine incorporated in the presence of exogenous mRNA

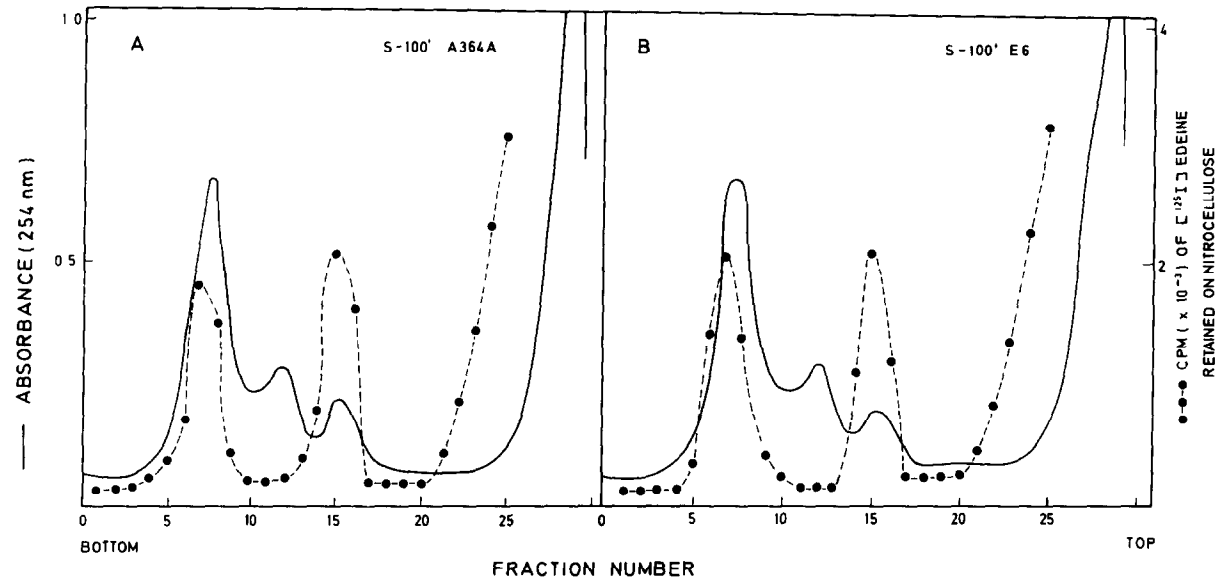

Fig.2. Binding of [^{125}I]edeine to ribosomes from (A) wild-type extract and (B) mutant extract. Incubation components, centrifugation and analytical procedures are described in the text.

the wild-type, suggesting that the ribosomes alone were not responsible for the capacity to resist the antibiotic effect as presented by the mutant. This ability probably involves other components of the protein synthesis initiation step. On the basis of these findings, the possibility of the mutant extract carrying an altered enzyme capable of degrading edeine thereby resulting in an extract resistant to the antibiotic was ruled out.

Further investigations should help define factors mediating the mutant resistance to edeine which, as a consequence, could contribute to the understanding of the yeast peptide initiation event.

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