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A ribosome-associated inhibitor of in vitro nonsense suppression in [*psi*$^-$] strains of yeast

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All classes of tRNA-mediated nonsense suppression are much more efficient in yeast cell-free lysates prepared from a [*psi*$^+$] strain than in those prepared from an isogenic [*psi*$^-$] strain. Mixed [*psi*$^+$]/[*psi*$^-$] lysates do not support efficient suppression. Fractionation of the [*psi*$^-$] lysate demonstrated the presence of an inhibitor of in vitro suppression that is loosely associated with the 80 S ribosome. The data indicate that the inhibitor is a factor involved in the termination of translation in this simple eukaryote.

Nonsense suppression; [*psi*] factor; Ribosome; Translation termination; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

The efficiency of yeast ochre suppressor tRNAs can be modified by the cytoplasmically inherited [*psi*] factor. It was originally identified by its effect on the weak ochre suppressor *SUQ5* which is only detectable in [*psi*$^+$] strains [1]. In [*psi*$^-$] strains, *SUQ5* and a range of other ochre suppressors are much less efficient. Subsequent genetic and physical studies have demonstrated that the [*psi*] factor is a novel cytoplasmic genetic element in yeast [2,3].

The [*psi*] phenotype is also manifested in vitro with the efficiency of all three classes of suppressor tRNA (ochre, amber, UGA) being much more efficient in cell-free lysates prepared from a [*psi*$^+$] strain than in cell-free lysates prepared from a [*psi*$^-$] strain [4]. This is in contrast to in vivo observations where [*psi*] has no detectable effects on the efficiency of amber [5] or UGA suppressors (Cox, B.S., unpublished). Also, when a [*psi*$^+$] and a [*psi*$^-$] cell-free lysate are mixed little or no in vitro nonsense suppression is observed [4], again sharply contrasting with the in vivo observations where a diploid generated from a [*psi*$^+$] × [*psi*$^-$] cross invariably has a [*psi*$^+$] phenotype [1].

Here, we demonstrate that [*psi*$^-$] lysates contain a ribosome-associated inhibitor of in vitro nonsense suppression and propose that this inhibitor is the yeast protein release factor (RF) required for translational termination.

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2. MATERIALS AND METHODS

2.1. *Cell-free translation*

Cell-free S100′ lysates were prepared from either the *sup*$^+$ [*psi*$^+$] strain 465/4d or a [*psi*$^-$] derivative thereof as described in [6]. In vitro amber suppression assays were carried out as in [4,7].

2.2. *Fractionation of lysate*

Ribosomes were pelleted from S100′ lysates by ultracentrifugation (2 h, 48000 rpm) and the resulting post-ribosomal supernatant (PRS) carefully removed from the ribosome pellet (RIB).

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The RIB pellet was resuspended in 1.0 ml G25 buffer [100 mM KOAc, 20 mM Hepes (pH 7.4), 2 mM MgOAc, 2 mM dithiothreitol, 20% (w/v) glycerol] and repelleted in a second ultracentrifugation step (2 h, 48000 rpm). The washed RIB pellet was resuspended in 100 μl G25 buffer.

To remove ribosome-associated factors 50 μl of the RIB fraction was pelleted and resuspended in 100 μl KCl buffer (0.5 M KCl, 5 mM MgOAc, 20 mM Hepes, pH 7.4) and placed on ice for 30 min. The ribosomes were then repelleted, the supernatant carefully removed and the remaining washed ribosomes resuspended in 50 μl G25 buffer. All components were stored at $-70°C$ until required. In the translation mixtures all components were separately treated with micrococcal nuclease, prior to mixing and adding template.

2.3. *Protein determination*

The protein concentration of all samples was determined according to Bradford [8].

3. RESULTS

The efficiency of amber suppression in vitro was assayed by determining the level of translational read-through of the amber termination codon of the brome mosaic virus (BMV) coat protein cistron, in a yeast cell-free system [7]. Two read-through polypeptides (CP′, CP′′) are synthesized in the presence of amber suppressor tRNA (fig.1). CP′′ is synthesized as a result of an endogenous UGA suppressor tRNA activity in the yeast cell-free system [7].

The efficiency of amber suppression is much higher in a [*psi*$^+$] lysate than in a [*psi*$^-$] lysate and little or no amber suppression is seen when the two lysates are mixed in equal proportions prior to the addition of the suppressor tRNA and BMV RNA (fig.1). If, however, the [*psi*$^+$] and [*psi*$^-$] lysates were mixed 30 min after the addition of the suppressor tRNA and BMV RNA, amber suppression was still observed (not shown). This suggests that the [*psi*$^-$] lysate does not contain a post-translational modifying activity, e.g. a protease, specific for read-through polypeptides, but rather contains a factor which acts at the level of translation to inhibit termination read-through.

As a first step in characterizing the inhibitor we fractionated both the [*psi*$^+$] and [*psi*$^-$] cell-free

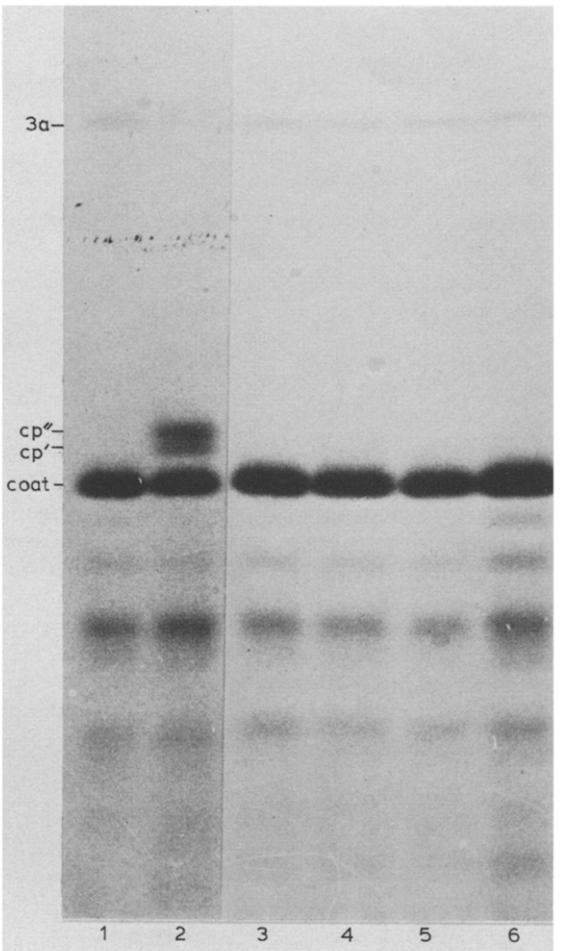

Fig.1. In vitro amber suppression in [*psi*$^+$] and [*psi*$^-$] lysates. A [*psi*$^+$] lysate (lanes 1,2), a [*psi*$^-$] lysate (lanes 3,4) and a mixed ([*psi*$^+$]/[*psi*$^-$]) lysate (lanes 5,6) were tested. Amber suppressor tRNA was added to the assays in lanes 2, 4 and 6.

lysates into a ribosome fraction (RIB) and a post-ribosomal supernatant fraction (PRS). Neither fraction contained significant protein synthetic activity alone, but when recombined activity was essentially restored (fig.2). The PRS and RIB fractions from both lysates were then mixed in various combinations and the efficiency of amber suppression determined (table 1). Detectable amber suppression was only observed when both fractions were derived from the [*psi*$^+$] lysate and the mixing experiments demonstrate that the inhibitor, present in [*psi*$^-$] lysates, is present in both the RIB and

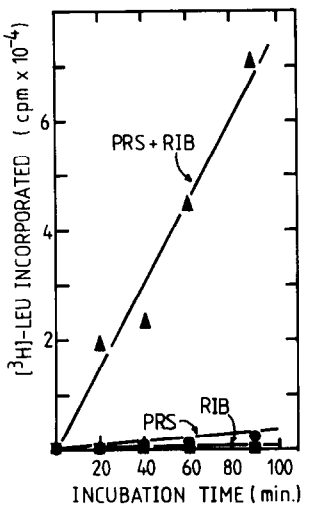

Fig.2. Protein synthetic activity of the fractionated [*psi*$^+$] lysate and its individual components. (■) Ribosome fraction alone; (●) post-ribosomal supernatant fraction alone; (▲) reconstituted lysate.

PRS fractions. The inhibitor, like a number of other protein synthesis factors, appears to be loosely associated with the ribosome.

The inhibitor was removed from [*psi*$^-$] 80 S ribosomes by incubating the ribosomes in a high salt buffer (table 2), a treatment which effectively removes all ribosome-associated protein synthesis factors [9]. The [*psi*$^-$] salt wash fraction strongly inhibited amber suppression whereas a [*psi*$^+$] salt wash fraction did not (table 2). Heating the unfractionated [*psi*$^-$] lysate or the [*psi*$^-$] RIB fraction at 65°C for 10 min, prior to addition to the [*psi*$^+$] lysate, destroyed their inhibitory activity (table 2), indicating that the inhibitor is a protein rather than a nucleic acid species.

We conclude from these experiments that the [*psi*$^-$] lysate contains a protein factor or factors loosely associated with the ribosome that severely restricts the ability of amber suppressor tRNA to translate the UAG codon in vitro. It is not associated with the mitochondrial ribosome or encoded by the mitochondrial genome because a cell-free lysate from [*psi*$^-$] [*rho*^{0}] (mitochondria-less) strain also contains this inhibitor (not shown).

Table 1

Efficiency of in vitro amber suppression in fractionated [*psi*$^+$] and [*psi*$^-$] cell-free lysates and in lysates containing mixed components

Origin of lysate components		% amber suppression
PRS	RIB	
+	+	29.7
−	−	1.2
+/−	+	5.8
+/−	−	0.1
+	+/−	6.9
−	+/−	4.5
+	−	4.9
−	+	1.7

+, [*psi*$^+$] lysate; −, [*psi*$^-$] lysate

Table 2

Analysis of the inhibitor of in vitro amber suppression found in [*psi*$^-$] lysates

Component added	Amount of protein added (μg)	% amber suppression
None	−	36.0
[*psi*$^-$] lysate		
Untreated	8.3	7.0
Heat-denatured	8.3	37.1
[*psi*$^-$] ribosomes		
Untreated	3.4	9.6
Heat-denatured	3.4	38.6
Washed with 0.5 M KCl	3.4	24.9
0.5 M KCl wash fraction		
[*psi*$^+$] ribosomes	10.0	35.8
[*psi*$^-$] ribosomes	10.0	8.1

In all incubations 52 μg protein from the [*psi*$^+$] total lysate was present

4. DISCUSSION

The efficiency of nonsense suppression, both in vivo and in vitro, reflects the competition between the suppressor tRNA and the normal termination mechanism, for the termination codon. Studies to examine such a competition have been carried out in *E. coli* cell-free lysates where, by varying the amounts of suppressor tRNA or termination

release factor (RF), the levels of tRNA-driven suppression varied as would be predicted by such a competition [10,11]. By implication, physiological or genetic factors that increase the efficiency with which a suppressor tRNA translates its cognate termination codon must either impair the normal termination mechanism, or increase the efficiency with which the suppressor tRNA binds to the termination codon on the ribosome. The cytoplasmically inherited [*psi*] factor represents a novel genetic modifier that influences this competition; the suppressor tRNA is more efficient in a [psi^+] strain than in a [psi^-] strain [1], the same holding true for in vitro nonsense suppression [4]. In addition, antibiotic-induced termination errors (but not missense errors) are elevated in [psi^+] strains and lysates [12,13].

In this paper we report the existence of an inhibitor in [psi^-] strains that markedly reduces the efficiency of amber suppression in cell-free lysates prepared from an isogenic [psi^+] strain. Similar results were obtained with in vitro UGA and ochre suppression (not shown). The inhibitor is loosely associated with the 80 S ribosome, can be removed by washing the ribosome with high salt and its inactivation by heat (65°C for 10 min) strongly suggests that it is a protein rather than a nucleic acid.

We have previously proposed that the observed differences in the efficiency of nonsense suppression between [psi^+] and [psi^-] cell-free lysates is at the level of termination rather than suppressor tRNA [4,13]. The inhibitor we have identified in [psi^-] lysates could therefore be the normal yeast release factor (RF). The mechanism of translation termination in yeast is currently unknown, but by analogy with higher eukaryotes, should contain a single RF that binds transiently to the 80 S ribosome and is capable of recognising all three termination codons, unlike in *E. coli* where at least two RFs exist (review [14]). Therefore, one possible mechanism to explain our observations is that [psi^+] strains synthesize either a defective RF or reduced levels of the normal RF and that by addition of [psi^-] lysate one restores an effective concentration of good RF, thus shifting the competition for the termination codon back in favour of the termination mechanism. To test this model we are currently isolating and characterising RFs from both [psi^+] and [psi^-] yeast strains.

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