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Nonsense suppression in eukaryotes: the use of the Xenopus oocyte as an in vivo assay system

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ABSTRACT

Amber, ochre, and opal nonsense suppressor tRNAs isolated from yeast were injected into Xenopus laevis oocytes together with purified mRNAs (globin mRNA from rabbit, tobacco mosaic virus-RNA). Yeast opal suppressor tRNA is able to read the UGA stop codon of the rabbit β-globin mRNA, thus producing a readthrough protein. A large readthrough product is also obtained upon coinjection of yeast amber or ochre suppressor tRNA with TMV-RNA. The amount of readthrough product is dependent on the amount of injected suppressor tRNA. The suppression of the terminator codon of TMV-RNA is not susceptible to Mg++ concentration or polyamine addition. Therefore, the Xenopus laevis oocyte provides a simple, sensitive, and well buffered in vivo screening system for all three types of eukaryotic nonsense suppressor tRNAs.

INTRODUCTION

Nonsense mutations and their suppressors have played a key role in the study of gene expression in bacteria and yeast[1,2], but despite great efforts, no nonsense suppressor tRNAs have been identified so far in higher eukaryotes[1]. A crucial part in the search for nonsense suppressors is the assay system employed for screening. Several eukaryotic, in vitro protein synthesis systems have been developed and used for screening purposes[3-5], but most of these are susceptible to changes in the components (see below) and are thus prone to artifacts[6-8]. In order to eliminate this problem microinjection techniques have been used to introduce the tRNAs to be assayed directly into cultured cells[1,9]. However, only a few nonsense

mutants are known[1], and some of these highly specialized cells (e.g. the IF 3 mutant of mouse myeloma) are not able to use the injected suppressor tRNAs in their protein synthesizing machinery (M.Bienz, unpublished results).

The usefulness of the Xenopus laevis oocyte as a living test tube for various purposes has been definitely demonstrated[10]. Messenger RNAs injected into the oocytes are translated with high efficiency[11-13]. Furthermore, injected yeast tRNAs will be aminoacylated[14] and the amino acids will be incorporated into proteins[15]. Taking advantage of these facts we have injected amber, ochre, and opal nonsense suppressor tRNAs isolated from yeast together with purified mRNAs (globin mRNA from rabbit, tobacco mosaic virus (TMV)-RNA) into oocytes. In this paper we demonstrate that the yeast opal suppressor tRNA is able to read the UGA stop codon of the rabbit β-globin mRNA, thus producing a readthrough protein. A large readthrough product is also obtained upon coinjection of yeast amber or ochre suppressor TMV-RNA. Hence, the Xenopus laevis oocyte can be used as an assay system for eukaryotic nonsense suppressor tRNAs.

MATERIALS AND METHODS

Preparation of mRNAs and suppressor tRNAs: Rabbit globin mRNA was prepared according to Aviv and Leder[16] or Marbaix et al.[17], TMV-RNA has been extracted from the virus as described by Marcus et al.[18]. Opal suppressor $tRNA^{Ser}$ was purified to 80% homogeneity from a S.pombe strain as described earlier[19]. Amber or ochre suppressor $tRNA^{Tyr}$ were partially purified to 30% homogeneity from the S.cerevisiae strains SUP5-a and SUP5-o[20] by one-dimensional gel electrophoresis[21], and after staining with ethidium bromide, by elution of the $tRNA^{Tyr}$-containing band[22]. The same procedure was used for the purification of ochre suppressor $tRNA^{Ser}$ from S.pombe to 30% homogeneity.

Injection and labeling of Xenopus laevis oocytes; polyacrylamide gel electrophoresis: Samples of 10 Xenopus laevis oocytes were injected with one of the following: 5 ng globin mRNA or 100 ng TMV-RNA, 5 ng opal suppressor $tRNA^{Ser}$, 15 ng amber or ochre suppressor $tRNA^{Tyr}$ or 15 ng ochre suppressor $tRNA^{Ser}$ or varying tRNA concentrations as indicated in the figures 2 and 3. These concentrations represent the effective amount of $tRNA^{Ser}$ or $tRNA^{Tyr}$ respectively since the purification factor was taken into account. After injection, the oocyte samples were incubated in Barth's medium[23] at 18° C over night and labeled with 70 µCi [^{35}S]-methionine for TMV-RNA or with 20 µCi [^{3}H]-histidine for globin. The use of histidine for the labeling of globin allows an efficient detection of the globin readthrough because of the extremely high histidine content of globin in comparison with the background proteins of the oocyte. Extraction of soluble proteins was performed according to Knowland[12], omitting the DNAse and RNAse digestion steps. Sample preparation and one-dimensional SDS-gel electrophoresis was carried out after Laemmli[24], using a low bisacrylamide concentration in the separation gel (acrylamide:bisacrylamide 75:1) in order to get optimal separation of the readthrough products from the background proteins. The acrylamide concentration was 15% for globin and 7,5% for TMV-RNA. In the case of [^{3}H]-labeling, fluorography was done according to Bonner and Laskey[25]. For the immunoprecipitation the oocyte extracts were incubated with anti-rabbit globin over night at 4° C, the precipitates were collected by centrifugation, subsequently washed three times with 25 mM Tris-HCl (pH 7.5), 10 mM EDTA, 0.35 M NaCl, 1% Triton X-100 and resuspended in sample buffer[24].

RESULTS AND DISCUSSION

Rabbit β-globin mRNA terminates with a UGA codon followed by 92 nucleotides and a poly(A) tail[26,27]. Kohli et al.[19] were able to suppress this terminator codon by the addition of a UGA suppressor tRNA of Schizosaccharomyces pombe . The resulting elongated protein (MW 18.5 K, natural protein 16 K) was assayed

on a one-dimensional SDS-polyacrylamide (PAA) gel[19]. Since efficient translation of β-globin mRNA is possible in Xenopus oocytes[11], and since yeast tRNA can effectively participate in the oocyte protein synthesis[14,15,28], we reasoned that a readthrough protein might also be synthesized in this in vivo system.

The results of the experiment confirm this idea (Fig. 1a). Upon injection of 5 ng globin mRNA per oocyte, the 16 K MW protein (β-globin) is clearly visible on a 15% PAA gel in comparison with the uninjected control. Although the injected globin mRNA contains the messengers for the α and the β chain, almost none of the α-messenger is translated in the absence of hemin[11,29]; consequently the synthesized protein is the β-globin chain. Additional injection of a purified UGA suppressor $tRNA^{Ser}$ from yeast leads to a 18,5 K MW readthrough product. This demonstrates the efficient participation of the foreign tRNA in the protein synthesis of the Xenopus oocyte. Neither amber nor ochre $tRNA^{Tyr}$, nor ochre $tRNA^{Ser}$ (all isolated from yeast) lead to readthrough products. Control experiments performed with immunoprecipitation with goat antiglobin show unambiguously that rabbit β-globin and its readthrough product have been synthesized in the presence of the UGA suppressor $tRNA^{Ser}$ (Fig. 1b).

Tobacco mosaic virus-RNA can be translated into a 110 K MW protein both in Xenopus oocytes[12] and in a reticulocyte in vitro translation system[6]. Traces of a 160 K MW protein can be observed in the reticulocyte system and the amount can be increased by the addition of Mg^{++} ions[6], polyamines[7], or partially purified amber or ochre suppressor tRNAs[6]. It has been concluded that this product arises by the readthrough of a leaky amber termination codon using a mechanism which has been described for some prokaryotic viruses[30-33]. Analogous to the ß-globin readthrough, TMV-RNA can give rise to the 160 K MW protein after it is injected into oocytes together with amber suppressor $tRNA^{Tyr}$, an ochre suppressor $tRNA^{Tyr}$, or an ochre suppressor $tRNA^{Ser}$, all from yeast (Fig. 1c). Al-

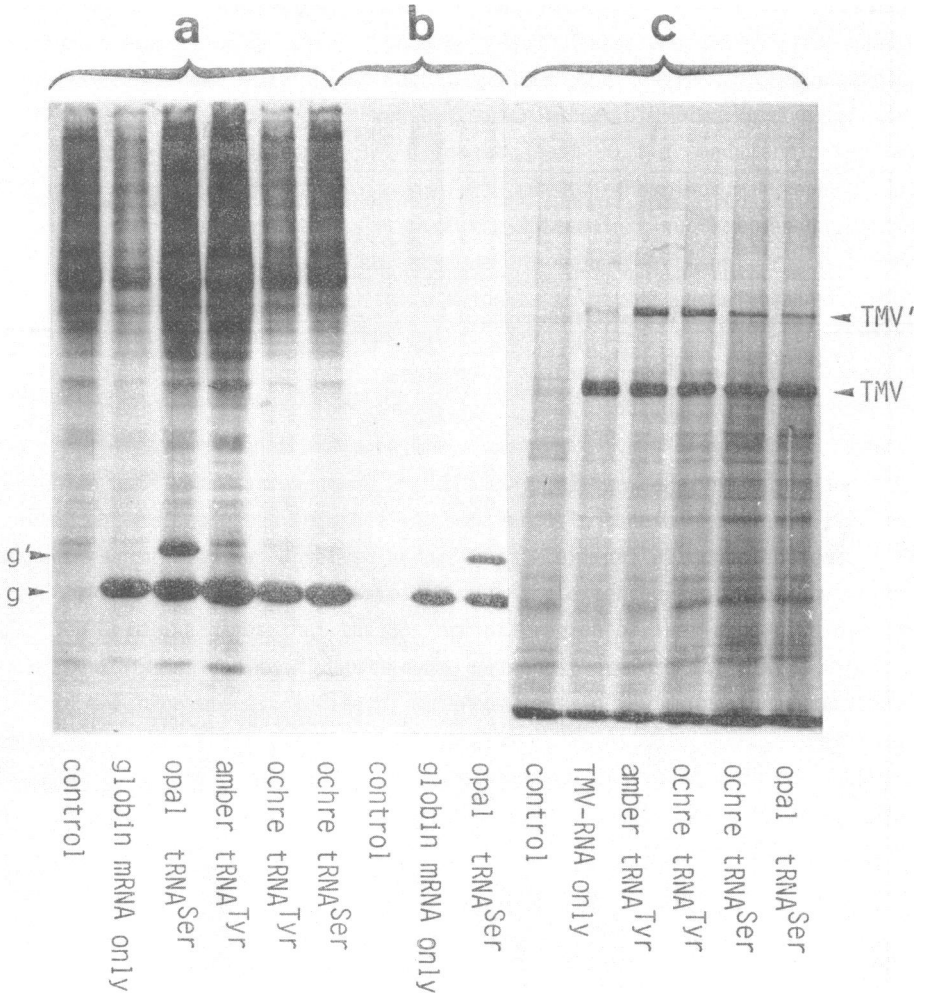

Figure 1: Autoradiographs of translational products of Xenopus laevis oocytes after globin mRNA (a), after globin mRNA and subsequent immunoprecipitation with goat antiglobin (b), or TMV-RNA (c) injection, and concomitant yeast suppressor tRNA injection. Arrows point to the globin (g) and to the globin readthrough product (g') in Fig. 1a, to the 110 K (TMV) and to the 160 K (TMV' = readthrough product) protein in Fig. 1c.

though the response to the ochre $tRNA^{Ser}$ is weak (20%), it is clearly present, whereas no increase in the amount of the readthrough product above the natural level (7-11%) is observable

with opal $tRNA^{Ser}$ (11%) which effectively reads the UGA terminator. In the *in vitro* system the 160 K MW protein also occurs in small amounts without the addition of suppressor tRNA[6]. This confirms the assumption that the 160 K MW protein is a readthrough product of the 110 K MW protein. However, the effect of the injected suppressor tRNA is strong enough to serve as an indication for amber or ochre suppressor activity.

The amount of the readthrough product is dependent on the amount of injected eukaryotic suppressor tRNA for both β-globin mRNA (Fig. 2a) and TMV-RNA (Fig. 2b). The quantitative data are summarized in Fig. 3. A minimum of 1 ng/oocyte is required for the opal $tRNA^{Ser}$, 10 ng/oocyte for the ochre $tRNA^{Ser}$, and 2 ng/oocyte for amber or ochre $tRNA^{Tyr}$ in order to obtain unambiguous results (one oocyte is about 1 µl). The difference between the two ochre suppressors reflects the fact that the proportion of the actual suppressor tRNA in the probe is only 1:20 for ochre $tRNA^{Ser}$ and 1:8 for ochre or amber $tRNA^{Tyr}$, since there are 20 genes coding for $tRNA^{Ser}$ and 8 genes coding for $tRNA^{Tyr}$ and only one of each is mutated to a suppressor. This is also the cause of the lower readthrough efficiency of ochre $tRNA^{Ser}$. Saturation is achieved upon injection of 2.5 µg of total tRNA/oocyte for both systems (not shown). This represents about 12 times the concentration of total tRNA contained in one oocyte. Injection of greater amounts leads to inhibition of protein synthesis. The efficiency of the terminator readthrough can be calculated from the ratio of readthrough protein formed in the presence of suppressor tRNA. For such an estimation, the molar ratios of the radioactive amino acid must be taken into account. However, this can only be done for the rabbit β-globin, since the amino acid compositions of the TMV 110 K and 160 K MW protein are not known.

As mentioned above, suppression of the terminator codon of TMV-RNA *in vitro* is susceptible to Mg^{++} concentration[6] and polyamine addition[7], whereas none of these effects is observed in the *Xenopus* oocyte (Fig. 2b, last four panels; 2.1 mM Mg^{++} 10%, and 100 µM spermidine 11% readthrough). Therefore, the *in*

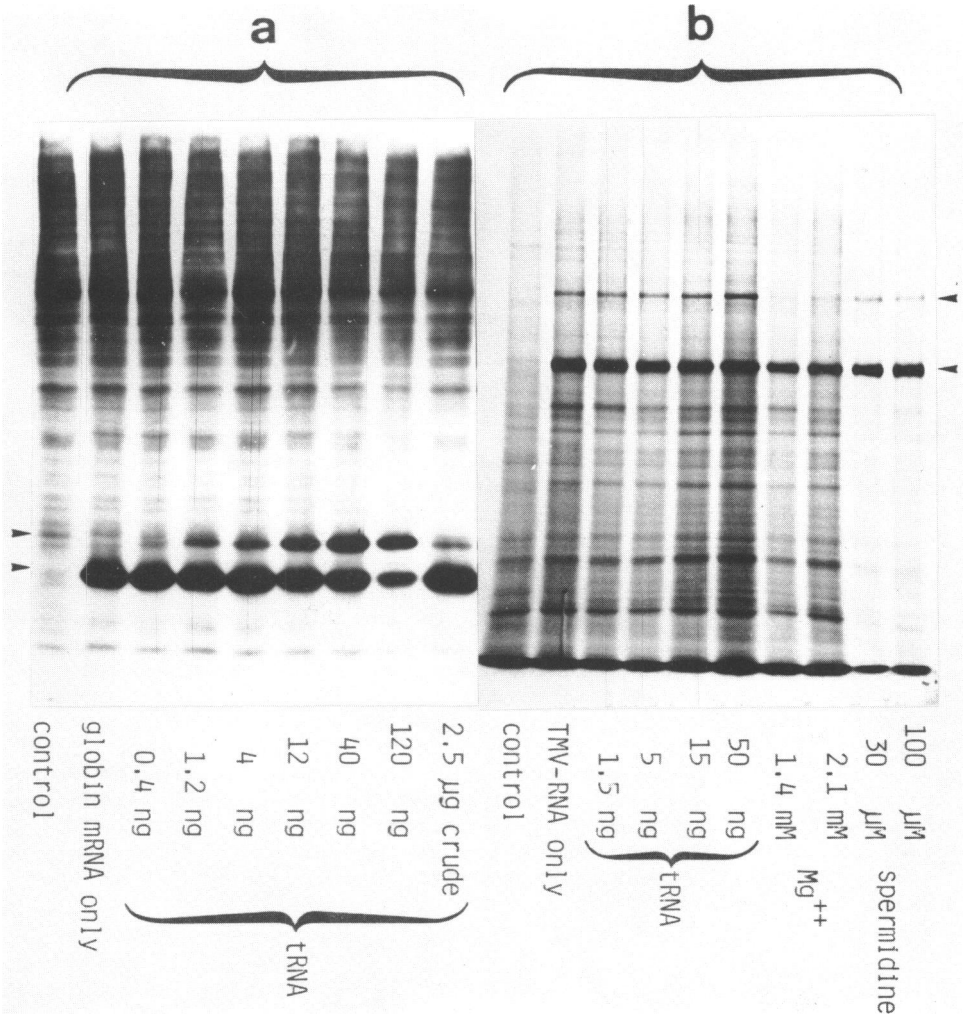

Figure 2: Autoradiographs of translational products after injection of globin mRNA (a) or TMV-RNA (b) into Xenopus oocytes and concomitant injection of suppressor tRNA in increasing amounts, as indicated in the figures, to demonstrate the dosage dependence of the amount of the readthrough product on tRNA concentration (lanes 3-7 in a and b). Opal suppressor $tRNA^{Ser}$ was used in Fig. 2a; ochre suppressor $tRNA^{Ser}$ was used in Fig. 2b, in order to show the minimal concentration threshold of an inefficient suppressor tRNA for a recognizable readthrough. Fig.2a, lane 8: crude tRNA from S.pombe opal suppressor strain. The effect of Mg^{++} and spermidine on TMV-RNA readthrough is shown in Fig. 2b, lanes 8-11. Mg^{++} was not only injected but also added to the incubation medium.

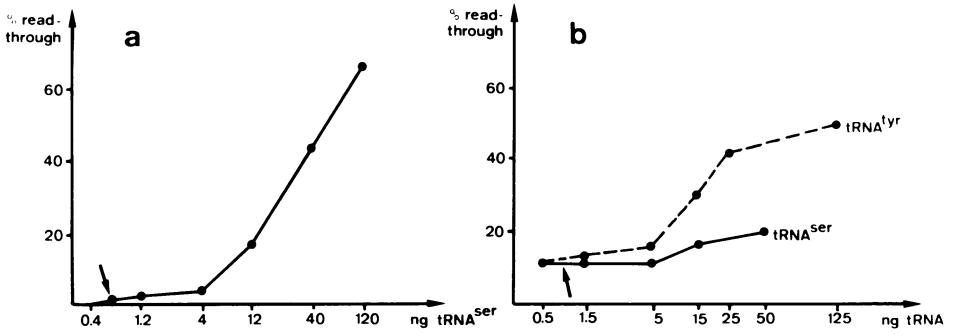

Figure 3: Dependence of the amount of readthrough products on the concentration of the suppressor tRNAs. The graph values correspond to the values of the gels in Figs. 2a and b. In Fig. 3a the ordinate gives the effective values of readthrough percentage since the histidine content of the readthrough protein has been taken into account (β-globin 9 his, readthrough protein 10 his). This calculation cannot be performed for the TMV proteins (Fig. 3b), because the amino acid compositions are not known. Hence the radioactivity of the 110 K MW protein has been used directly. On the abscissa, the concentration of the tRNAs is given in a logarithmic scale in order to visualize the lower part of the curves, i.e. the treshold of minimal tRNA concentration. Again, the concentration values represent the effective amount of $tRNA^{Ser}$ injected into one oocyte; the purification factor is taken into account. The arrow in Fig. 3a points to the value of readthrough product after injection of crude tRNA from the opal suppressor strain of S.pombe. The arrow in Fig. 3b indicates to the background level of 11% readthrough which is obtained after coinjection of 1.4 mM or 2.1 mM magnesium acetate or of 30 uM or 100 mM spermidine together with TMV-RNA.

vivo system seems to be more effectively buffered against the influence of these substances with respect to suppression. Furthermore, the oocyte provides optimal aminoacylation conditions which may be needed for a detection of suppressor effects.

A comparison of the sensitivity of the Xenopus oocyte with the reticulocyte system shows that about one third to one half the amount of tRNA is needed to produce a recognizable readthrough product for β-globin and TMV-RNA in the oocyte (Kohli et al.[19], and not shown), i.e. the in vitro system is only 30-50% as efficient in comparison with the in vivo system. This is despite the background label due to the Xenopus oocyte

proteins. Furthermore, a readthrough protein is more easily observed in the Xenopus oocyte in comparison with the in vitro systems if unfractionated suppressor tRNA is used (Kohli et al.[19]; Fig. 2a, last panel). The fact that the $tRNA^{Ser}_{S.pombe}$ (Fig. 1c), which has been genetically described[34] as an inefficient ochre suppressor, is albe to readthrough the TMV-RNA stop codon in the oocyte at detectable level also demonstrates that weak suppressors can be detected in this assay system.

In conclusion we have shown that for all three types of nonsense suppressor tRNAs, the Xenopus laevis oocyte provides a simple, sensitive, and well buffered in vivo screening device which is less prone to artifacts than the in vitro protein synthesizing systems. This is the type of system which would be necessary for the successful search for eukaryotic suppressor tRNAs.

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