Volume 9 Number 15 1981 Nucleic Acids Research

Usage of the three termination codons in a single eukaryotic cell, the Xenopus laevis oocyte

M.Bienz[1], E.Kubli[1], J.Kohli[2], S.deHenau[3], G.Huez[3], G.Marbaix[3] and H.Grosjean[3]

[1]Zoological Institute, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received 5 May 1981

ABSTRACT

Oocytes from Xenopus laevis were injected with purified amber (UAG), ochre (UAA), and opal (UGA) suppressor tRNAs from yeasts. The radioactively labeled proteins translated from the endogeneous mRNAs were then separated on two-dimensional gels. All three termination codons are used in a single cell, the Xenopus laevis oocyte. But a surprisingly low number of readthrough polypeptides were observed from the 600 mRNAs studied in comparison to uninjected oocytes. The experimental data are compared with the conclusions obtained from the compilation of all available termination sequences on eukaryotic and prokaryotic mRNAs. This comparison indicates that the apparent resistance of natural termination codons against readthrough, as observed by the microinjection experiments, cannot be explained by tandem or very close second stop codons. Instead it suggests that specific context sequences around the termination codons may play a role in the efficiency of translation termination.

INTRODUCTION

The genetic code is composed of 61 triplets coding for amino acids and 3 stop, or termination codons: UAG (amber), UAA (ochre), and UGA(opal). From the determined sequences of messenger RNAs and genes it can be concluded that all three stop codons are used in prokaryotic and eukaryotic cells[1], with the exception of mitochondria, where UGA specifies tryptophan[2,3].

It has been proposed that the stop codons might have additional specific functions and meanings. The low efficiency and deleterious effects of ochre suppressor tRNAs have led to the suggestion that UAA is the prevalent termination signal in E.coli[4]. Also, Kaplan[5] has estimated that the ochre codon is used 25 times more often than the amber codon in E.coli. In eukaryotes

two findings have been interpreted in favor of a special use of the termination codons. Firstly, natural readthrough of putative amber codons might occur in animal and plant cells infected with murine oncorna viruses and TMV[6,7]. Furthermore, a Herpes simplex virus amber nonsense mutant has been found to be leaky, whereas an opal mutant is not[8]. These authors propose that UAA and UGA are the usual terminators in eukaryotes. Secondly, a natural UGA suppressor activity not associated with viral infections has been reported by Geller and Rich[9]. In conclusion it has been suggested that the codon UAA would mean that translation must stop, whereas UAG and UGA would allow occasional readthrough[9].

In the light of these findings it is of interest to study the stop codons used in the population of mRNAs of a single cell. We have therefore investigated the distribution of the stop codons used by the mRNA population of the Xenopus laevis oocyte and also compiled all available mRNA and gene sequences[1]. In a preceding paper[10] we have shown that suppressor tRNAs from yeasts, when microinjected into a Xenopus laevis oocyte together with a messenger RNA possessing the corresponding termination codon, lead to the production of a readthrough protein due to suppression of the stop codon of the messenger RNA. In this paper, we have microinjected each of the three types of nonsense suppressor tRNAs isolated from S.cerevisiae and S.pombe alone or in combinations of two. The translation products were analyzed by electrophoresis on two-dimensional polyacrylamide gels and compared with an uninjected control. Readthrough events were observed, but at low frequency. A quantitative estimation of the terminators used in the Xenopus oocyte was attempted. The results are evaluated with the help of compilation of termination sequences[1], where we also discuss some further findings and data from the literature.

MATERIALS AND METHODS

Sources and preparations of tRNAs: The UGA suppressor $tRNA^{Ser}$ (anticodon xU*CA[11], xU* is partly mcm^5U partly s^2U) was purified to 80% homogeneity from the S.pombe strain sup3-e as described earlier[12]. The ochre suppressor tRNA from S.pombe sup3-i was enriched by BD-cellulose column chromatography to approximately 10% purity (J.Kohli, unpublished). Crude tRNA preparations from

S.cerevisiae strains SUP5-a (amber, anticodon CΨA[13]) and SUP5-o (ochre) were provided by Drs.P.Piper and J.Celis (Aarhus). From both batches $tRNA^{Tyr}$ has been purified to 20 - 30% homogeneity by one-dimensional gel electrophoresis[14], subsequent staining with ethidium bromide and elution of the $tRNA^{Tyr}$ containing band[15]. Since the amount of readthrough product is proportional to the concentration of the suppressor tRNA in an injected tRNA probe[10] the degree of purity of the sample has no influence on our assay system. Pure $tRNA^{Phe}$ (anticodon GmAA, Gm is 2'-o-methyl G) from yeast was purchased from Boehringer (Mannheim) and pure cytoplasmic $tRNA^{Trp}$ (anticodon CmCA, Cm is 2'o-methyl C) was a gift from Dr.G. Keith (Strasbourg).

Injection and labeling of Xenopus oocytes: Xenopus laevis oocytes were injected with suppressor tRNAs: 5 ng/oocyte for the UGA suppressor $tRNA^{Ser}$, 5 - 15 ng/oocyte for the UAG and UAA suppressor $tRNA^{Tyr}$ and 2 ng/oocyte for the UAA suppressor $tRNA^{Ser}$, 5 ng/oocyte for $tRNA^{Trp}$ and $tRNA^{Phe}$, i.e. the purification factor was taken into account. For each sample, 10 oocytes were labeled in 100 µl Barth's solution[16] with 70 µCi [^{35}S]-methionine (Radiochemical Centre, Amersham, specific activity 730 Ci/mM) for 20 hours at 18° C. Preparation of the protein samples was done according to Gurdon et al.[17], omitting the RNase and DNase digestion steps.

Two-dimensional gel electrophoresis: The analysis of the proteins on two-dimensional gels was done essentially according to O'Farrell[18]. For the fluorography the procedure of Bonner and Laskey[19] was followed.

RESULTS

Rationale and evaluation techniques for the experimental determination of the abundance of the three termination codons used in the mRNA population of the Xenopus oocyte: The preceding paper[10] demonstrates that the injected yeast suppressor tRNAs participate actively in the protein synthesis of the oocyte. Therefore, we thought it possible to readthrough the natural stop codons of the oocyte mRNAs and thus increase the size of the synthesized proteins. The resulting differences could then be assayed on two-dimensional polyacrylamide gels[18] which offer a sensitive test for alterations in proteins. Furthermore, we assume that each

suppressor induced change represents a readthrough of a natural stop codon of the type corresponding to the injected tRNA suppressor. In other words, counting the changes induced by amber, ochre, or opal suppressors should give an estimation of the relative abundance of amber, ochre, and opal termination codons in the mRNA population of the Xenopus oocyte. The mRNA population assayed by this procedure constitutes the mRNAs actively engaged in translation in a eukaryotic cell. The oocytes used in these experiments were all obtained from the same frog.

Yeast suppressor tRNAs have been injected separately or in combinations of two into oocytes. The following suppressor tRNAs were used: amber and ochre suppressor $tRNA^{Tyr}$ from S.cerevisiae, ochre suppressor $tRNA^{Ser}$, and opal suppressor $tRNA^{Ser}$ from S.pombe. The suppressor tRNA concentration (2 - 15 ng/oocyte, purification factor taken into account. This corresponds roughly to 1/20 to 1/3 of the total amount of tRNA in a Xenopus oocyte[20]) was chosen such as to produce clearly observable readthrough proteins in the experiments described elsewhere (Fig. 2 Bienz et al.[10]) with coinjected mRNA. After injection the oocytes were labeled for 20 hours with [^{35}S]-methionine, the soluble proteins extracted and analyzed on two-dimensional gels[18]. Following autoradiography the observed changes were classified upon visual inspection as either major or minor: Major means that a new strong spot appears, or that there is a reduction of an already existing strong spot. Minor means that a faint spot appears or that an already existing faint spot disappears. It is difficult to correlate an affected protein with its readthrough product, since it is impossible to predict the changes in the characteristics imposed on an unknown protein by the readthrough event and hence the future location on the two-dimensional gel. For this reason we did not try to count newly appearing and disappearing (or decreasing) spots individually. Elongation of a given protein may result in either one or both types of changes depending on the efficiency of the readthrough. For the evaluation, all patterns were projected on the same control gel. This allows the identification of common changes induced by different suppressor tRNAs. The pattern comparison was done with a sample of proteins corresponding to 150 strong and 450 weak spots on the control gels from uninjected oocytes all origi-

nating from the same frog. The comparison of films was repeated several times to minimize uneven judgement by eye. Also the protein pattern of oocytes injected with saline, yeast $tRNA^{Phe}$ or $tRNA^{Trp}$ served as further controls. The method used resulted in highly reproducible gel patterns. Autoradiograms from independent injections and gels are displayed in Figures 1 and 3, where some of the reference spots are marked with arrows in the upper right corner of the respective films. It is important to realize that the detailed evaluation can only be done with the original autoradiograms. This is due to the fact that most of the observed alterations are minor changes (see below). Hence, the photographs of Fig. 1 and 3 provide a general impression of the complexity of the pattern and only some major and suppressor-typical changes are indicated.

Description of the results: The protein synthesis pattern of the uninjected oocytes is shown in Fig. 1a. About 600 protein spots are reproducibly resolved by the two-dimensional gel electrophoresis technique of O'Farrell[18]. Saline injected oocytes reveal only 3 minor changes (Fig. 1b) whereas yeast $tRNA^{Phe}$ or $tRNA^{Trp}$ produce each about 20 minor changes (Fig. 1c; $tRNA^{Phe}$ pattern not shown) in comparison with the uninjected oocytes (Fig. 1a). This demonstrates that the pattern is only slightly affected by the injection per se or the introduction of non-suppressor tRNAs. The very few changes induced by injection of yeast cytoplasmic $tRNA^{Trp}$ do not coincide with the changes after injection of the opal suppressor tRNA (Fig. 1c and 1f). Instead a typical "amber" spot can be observed (Fig. 1c and 1d) besides other untypical changes. Thus yeast cytoplasmic $tRNA^{Trp}$ has no detectable suppressor activity on natural UGA termination codons under *in vivo* conditions (see also Grosjean et al.[21]).

Some significant alterations are observed after injection of the yeast suppressor tRNAs (Fig. 1d - f). But surprising to us was the low number of total observed changes (159). Since the appearence of new spots and disappearence of preexisting spots can be due to the same readthrough event the effective number of readthrough events must be lower than 26%. Judging form our previous experience with foreign mRNA[10] we expected more changes. This refractory behaviour against readthrough by the termination codons

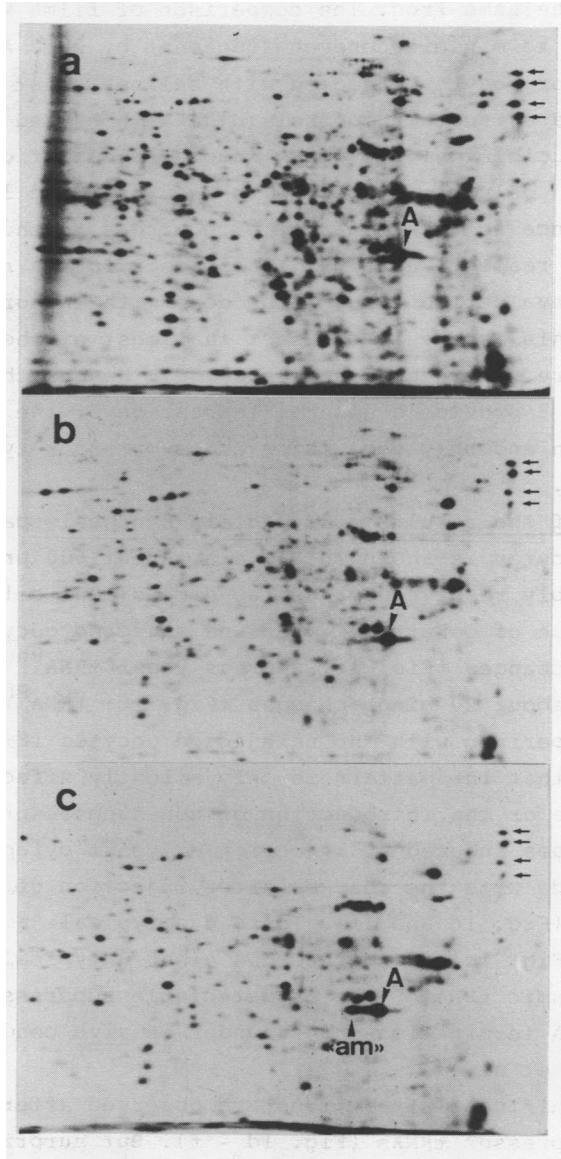

Figure 1: Autoradiographs of two-dimensional polyacrylamide gels of [^{35}S]-labeled oocyte proteins. First dimension: electrofocusing, pH range 5 - 7 (from right to left); second dimension: 10% polyacrylamide-SDS (top to bottom). a.) Uninjected control oocytes. b.) Injected with Barth's solution without antibiotics. c.) Injected with cytoplasmic $tRNA^{Trp}$ from yeast. Four constant spots are labeled by arrows in the upper right corner of the gels. The actin region is indicated by A.

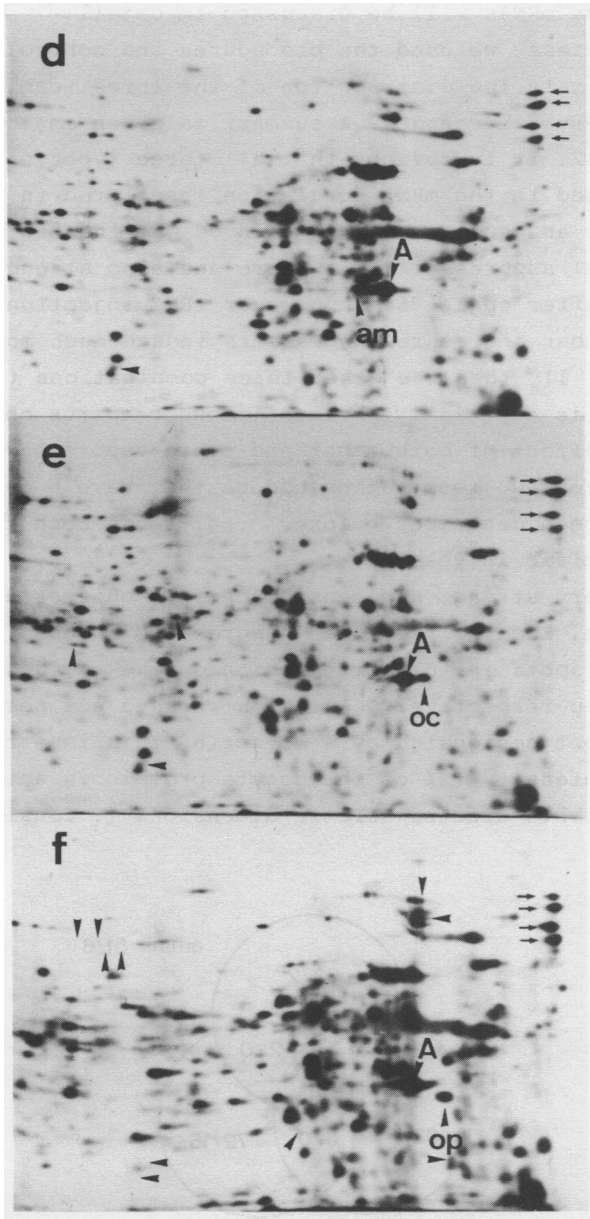

Figure 1 (cont.): d.) Injected with $tRNA^{Tyr}_{amber}$. e.) Injected with $tRNA^{Tyr}_{ochre}$. f.) Injected with $tRNA^{Ser}_{opal}$. Prominent suppressor specific spots are indicated by an am(amber), oc(ochre) and op (opal) respectively. Some other changes induced by suppressor tRNA injections are labeled by additional arrows.

of endogeneous mRNAs will be discussed in detail.

Nevertheless, we used the procedures and controls described above to estimate the distribution of the three termination codons from the observable changes. A summary is given in the Venn-diagram of Fig. 2. It is obvious that all three types of termination codons are used in the mRNA population translated in the Xenopus oocyte. About an equal amount of changes are induced by amber (51) and ochre (53) suppressor tRNAs. More and also stronger changes are counted after opal (78) suppressor tRNA injection. Fig. 2 shows that amber and ochre suppressors induce much more common changes (16 + 1), than the other three combinations (3 + 1, 2 + 1, and 1). This is especially obvious if one compares only the strong changes. An effect of both amber and ochre suppressor tRNAs from S.cerevisiae on two termination codons that have not yet been sequenced has been described before[6,7], although both these suppressors are specific in yeast[22].

A few very strong, suppressor specific changes deserve special consideration. Each of the three suppressors induces the appearance of strong spots in the actin region of the gel designated by am, oc, and op respectively. Although we have not positively identified the spot designated by A as actin, this is strongly suggested by its intensity (2% of the oocyte protein is actin) and its behaviour in both electrophoretic dimensions[23]. Considering the

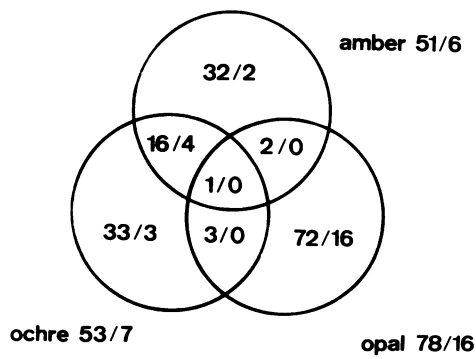

Figure 2: Venn-diagram of the results obtained with single suppressor tRNA injections. The first number refers to the total number of changes observed (600 spots screened), the second to changes in the strong spots only (150 spots screened)

fact that all three suppressor specific spots of this region are very strong they may be readthrough products of the various actin proteins. A few other strong deviations from the normal pattern are indicated by arrows in Fig. 1d - f.

A comparison of the patterns produced after injection of two different ochre suppressor tRNAs ($tRNA^{Tyr}$ and $tRNA^{Ser}$) revealed at least 60% common changes (not shown). The total number of induced changes in comparison with the control gel is similar. This supports the conclusion that the observed changes are dependent on the termination codon specificity of the suppressor and not on the tRNA species. Furthermore, the number of suppressor-induced changes seems not critically correlated with the efficiency of the suppressors in vivo in yeast.

The results of an additional experiment are represented in Fig. 3 and Table 1. Here oocytes were injected with a mixture of

Suppressor tRNA injected	Changes observed				
	Coinciding with single inj.			Double inj. specific	Total
	amber	ochre	opal		
amber+ochre	16(8)	13(7)	8(2)	33(9)	58(20)
single inj. (am + oc)	51(6)	53(7)	*6(0)		87(9)
amber+opal	21(7)	12(5)	24(13)	66(19)	107(38)
single inj. (am + op)	51(6)	*20(4)	78(16)		126(22)
ochre+opal	8(3)	11(2)	18(11)	41(19)	73(33)
single inj. (oc + op)	*19(4)	53(7)	78(16)		128(23)
Total double injections specific				140(47)	

Table 1: Changes observed after injection of mixtures of suppressor tRNAs. The numbers indicate the observed changes with the number of strong changes in brackets. For comparison the changes observed after single suppressor tRNA injections are given too. * overlaps.

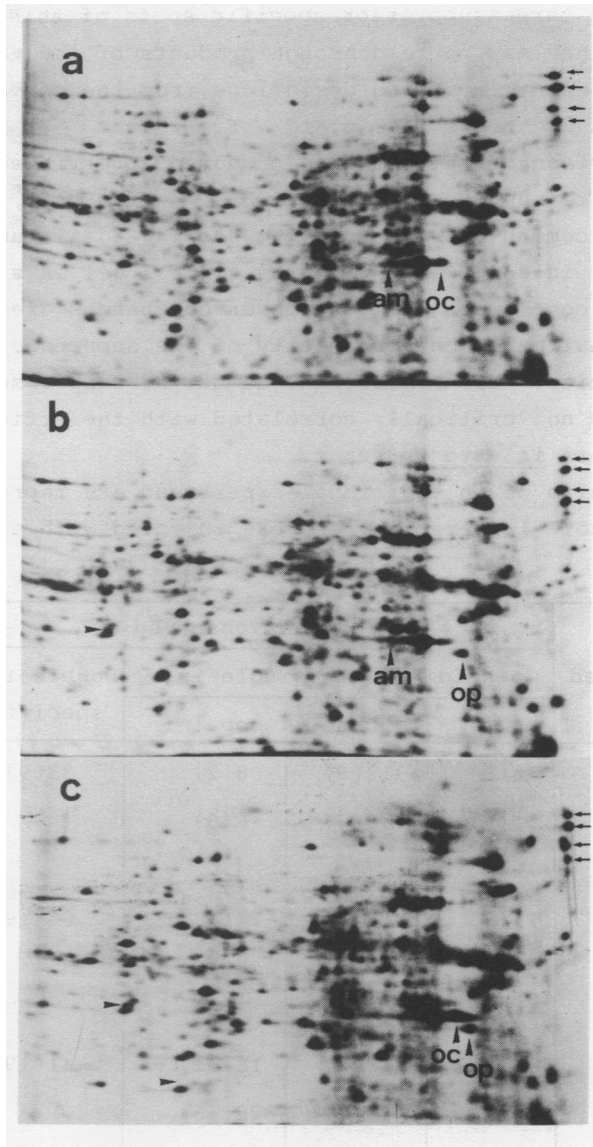

Figure 3: Autoradiographs of two-dimensional polyacrylamide gels of [^{35}S]-labeled oocyte proteins after injection of two suppressor tRNAs at the same time.
a.) $tRNA^{Tyr}_{amber}$ + $tRNA^{Tyr}_{ochre}$. b.) $tRNA^{Tyr}_{amber}$ + $tRNA^{Ser}_{opal}$.
c.) $tRNA^{Tyr}_{ochre}$ + $tRNA^{Ser}_{opal}$. Each of the suppressor specific spots (compare with Fig. 1) is clearly visible. Labeling and electrophoresis conditions as indicated in Fig. 1.

two suppressor tRNAs of different specificity. The patterns contain a part but never all the changes induced by individual suppressor tRNAs. Rather good agreement is observed if only major changes are considered. For example in the actin region, labeled by am, oc, and op, the suppressor specific spots can be seen according to the combinations of the injected suppressor tRNAs. As summarized in Table 1, there is, however, a set of changes specific for each type of double injection: The combinations of suppressor tRNAs amber/ochre, amber/opal, and ochre/opal give rise to 33(9), 66(19), and 41(19) specific changes. We interpret this set of changes as two consecutive readthrough events at the natural termination codon and a following in phase stop codon. For the detection of these double readthrough changes as well as the single readthrough discussed above, the distances between in phase termination codons in the 3' untranslated region of the studied mRNAs is of paramount importance. We discuss our data in the light of an estimate of these distances based on determined mRNA sequences[1]. It remains to be mentioned that a readthrough polypeptide should be resolved from the uncharged protein on our gels by one net change in charge (first dimension, electrofocusing) or the increased molecular weight caused by the addition of 5 - 15 amino acids depending on the size of the proteins (second dimension SDS-gel).

DISCUSSION

Experiments aimed at the determination of the distribution of the different polypeptide chain termination codons and the occurence of tandem stop codons have been carried out in E.coli by Kaplan[5] and by Lu and Rich[24]. They studied the effect of tRNA suppression on the C-terminus of the proteins translated in vivo. Nothing similar has been tried for eukaryotes. In our approach we inject isolated suppressor tRNAs from yeast into a living eukaryotic cell (the Xenopus laevis oocyte) and observe the changes at the C-terminus of the proteins by two-dimensional electrophoresis[18]. Although there exists no direct evidence that the changes induced by the injected suppressor tRNAs are all due to readthrough events, several strong arguments can be listed in favour of this interpre-

tation. First, we have demonstrated before that all the suppressor tRNAs used are highly active in the oocyte in the reading of the termination codons of coinjected foreign mRNAs[10]. Second, amber and ochre suppressor tRNAs share many more common changes (interpretable as wobble, Fig. 2) than the other three combinations. Third, two different ochre suppressor tRNAs ($tRNA^{Tyr}$ and $tRNA^{Ser}$) induce about the same amount of total changes. Forth, injections of $tRNA^{Phe}$ and cytoplasmic $tRNA^{Trp}$ (Fig. 1 f) induce essentially no changes, except minor ones in comparison with the suppressor tRNAs. As can be seen in the Venn-diagram (Fig. 2) all three termination codons are used in the Xenopus laevis oocyte.

A main result of the experiments is the low frequency of observable changes upon injection of suppressor tRNAs: less than 26% of the 600 mRNAs are visibly affected by injection of large amounts of individual suppressor tRNAs.

Important for the discussion of our results are the following points:

(1) What is the frequency of occurence of second in phase termination codons in oocyte mRNAs and at what distances are they found from the natural termination codons? Are very close second stops or even tandem termination codons an explanation for the scarcity of observed readthroughs? That a second stop codon is essential for the avoidance of readthrough events or products that are lethal for the phage MS2 has recently been demonstrated by Iserentant et al.[25].

(2) It has been demonstrated clearly that the sequence context around a nonsense triplet created by mutation may strongly influence the efficiency of suppression in vivo[26-29]. Similarly the context of a natural termination codon may favour chain release over readthrough or vice versa[26,30]. In vitro evidence pointing in this direction comes from the behaviour of opal suppressor $tRNA^{Ser}$ from S.pombe, which even at very high concentrations was able to read the UGA terminator of rabbit β-globin mRNA at the best to 60%[12] but suppresses in the same protein synthesis system five UGA codons on a mitochondrial mRNA with high efficiency[31].

(3) Several publications demonstrate or suggest not only that some termination codons are leaky but that the corresponding read-

through proteins are essential for the organism[6,7,32-34]. Thus there may be different classes of termination sequences. One extreme type would prevent readthrough completely (second in phase stops, unfavourable context) whereas the other extreme would allow frequent readthrough (favourable context). Several authors have proposed different meanings for the three termination codons[4,9,32], as for instance UAA being the prefered natural termination codon or UGA being frequently a leaky terminator.

(4) All the above (3) mentioned readthrough proteins have been interpreted and in several cases also proved to originate by insertion of an amino acid in response to a stop codon. But it was demonstrated that frameshift mutations are appreciably leaky in E.coli[35,36] and that certain normal tRNAs promote site specific frameshifts in vitro with MS2 as mRNA[37]. Leaky +1 and -1 frameshift mutations have recently been characterized in the mitochondrial genome of yeast[38]. Hence elongated polypeptides need not necessarily originate by termination suppression.

To reduce the uncertainties that hinder the interpretation of our results, we decided to study the termination sequences of all sequenced mRNAs and genes. In a separate publication[1] we present a compilation of all termination sequences (207) presently available and some conclusions concerning the frequency and the sequence contexts of the three termination codons, the occurence and distance of second stops and some differences between prokaryotes and eukaryotes. We are confident that some generalizations from the sample of eukaryotic sequences (mainly from vertebrates, their viruses, and yeast) also hold for the Xenopus oocyte.

The low frequency of observable readthrough events after injection of suppressor tRNAs cannot be explained by a high frequency of second stops very close to the termination codons[1]. The average distance of the termination codon to the next stop codon in phase, the begin of poly(A) or the 3' end is 16 triplets on cellular mRNAs of eukaryotes. And more than 60% of the mRNA sample contains a second termination codon at a distance longer than 9 triplets which should have given an observable readthrough product in our two-dimensional electrophoresis system. On the other hand double injections create new readthrough events not observed

with single injections (Table 1). But a fraction of them probably results from further elongation of readthrough products already observed with the single injections, because less than half of the changes detected after single injections are found again in the relevant double injections (Table 1). This may indicate that second stops are easier to read for a suppressor tRNA than the actual termination codons, which may be especially protected against readthrough.

Most of our knowledge on the efficiency of stop codons comes from studies of nonsense mutations in the protein coding part of a gene; however, it remains to be demonstrated that these results are relevant for natural stop codons. The present work suggests that a natural stop codon is particularly efficient in polypeptide chain termination and less prone to suppression than expected. From the evolutionary point of view, we may speculate that selective pressure on the nucleotides surrounding the natural stop codons has played a role in the regulation of the efficiency of polypeptide chain termination in each mRNA. Such a regulatory effect may be the result of mRNA sequence or structural changes (or both) which have a higher affinity for release factors, or an increased binding capability with potential suppressor tRNA. It is also possible that these changes could affect some other as yet undetermined mechanism(s) of polypeptide chain termination. For certain mRNAs, a tight control at the stop codon may have developed. Consequently, protein synthesis would necessarily stop at that codon; while for other mRNAs a certain degree of leakiness would be tolerated, or even be obligatory[32].

The analysis of our suppressor tRNA injection data (Fig. 2, Table 1) shows that the opal suppressor is more efficient in the creation of elongated proteins in the frog oocytes than amber and ochre suppressors. Whether this is explained by the differential susceptibility to readthrough of the three termination codons[9] or by the real distribution of the termination codons in the Xenopus laevis oocyte remains undecided. What is clear is that all three termination codons are used in a single cell, the Xenopus oocyte. However, the termination codons susceptible to readthrough may also be prone to leaky termination.

Our observation that the Xenopus laevis termination codons

are resistant to readthrougn is supported by the finding that the UAA termination codon of the major β-globin mRNA from Xenopus cannot be suppressed in the oocyte by injected ochre suppressor tRNA (M.Bienz, unpublished). Furthermore, the context of the termination codon of β-globin mRNA[39] agrees with the statistically predicted sequence[1], thus confirming the tight control of the termination codons of eukaryotic mRNAs. It appears rewarding to perform similar studies with other sequenced mRNAs in order to test whether the observed statistical tendency correlates with enhanced efficiency of translation termination.

ACKNOWLEDGEMENTS

This work was supported by grants from the Belgian government "Actions concertées" and "Fonds national de la recherche scientifique" and from the Swiss National Science Foundation to E.K. and J.K. and the Hescheler-Stifung to E.K.. We would like to thank Prof.H.Chantrenne in whose laboratory part of this work was done, Dr.R.Grantham for reading the manuscript, and EMBO for a short term fellowship to M.B. during which this work was initiated.

[2]Institute of General Microbiology, University of Berne, Altenbergrain 21, CH-3013 Bern, Switzerland

[3]Laboratoires de Chimie Biologique, Université de Bruxelles, 67, Rue des Chevaux, B-1640 Bruxelles, Belgium

REFERENCES

1 Kohli, J., and Grosjean, H. (1981) Molec.gen.Genet., in press.
2 Barrell, B.G., Anderson, S., Bankier, A.T., DeBruijn, M.H.L., Chen, E., Coulson, E.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R., and Young, I.G. (1980) Proc.Natl.Acad.Sci. USA 77,3164-3166.
3 Fox, F.T. (1979) Proc.Natl.Acad.Sci. USA 76,6534-6538.
4 Brenner, S., Stretton, A.O.W., and Kaplan, S. (1965) Nature 206,994-998.
5 Kaplan, S. (1973) Molec.gen.Genet. 120,191-200.
6 Philipson, L., Anderson, P., Olshevsky, U., Weinberg, R., and Baltimore, D. (1978) Cell 13,189-199.
7 Pelham, H.R.B. (1978) Nature 272,469-471.
8 Cremer, K.J., Bodemer, M., Summers, W.C., and Gesteland, R.F. (1979) Proc.Natl.Acad.Sci. USA 76,430-434.
9 Geller, A.I., and Rich, A. (1980) Nature 283,41-46.
10 Bienz, M., Kubli, E., Kohli, J., deHenau, S., and Grosjean, H. (1980) Nucl.Acids Res. 8,5169-5178.
11 Rafalski, A., Kohli, J., Agris, P., and Söll, D. (1979) Nucl.Acids Res. 6,2683-2695.

12 Kohli, J., Kwong, T., Altruda, F., Söll, D., and Wahl, J. (1979) J.Biol.Chem. 254,1546-1551.
13 Piper, P.W., Wasserstein, M., Engbaek, F., Kaltoft, K., Celis, J.E., Zeuthen, J., Liebman, S., and Sherman, F. (1976) Nature 262,757-761.
14 Fradin, A., Gruhl, H., and Feldmann, H. (1975)FEBS Letters 50,185-189.
15 Maxam, A.M., and Gilbert, W. (1977) Proc.Natl.Acad.Sci. USA 74,560-564.
16 Gurdon, J. (1968) J.Embryol.exp.Morph. 20,401-414.
17 Gurdon, J.B., DeRobertis, E.M., and Partington, G. (1976) Nature 260, 116-120.
18 O'Farrell, P.H. (1975) J.Biol.Chem. 250,4007-4021.
19 Bonner, W.M., and Laskey, R.A. (1974) Europ.J.Biochem. 46,83-88.
20 Clarkson, S.G., Birnstiel, M.L., and Serra, V. (1973) J.Mol.Biol. 79,391-410.
21 Grosjean, H., deHenau, S., Sibler, A.P., Martin, R., Dirheimer, G., Keith, G., and Kohli, J. (1981) Arch.internat.Physiol.Biochem. 89, 58-59.
22 Hawthorne, D.C., and Leupold, U. (1974) Curr.Top.Microbiol.Immunol. 64,1-47.
23 Harsa-King, M.L., Bender, A., and Lodish, H. (1979)in:Eucaryotic gene regulation, R.Axel et al., eds., Academic Press, New York, p. 239.
24 Lu, P., and Rich, A. (1971) J.Mol.Biol. 58,513-531.
25 Iserentant, M., VanMontagu, M., and Fiers, W. (1980) J.Mol.Biol. 139,243-263.
26 Salser, W., Fluck, M., and Epstein, R. (1969) Cold Spring Harbor Symp.Quant.Biol. 34,513-520.
27 Yahata, H., Ocada, Y, and Tsugita, A. (1970) Molec.gen.Genet. 106, 208-212.
28 Feinstein, S.I., and Altman, S. (1977) Genetics 170,155-159.
29 Bossi, L., and Roth, J.R. (1980) Nature 286,123-127.
30 Engelberg-Kulka, H. (1981) Nucl.Acids Res. 9,983-991.
31 DeRonde, A., VanLoon, A.P.G.M., Grivell, L.A., and Kohli, J. (1980) Nature 287,361-363.
32 Weiner, A.M., and Weber, K. (1973) J.Mol.Biol. 80,837-875.
33 Yates, J.L., Gette, W.R., Furth, M.E., and Nomura, M. (1977) Proc. Natl.Acad.Sci. USA 74,689-693.
34 Engelberg-Kulka, H. , Dekel, L., Israeli-Reches, M., and Belfort, M. (1979) Molec.gen.Genet. 170,155-159.
35 Atkins, J.F., Elseviers, D., and Gorini, D. (1972) Proc.Natl.Acad.Sci. USA 69,1192-1195.
36 Gallant, J., and Foley, D. (1980) in: Ribosomes: Structure, function and genetics. Chambliss et al., eds.,Baltimore Univ.Press. p. 615.
37 Atkins, J.F., Gesteland, R.F., Reid, B.R., and Anderson, C.W. (1979) Cell 18,1119-1132.
38 Fox, D.T., and Weiss-Brummer, B. (1980) Nature 288,60-63.
39 Williams, J.G., Kay, R.M., and Patient,R.K. (1980) Nucl.Acids Res. 8,4247-4258.