Hypothesis

Does translational ambiguity increase during cell differentiation?

Marguerite Picard-Bennoun

Laboratoire de Génétique de l'Université Paris XI et laboratoire associé au CNRS (86), Bâtiment 400, 91405 Orsay Cedex, France

Received 24 September 1982

Several observations made in the fungus *Podospora anserina* suggest that translational ambiguity may increase, and possibly must increase, at specific stages of the life cycle. Such changes in the properties of the translational apparatus seem to occur as well in the yeast *S. cerevisiae* and in the alga *C. reinhardii*. A slight increase of the misreading level would allow readthrough or frameshifting necessary to synthesise regulatory proteins in low amout at key points of cellular differentiation.

Translational fidelity *Cell differentiation* *Lower eukaryotes* *Ribosomal mutants*

Eukaryotic as well as prokaryotic cells exhibit a low but significant level of errors during translation. The few estimates available in vivo suggest that the error level might be in the range of $10^{-3} - 10^{-4}$ amino acids misincorporated per codon translated. Besides these missense errors, errors of frameshifting and readthrough of termination codons have been reported (review [1]).

Viruses are known to take advantage of translation errors in both prokaryotic and eukaryotic systems. Thus, the growth of certain viruses was found to be dependent on either the partial readthrough of UGA [2,3] or UAG [4,5] termination codons or on a frameshift of the ribosomes [6]. Significantly the growth of some phages is inhibited in *Escherichia coli* mutant strains whose ribosomes exhibit extreme fidelity [7,8].

There is at present no case where it has been shown that the cell uses such a process to its own advantage, although it has been suggested that the regulation of the tryptophan operon of *E. coli* might depend on UGA readthrough [9].

Here I discuss several observations made in the fungus *Podospora anserina* that suggest that translational ambiguity may increase, and possibly must increase, at key points during cellular differentiation. Such changes in the properties of the translational apparatus seem to occur also in other lower eukaryotes. It seems possible that a slight increase of the misreading level would allow readthrough or frameshifting necessary to synthesise regulatory proteins in low amount at specific steps of cellular differentiation.

Mutations which appear to increase translational ambiguity have been obtained in *Podospora* [10]. They act in vivo as omnipotent informational suppressors similar to those described in yeasts [11]. Biochemical analysis of these mutants ([12] and unpublished data) shows that they are very much like the ribosomal ambiguity mutations of *E. coli* [13]. *Podospora*'s ribosomal suppressors can be classified according to their efficiency in vivo. Strains carrying a highly efficient suppressor are unable to differentiate female organs. Several arguments show that this defect is directly related to the level of translational errors.

(i) While suppressors of low efficiency do not cause female sterility, double-mutant strains carrying two such suppressors, which are subject to an additive suppressive effect, do not develop female organs [10].

(ii) All the mutations which restore female fertili-

Published by Elsevier Biomedical Press
001457593/82/0000—0000/$2.75

ty, in these strains decrease suppressor efficiency [14].

(iii) When paromomycin, which is known to increase translational errors in vivo and in vitro in *Podospora* [12,15,16], is added to the culture medium at low concentrations, production of female organs is reduced. Those female organs which do develop in the presence of the antibiotic are unable to promote the whole process of sporulation. Cycloheximide, used as a control, produced no effect on female organ differentiation even at doses that slow the growth rate by a factor of two [15].

Antisuppressor mutations similar to those in yeasts [17–19] have been characterized in *Podospora* [14,20]. They decrease the efficiency of (tRNA) nonsense and ribosomal suppressors, possibly by slowing down the translational error rate. Biochemical analysis has shown [12,16] that they are similar to the restrictive ribosomal mutations of *E. coli* (review [13]). Some of these antisuppressor mutations strongly disturb either female organ differentiation or sporulation. The direct relationship between these defects and the level of misreading in these strains is not yet so clearly ascertained as for the suppressor bearing strains. However, paromomycin completely relieves the sporulation defect in strains carrying the restrictive *AS7* mutations (Coppin-Raynal, personal communication). Furthermore, preliminary analysis of mutations able to restore sporulation in these strains suggest that some of these mutations may enhance the ambiguity level (Dequard-Chablat, personal communication).

These two sets of observations show that the cellular differentiation involved in sexual reproduction of *Podospora* (development of female organs and sporulation) occurs only within a certain range of misreading frequencies. These processes are impaired both when the error level is too high and when it is too low, even though under these extreme conditions vegetative growth continues.

Other observations suggest that translational fidelity might be decreased during cell differentiation not only in *Podospora* but also in the yeast *Saccharomyces cerevisiae* and in the alga *Chlamydomonas reinhardii*.

In the yeast, it was observed [21] that some nonsense suppressor mutations disturb sporulation when the cells are homozygous for the suppressors. Physiological analysis led the authors to assume that these defects are caused by increased efficiency of suppression. In *Podospora*, we were able to show that this (putative) increase of nonsense suppression efficiency during sporulation is controlled at the ribosomal level. The *su4* mutations appear to produce a (tRNA) nonsense suppressor [14]. Sporulation is blocked at a very precise stage in strains homozygous for these mutations but is restored when translational ambiguity is decreased by an antisuppressor mutation [14]. Strains heterozygous for the *su4* mutations are able to sporulate except if translational ambiguity is increased by a ribosomal suppressor (unpublished). In *Chlamydomonas*, P. Bennoun (personal communication) observed that several nuclear mutants defective in photosynthesis are leaky when the cells have differentiated into gametes but not during vegetative growth. A functional leakiness induced by gametogenesis per se appears unlikely. Thus, the observations made in *Podospora, S. cerevisiae* and *Chlamydomonas* may be readily interpreted, as a whole, if one assumes that translational fidelity is decreased during gametogenesis and/or sporulation.

It may be relevant to recall that the cellular processes needed for sexual reproduction in these three lower eukaryotes are induced by starvation, in particular by nitrogen starvation. It is well established that bacteria have developed a mechanism, known as the stringent response, as defence against miscoding generated by amino acid starvation (review [22]). We do not know, at this time, whether eukaryotic cells have analogous mechanisms. Nevertheless, gametogenesis in *Chlamydomonas* [23] and sporulation in *S. cerevisiae* [24] are both characterized by extensive turn-over of vegetative ribosomes and synthesis of new ribosomes. There is no evidence that these newly synthesised ribosomes differ from those made in vegetative cells. However, in two other lower eukaryotes, *Dictyostelium* [25] and *Tetrahymena* [26], cell differentiation is accompanied by changes in ribosome structure and function.

The translational leakiness associated with gametogenesis in *Chlamydomonas* reveals new decoding properties of the translational apparatus when compared to vegetative cells. This may be

achieved by a passive increase of translational errors caused by starvation through depletion of some aminoacyl-RNA, by synthesis of new species of tRNAs (or specific modifications of pre-existing ones) leading to new decoding properties, and/or by changes at the ribosomal level causing an enhanced misreading.

Defects in sporulation caused by some tRNA nonsense suppressors in *S. cerevisiae* and in *Podospora* can be explained if these tRNAs carry out some termination codon readthrough which is not deleterious during growth but which is lethal to the process of sporulation. In the same way, nonsense readthrough or ribosomal frameshifting induced by ribosomal suppressors might inhibit cell differentiation without affecting cellular growth.

The physiological defects associated with the high fidelity mutations (antisuppressors) suggest that termination codon readthrough or frameshift of some messages is necessary for differentiation, at least in *Podospora*. Termination codon readthrough occurring in normal cells has been demonstrated only once but it is strongly suggested in another case. Thus, Geller and Rich [27] reported a UGA suppressor activity which produces a β-haemoglobin readthrough protein in rabbit reticulocytes. It is noteworthy that the only regulatory genes whose sequences are known in eukaryotes are those with control mating-type and sporulation in *S. cerevisiae*. One of them contains a UGA codon in its reading frame [28]. In vitro mutagenesis and transformation experiments have demonstrated that readthrough of this codon is necessary for sporulation to proceed [29].

Control of gene expression at the level of termination might be used at key points in cellular development by the synthesis of specific tRNAs [27]. However, it is not known whether the $tRNA^{Trp}$ isolated from rabbit reticulocytes and the UGA suppressor serine tRNA obtained from bovine liver [30] are synthesised only in specific cellular types. on the other hand, Bienz and Kubli [31] suggested that this kind of regulation might be performed by a tRNA modification enzyme.

In summary, there is evidence that readthrough or frameshifting is needed in lower eukaryotes for the synthesis of regulatory proteins at specific stages of the life cycle. We suggest that this phenomenon is controlled through modulation of translational fidelity at the ribosomal level.

ACKNOWLEDGEMENTS

I am much indebted to all the people of my laboratory for contribution to this work and stimulating discussions. I thank Dr P. Bennoun and Dr. C.G. Kurland for encouragement and critical reading of the manuscript. This work was supported by grants of the CNRS (ATP Microbiologie), the DGRST (Action Concertée: Relations entre macromolécules biologiques), NATO (grant 1637) and the Fondation pour la Recherche Médicale.

REFERENCES

[1] Buckingham, R.H. and Grosjean, H. (1982) in: Accuracy in molecular Biology (Galas, D.J. ed.) Dekker, New York, in press.
[2] Weiner, A.M. and Weber, K. (1971) Nature New Biol. 234, 206–209.
[3] Yates, J.L., Gette, W.R., Furth, M.E. and Nomura, M. (1977) Proc. Natl. Acad. Sci. USA 74, 689–693.
[4] Pelham, H.R.B. (1978) Nature 272, 469–471.
[5] Philipson, L., Andersson, P., Olshevsky, U., Weinberg, R., Baltimore, D. and Gesteland, R. (1978) Cell 13, 189–199.
[6] Kastelein, R.A., Remaut, E., Fiers, W. and Van Duin, J. (1982) Nature 295, 35–41.
[7] Engelberg-Kulka, H., Dekel, L., Israeli-Reches, M. and Belfort, M. (1979) Mol. Gen. Genet. 170, 155–159.
[8] De Mars Cody, J. and Conway, T.W. (1981) J. Virol. 37, 813–820.
[9] Engelberg-Kulka, H., Dekel, L. and Israeli-Reches, M. (1981) Biochem. Biophys. Res. Commun. 98, 1008–1015.
[10] Picard, M. (1973) Gen. Res. (Cambridge) 21, 1–15.
[11] Hawthorne, D.C. and Leupold, U. (1974) Curr. Top. Microbiol. Immunol. 64, 1–47.
[12] Coppin-Raynal, E. (1982) Curr. Genet. 5, 57–63.
[13] Piepersberg, W., Geyl, D., Hummel, H. and Böck, A. (1980) in: Genetics and evolution of transcriptional and translational apparatus, pp. 359–377, Kodansha Scientific, Tokyo.
[14] Picard-Bennoun, M. (1976) Mol. Gen. Genet. 147, 299–306.
[15] Coppin-Raynal, E. (1981) Biochem. Genet. 19, 729–740.
[16] Picard-Bennoun, M. (1981) Mol. Gen. Genet. 183, 175–180.

[17] Thuriaux, P., Minet, M., Hofer, F. and Leupold, U. (1975) Mol. Gen. Genet. 142, 251–261.
[18] Liebman, S.W. and Cavenagh, M. (1980) Genetics 95, 49–61.
[19] Ishiguro, J. (1981) Curr. Genet. 4, 197–204.
[20] Coppin-Raynal, E. and Le Coze, D. (1982) Genet. Res. (Cambridge) in press.
[21] Rothstein, R.J., Espositio, R.E. and Espositio, M.S. (1977) Genetics 85, 35–54.
[22] Gallant, J.A. (1979) Annu. Rev. Genet. 13, 393–415.
[23] Martin, N.C., Chiang, K-S. and Goodenough, U.W. (1976) Dev. Biol. 51, 190–201.
[24] Pearson, N.J. and Haber, J.E. (1977) Mol. Gen. Genet. 158, 81–91.
[25] Ramagopal, S. and Ennis, H.L. (1981) Proc. Natl. Acad. Sci. USA 78, 3083–3087.
[26] Hallberg, R.L., Wilson, P.G. and Sutton, C. (1981) Cell 26, 47–56.
[27] Geller, A.I. and Rich, A. (1980) Nature 283, 41–46.
[28] Astell, C.R., Alstrom-Jonasson, L., Smith, M., Tatchell, K., Nasmyth, K.A. and Hall, B.D. (1981) Cell 27, 15–23.
[29] Tatchell, K., Nasmyth, K., Hall, B.D., Astell, C.R. and Smith, M. (1981) Cell 27, 25–35.
[30] Diamond, A., Dudock, B. and Hatfield, D. (1981) Cell 25, 497–506.
[31] Bienz, M. and Kubli, E. (1981) Nature 294, 188–190.