A comparison of transcriptional linkage of tRNA cistrons in yeast and E. coli by the ultraviolet light technique

Horst Feldmann

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestrasse 33, 8000 München 2, GFR

Received 23 May 1977

ABSTRACT

The ultraviolet light mapping technique was employed to determine the lengths of tRNA cistrons in yeast. The applicability of the method was first tested in the E. coli system, in which the mapping positions for some tRNA cistrons and the ribosomal 5S RNA genes as well as the existence of multimeric transcription units for tRNAs are known. Rates of the synthesis of the tRNAs and small rRNAs after irradiation with various doses of UV light were determined by pulse labeling and quantitation of the RNA species after twodimensional gel electrophoreses. The small ribosomal RNAs served for internal calibration in the estimation of the target sizes. Our results suggest that - in contrast to the prokaryotic system - in yeast the majority of the tRNA genes are not linked into transcriptional units.

INTRODUCTION

There is now good evidence from direct and indirect approaches that in E. coli the genomic organization of the various tRNA genes differs. For some specific tRNAs the genes may be arranged in single transcription units. The majority of the tRNA genes, however, are found to be arranged as multimeric transcriptional units. The resulting precursor molecules, which may contain a set of similar or of different tRNA molecules in tandem array, are then subjected to a complex maturation process [reviewed e.g. in ref. 1]. A similar situation applies to the specific tRNAs encoded by the E. coli phages T2 and T4 [2]. A third type of tRNA genes in E. coli was found to be interspersed into the ribosomal RNA transcription units [3], and the processing of the transcription products may differ from that of the other precursor tRNAs.

In eukaryotes, the tRNA genes show a higher redundancy
than in prokaryotes [e.g. 4,8], which in special cases like in Xenopus laevis oocytes can amount to several hundred copies [5]. It has also been demonstrated in the latter case that tRNA genes together with nontranscribed spacers form clusters of serially repeated sequences [4,9]. A similar situation may apply to some of the tRNA genes in yeast[8].

In this context, the question arises as to whether genes in eukaryotes may also be transcribed from oligocistronic genomic entities like in prokaryotic systems. For the tRNA genes from Xenopus laevis mentioned above, this possibility seems to be rather unlikely [9]. As yet, no direct proof exists in other eukaryotes. The fact that multimeric precursor tRNA molecules have not been observed in Bombyx mori [10], human KB cells [11], or in yeast [12], may be taken as an indication that larger transcription units containing several tRNA genes are absent. It cannot be ruled out, however, that the original transcription products are longer and have been partially degraded during isolation.

One approach to follow this question more directly, is the use of the ultraviolet light technique for mapping transcriptional linkage. Measurements which render information on the arrangement of cistrons controlled by a common promoter, have been successfully carried out for instance for T-phage gene products [13,14], for ribosomal proteins in E. coli [15], and for the ribosomal RNAs in E. coli [16] and in higher organisms [17,18].

We have applied this technique for an estimation of the lengths of tRNA transcriptional units in yeast, in comparison with those in E. coli. The implications of the method for this special problem will be discussed below.

**MATERIALS AND METHODS**

\[^{3}H\]uracil (51 Ci/mMol) and \[^{14}C\] uracil (61 Ci/Mol) were purchased from the Radiochemical Centre, Amersham, England.

E. coli strain B8-1 was kindly given by Dr. M. Schweiger. Cells were grown in medium M9 (1% glucose) at 30°C [14]. UV irradiation experiments were performed at a cell density equivalent to 0.4 A\(_{620}\) nm - units.
Yeast strain P 1 (4094 - Bg, ade 2, ura 1) was kindly
given by Dr. A. Fradin. Cells were pregrown in yeast nitrogen
base (DIFCO) medium, brought to pH 5.8, with the addition of
2% glucose and 20 mg/ml each of adenine and uracil at 28°C.
Before labeling with radioactive uracil, the cells were cen-
trifuged and resuspended in fresh medium (without uracil) to
give the original cell density. UV irradiation experiments
were performed at a cell density equivalent to 1.2 A620 units.

All UV irradiation experiments were carried out with
10-15 ml aliquot samples in Petri dishes, exposed with gentle horizontal shaking to a low pressure mercury lamp (Hanau,
Quarzlampen, type 5241, 22W, NN15/44VK) at 40 cm distance
for various times. These and the following steps were carried
out in dim yellow light. Two labeling procedures were employed:
(i) cells were pre-labeled with [3H] uracil (2-5 μCi/ml)
(20 min for E. coli, 80 min for yeast). The culture was then
centrifuged, washed once with fresh medium and resuspended
in fresh medium to the appropriate cell density. Aliquots
were removed for UV irradiation, two aliquot samples remained
unirradiated. After irradiation (and in the case of yeast
after an additional 10 min) all of the samples were incuba-
ted with [14C] uracil to a concentration of 0.1-0.25 μCi/ml
for approximately half a generation time. After this, 0.25
mg/ml of unlabeled uracil was added, the samples were chilled
on ice and centrifuged. (ii) A culture was divided into two
portions. One part was used to prepare the aliquot samples
for UV irradiation and the labeling with [14C] uracil. The
other part of this culture was concomitantly labeled with
[3H] uracil. Aliquots of this culture were then mixed with
non-irradiated or the irradiated [14C] uracil labelled samp-
les, respectively. The combined samples were then treated
further as outlined above.

Low molecular weight RNA was prepared from the samples
by the phenol method and aliquots submitted to two dimen-
sonal gel electrophoresis [19]. For the first dimension, the
10% gels had slots of 10 x 0.3 mm. After the run, two or
three strips (0.8 cm wide) were polymerized onto the same

2833
20% gel for the second dimension. After staining the gels, spots in identical positions were cut out and their $^3$H- and $^{14}$C-radioactivities determined after combustion in a sample oxidizer (OXIMAT) by scintillation counting.

RESULTS AND DISCUSSION

The ultraviolet light technique is based on the observation that certain lesions induced in the DNA by UV light cause premature termination of transcriptions at those sites and that no reinitiation occurs beyond the lesion up to the next promotor. Rates of the synthesis of gene products after irradiation with various doses of UV light could be determined by pulses with labeled uridine and quantitation of the RNA species after polyacrylamide gel electrophoresis. Errors originating with the isolation procedure were minimized by internal standardization through prelabelling the mature RNA species with a different label [e.g. 18]. Thus, a prerequisite for such determinations is, that the gene products can be separated and analysed with sufficient accuracy.

Once the mapping positions (distances from the promotor) of the cistrons for specific RNA species are known, and the reduction rates of their synthesis after UV irradiation can be measured, these data can serve for an internal calibration in order to determine the lengths of the transcriptional units for other RNA species in the same organism. This approach was chosen to measure the lengths of transcriptional units for a number of tRNAs in yeast, taking the small ribosomal RNAs as references.

The separation of the tRNA species was achieved by the twodimensional gel electrophoretic system we recently described [19]. The reliability of the method was first tested in the E. coli system, where the target sizes for those tRNA cistrons interspersed into the ribosomal RNA cistrons and for the 5S RNA could be measured and be compared with mapping data [3].

1. Target sizes for tRNA genes in E. coli.

The experiments were carried out with E. coli strain B$_{s-1}$ which is incapable of dark repair of ultraviolet light
damage. Cells were grown to an appropriate density (see Materials and Methods). Aliquot samples were removed from the culture, irradiated with various UV doses and incubated for approximately half a generation time with the addition of [\(^{14}\text{C}\)]uracil, non-irradiated samples were treated the same way. Two different methods of labeling the cells with [\(^{3}\text{H}\)]uracil were employed (see Materials and Methods) to obtain the necessary internal standards. The results of either set of experiments were the same. It may be mentioned that measurements for which the cells had been labeled with [\(^{32}\text{P}\)]phosphate alone were unsuccessful: no sufficient accuracy in the determination of total RNA extracted from the samples was reached. Furthermore, only the combustion of the gel samples after labeling with [\(^{3}\text{H}\)]-and [\(^{14}\text{C}\)]uracil, respectively, gave reliable results. Direct scintillation counting in commercial solvents proved to be unsatisfactory due to quenching effects.

After the soluble RNA had been extracted from each sample, it was submitted to electrophoresis on a 10% polyacrylamide gel, followed by electrophoresis on 20% gels in the second dimension. We have reported earlier that this system is capable of separating individual tRNA species and the small ribosomal RNAs with good reproducibility. This was shown by fingerprinting analyses of the tRNAs from a number of spots [19]. The technique has also been applied successfully in other laboratories. Care was taken that the gels in the second dimension were operated under identical conditions. In this way, nearly superimposable patterns were obtained, which then allowed to precisely cut out corresponding spots from each gel. The amino acid acceptance of the tRNAs in the E. coli system was determined only for a few of the spots according to the method described in ref. 19. We found tRNA\(^{\text{Glu}}\) and tRNA\(^{\text{Ile}}\) in similar positions on our gels (Fig. 1) as reported earlier by Lund et al. [3] in their two-dimensional system.

From the \(^{3}\text{H}\)- and \(^{14}\text{C}\)- radioactivity values in each spot, the reduction of the synthesis of a number of tRNA species as a function of ultraviolet light dose was determined (Fig. 1).
FIGURE 1
Reduction of synthesis of tRNA species and the 5S rRNA in E. coli as a function of ultraviolet light dose. The curves were calculated from several experiments of the type described in text. For convenience, only the experimental values for the 5S RNA and the tRNAs from spot 18 and 16, respectively, are shown. The other inactivation curves were also found to be linear. The numbers refer to the RNA species shown in the inset.
Inset: schematic representation of a typical pattern after twodimensional gel electrophoresis of the E. coli tRNAs which were used in the UV inactivation experiments (spot 18 is tRNA Ile, spot 19 is tRNA Glu, spot 1 is 5S RNA, spot 2 is an unidentified RNA species).

The relative amount of the RNA species can be calculated accurately, using the \(^{3}H\text{labelled RNAs as internal standards, from the expression:}^\)
\[
\% \text{ of control synthesis} = 100 \times \frac{\text{cpm} \left[ ^{14}\text{C} \right] \text{RNA}}{\text{cpm} \left[ ^{3}\text{H} \right] \text{RNA}} \text{ irr} \times \frac{\text{cpm} \left[ ^{3}\text{H} \right] \text{RNA}}{\text{cpm} \left[ ^{14}\text{C} \right] \text{RNA}} \text{ non-irr}
\]

whereby "irr" refers to one RNA species obtained from a sample irradiated with a certain dose, and "non-irr" to the RNA obtained from a non-irradiated sample. The accuracy of the data presented Fig. 1 can be estimated to be in the range of 5%. This figure was obtained by comparing several independent experiments. Furthermore, the expression

\[
\frac{\text{cpm} \left[ ^{3}\text{H} \right] \text{RNA}}{\text{cpm} \left[ ^{14}\text{C} \right] \text{RNA}} \text{ non-irr}
\]

for any RNA species had a standard deviation of ca. 5% in these experiments.

E. coli 5S ribosomal RNA has been mapped by different methods [e.g. 3,16] to be located at the 3'-distal end of the ribosomal RNA precursor which contains the 16S and 23S RNA sequences and has a molecular weight approximately \(2 \times 10^6\) (corresponding to a length of ca. 6000 nucleotides). Taking the reduction of the synthesis of this 5S RNA after UV irradiation as an internal calibration for the reduction of the synthesis of a number tRNA species, we arrive at the following conclusions. The synthesis of a group of tRNA species is reduced with a fast rate, the mapping position therefore may be calculated to be approximately 2000 nucleotides distant from the promoter. Among these are a tRNA\(^{\text{Glu}}\) and a tRNA\(^{\text{Ile}}\) [3]. This is in good agreement with the finding that the cistrons for these tRNAs are located in the spacer region between the 16S and the 23S RNA cistrons [3]. In addition, we found a number of tRNA species, the synthesis of which is reduced with an "intermediate" rate, and others which are inactivated rather slowly. From the slopes of the inactivation curves it can be calculated that the target lengths are variable in the range of 230-1500 nucleotide pairs. Our observation thus reflects the finding that most of the tRNAs in E. coli are derived from tRNA precursors of varying length.
which may contain from two to eight tRNAs [e.g. 20]. The method is not sensitive enough to allow more detailed analyses. For example, if several genes for the same tRNA were arranged in tandem and placed under the control of a common promotor, the inactivation curve would become concave [e.g. 18]. However, from theoretical considerations, for the given (relatively short) target sizes the initial slope of this inactivation curve - at low UV doses as used in our experiments - would be indistinguishable from that obtained for tandemly clustered cistrons of the same tRNA species, each controlled by its own promotor.

2. Target sizes for tRNA genes in yeast.

The ultraviolet light irradiation experiments with yeast cells were carried out similar to those with E. coli, except that higher cell densities and higher UV doses had to be employed. This was found to be necessary in order to obtain measurable reduction rates for the synthesis of the majority of the tRNA species. For the analyses those spots from the two-dimensional gels were selected which were known to represent individual yeast tRNA species [19]. Until recently, no reliable mapping data for the small ribosomal RNAs (5S and 5.8S RNA) which were most suitable for an internal calibration, were available. The assumption [17] that the cistrons for 5S RNA in yeast were part of the 17S and 26S rRNA transcription unit at the remote end of the promotor, were contradicted by the finding [22] that the 5S RNA is encoded by the heavy DNA strand, whereas the large rRNAs are encoded by the light strand. This observation was corroborated by mapping of the ribosomal RNA cistrons [23]. According to this, the 5.8S RNA cistrons in yeast are part of the transcriptional unit inserted in between the cistrons for the 17S and the 26S RNA species, meaning that the 5.8S cistrons are located between 1600 and 2000 nucleotide pairs distal from the promotor.

We found (Fig. 2) that the synthesis of 5.8S RNA was reduced considerably fast, but that the synthesis of nearly all of the tRNAs was reduced to a small extent, the rates being similar for most of the tRNAs; only one exception to
FIGURE 2
Reduction of synthesis of tRNA species in yeast as a function of ultraviolet light dose.
The curves were calculated from several experiments as described in text. For convenience, only the experimental data for the 5.8S RNA and three tRNA species are shown. The numbers refer to the spots shown in the inset. tRNA species 2 revealed the highest, tRNA species 14 the lowest inactivation rate among the tRNAs investigated (except 7). The curves for these other tRNAs fall in between the two borderlines (indicated by the hatching). The data for the 5.8S rRNA was obtained by cutting out the corresponding band from the 10% gels of each single experiment.
Inset: schematic representation of a typical two-dimensional gel electrophoretic pattern of the yeast tRNAs which were used in the UV inactivation experiments. Spot 2 is a tRNA^Leu^, spot 14 is a tRNA^Arg^.

this rule was observed (RNA from spot no. 7 in Fig. 2). As in the case of E. coli, the inactivation curves appear to be linear. Comparing the rates it must be assumed that the tar-
get lengths for most of the tRNAs are in the range of 200 nucleotide pairs. Although similar considerations may apply in this case as outlined above for the E. coli system, our findings together with two other observations suggest that there is only space for a single tRNA cistron in such a transcription unit: (1) the yeast tRNA precursors have a size between 100 and 145 nucleotides in length [12] and (2) no larger precursors (e.g. tandem precursors) have been observed [12, 19, 24].

In conclusion, this study has shown that the genomic organization of the cistrons for most of the tRNAs in yeast should be entirely different from that in a prokaryote system. Whereas in E. coli the majority of the tRNA cistrons are found to be linked into larger transcriptional units, this seems to be a rare event for the tRNA cistrons in yeast.

ACKNOWLEDGEMENTS

The skilled technical assistance of Mr. N. Deering is grateful acknowledged. Dr. M. Schweiger has kindly supplied E. coli strain B5-1; I am indebted him for many helpful discussions and initial help in the experiments. The Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie have supported this work.

REFERENCES

1 Altman, S. (1975), Cell 4, 21-29
9 Clarkson, S.G. and Kurer, V. (1976), Cell 8, 183-195
10 Chen, G.S. and Siddiqui, M.A.Q. (1975), J. Mol. Biol. 96, 153-170
13 e.g.: Sauerbier, W., Millette, R., and Hackett, R.B. (1970), Biochim. Biophys. Acta 209, 368-387
22 Øyen T. and Aarstadt K. (1975), FEBS lett. 51, 227-236
23 Philippsen, P., personal communication
24 Hall, B.D., personal communication