MAPPING OF YEAST tRNAs BY TWO-DIMENSIONAL ELECTROPHORESIS ON POLYACRYLAMIDE GELS

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1. Introduction

Various systems for two-dimensional electrophoresis on polyacrylamide gels have been successfully applied to the separation of ribosomal proteins, e.g. [1]. RNAs, e.g. [2,3] and RNA-fragments, e.g. [4–6].

In connection with our investigations of precursors to tRNA in yeast [7] we have looked for a two-dimensional gel electrophoresis system with high resolution capacity. The procedures described in the following, had initially been designed for the purpose, 1) to achieve a better resolution of precursor tRNA species and, 2) to find a simple mapping procedure that could be used to relate individual precursors with the mature tRNAs. Some further analytical and preparative applications of this system are outlined briefly.

2. Results and discussion

We have shown earlier [7] that low mol. wt RNA from yeast pulse-labeled with [^{32}P] phosphate can be resolved into a number of bands for tRNA and precursors to tRNA by electrophoresis on 10% polyacrylamide gels. The resolution of bands could be refined to some extent by employing a relatively long special spacer gel (see legend to fig. 1). Gels of this type (fig. 1a and 2) were capable of resolving the tRNA population into about 16 bands, and the precursors to tRNA into about 20 bands. Unlabeled, unfractionated tRNA from yeast was separated in the same manner as [^{32}P] tRNA, as seen after staining the gel. Under the same conditions, tRNAs from *E. coli* were separated to a lesser degree. Low mol. wt RNA from *E. coli* MRE 600 labeled for 1 min with [^{32}P] phosphate, in addition to the bands for tRNA, 4.5 S RNA, and 5 S RNA gave about 20 bands, presumably precursors to tRNA (not shown here).

In order to obtain a further separation, we tested several two-dimensional gel electrophoretic systems by combining a gel run in acidic conditions [14,15] with a gel at pH 8.3 for the second dimension. In any case, we obtained characteristic patterns for tRNAs, which are not documented here.

The best results, however, were obtained by combining the 10% gel with a 20% gel in the same buffer conditions at pH 8.3. Fig. 1b shows the two-dimensional separation of non-labeled tRNA from yeast, which could be resolved into approx. 40 different spots, most of them representing pure tRNA-species. The assessment shown in fig. 1b was accomplished in several ways: i) co-electrophoresis of purified [^{32}P] tRNAs with non-labeled bulk tRNA ($tRNA^{Met}_1$, $tRNA^{Met}_3$). ii) electrophoresis of non-labeled purified tRNA species and bulk tRNA on the same gel in the first and in the second dimension ($tRNA^{Phe}$, $tRNA^{Leu}$, $tRNA^{Val}$, $tRNA^{Ser}$, $tRNA^{Ala}$, $tRNA^{Tyr}$), iii) fingerprinting of spots from a two-dimensional gel electrophoresis with [^{32}P] tRNA ($tRNA^{Ser}_{1,2}$, $tRNA^{Ala}_1$), iv) electrophoresis of bulk tRNA, charged with one amino acid of high specific activity (^{3}H or ^{14}C) in two dimensions ($tRNA^{Ser}$, $tRNA^{Gly}$, $tRNA^{Ala}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Trp}$).

The aminoacyl tRNAs were stabilized prior to electrophoresis by transforming the NH_2-group of the bound amino acid into an OH-group through treatment with HNO_2 [12]. The half-lives of the resulting

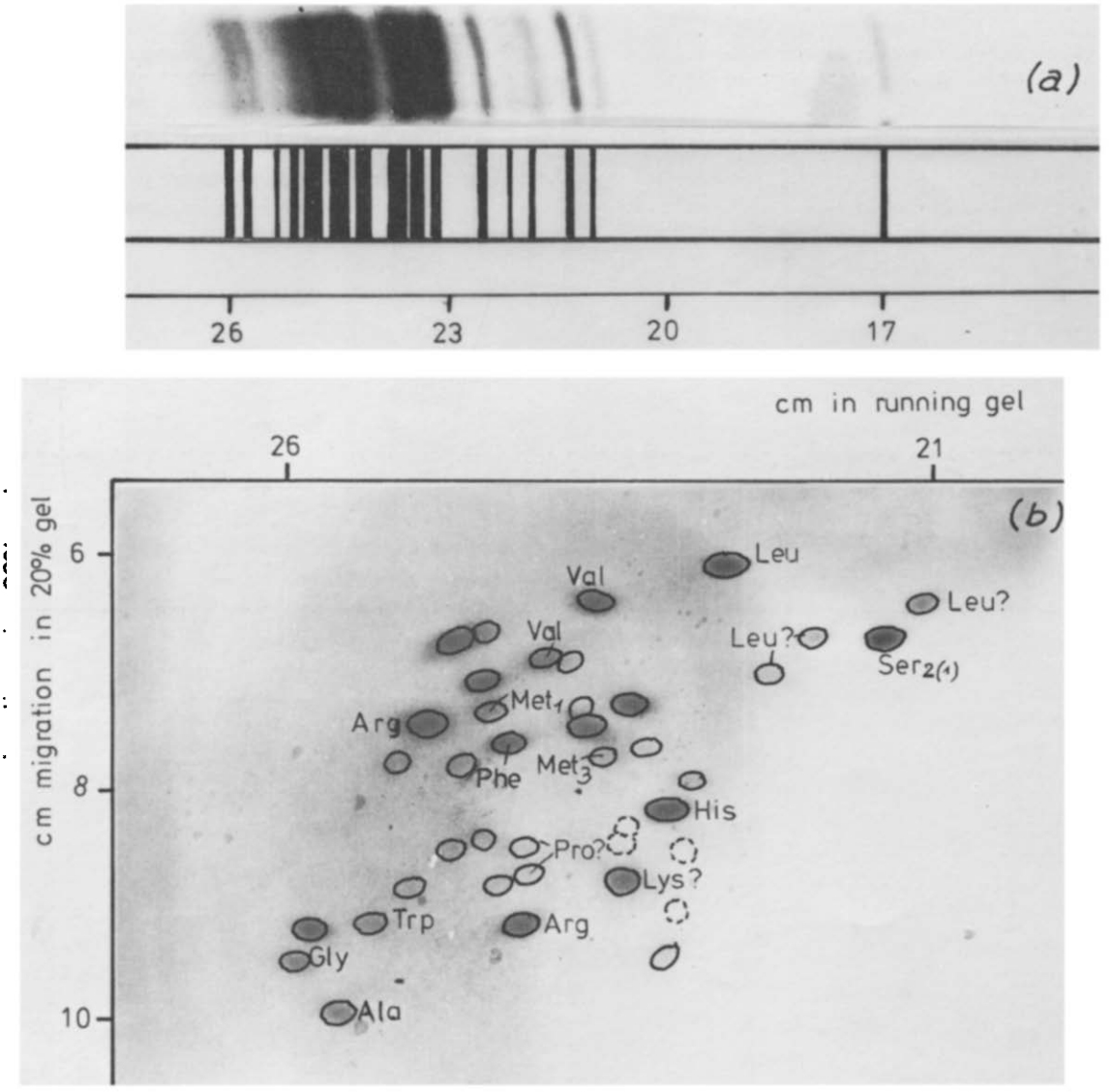


Fig. 1. Electrophoresis of yeast tRNA on polyacrylamide gels. a) First dimension: similar 10% gels were employed as described in [7], with the following modifications: 4 M urea in Tris–borate buffer, pH 8.3, length of gel 40 cm, spacer gel 5–6 cm high. Slot formers of appropriate dimensions (2–6 mm × 1–10 cm) were used. RNA samples were applied in a mixture of 60% sucrose, 4 M urea, 0.1 M sodium acetate buffer pH 4.5, 1% xylene cyanol FF. The blue marker is running slightly ahead of the fastest tRNA bands. b) Second dimension: 20% gels were prepared similarly as in [2], with the following modifications: 20% acrylamide, 0.8% *N,N'*-methylene-bis-acrylamide and 4 M urea. The same Tris–borate buffer was used as for the 10% gel. Slab gels were polymerized in a glas plate chamber (30–40 cm high) at 4°C, thus preventing the formation of gas bubbles between gel and cell surfaces. Gel strips from a run in the first dimension (0.5 to 2 cm wide) were carefully polymerized on top of a 20% gel by embedding it into a 10% gel mixture. Electrophoresis was carried out at 4°C and 15 V cm^{-1} for 48 to 72 hr. Monitoring and quantification of RNA bands of spots from the gels were done by the following techniques: (1) Labeled or unlabeled RNA: staining of gels with 1-ethyl-2[3-(1-ethylnaphtho[1,2d]-thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2d]-thiazolium bromide [8]. 10% gels were measured densitometrically as described earlier [7,8]: spots from 20% gels were cut out with a small punching blade (2 × 3 mm) and the optical density measured in a modified Zeiss PL4 photometer at 578 nm, using the punching blade as a frame. (2) [^{32}P] RNA: autoradiography, densitometry of the film [7], counting of gel slices in 1 ml H_2O after Cerenkov [9,10]. (3) RNA labeled with

Legend fig. 1 continued:

^{3}H and/or ^{14}C: measuring of radioactivity by liquid scintillation counting after combustion of gel slices in an Oximat sample oxidizer. Aminoacyl-tRNAs were prepared as described in [11] using a crude synthetase preparation from yeast, kindly given by Dr Hirsch of this laboratory. 10 A_{260}-units of yeast tRNA (Boehringer) each were charged with the following amino acids (Radiochemical Centre, Amersham, specific radioactivity in brackets) [^{14}C] arginine (158), [^{14}C] serine (171), [^{3}H] alanine (500), [^{14}C] histidine (327), [^{14}C] glycine (190), [^{3}H] tryphophane (1000). The samples were treated with HNO_2 in 1 ml solution following the procedure of Hervé and Chapeville [12]. Fingerprints of [^{32}P]tRNAs (after digestion with T1 or pancreatic RNAase) and subsequent analyses of the oligonucleotides were done according to [13]. The assessment of tRNA species (as indicated by the amino acids) is described in the text.

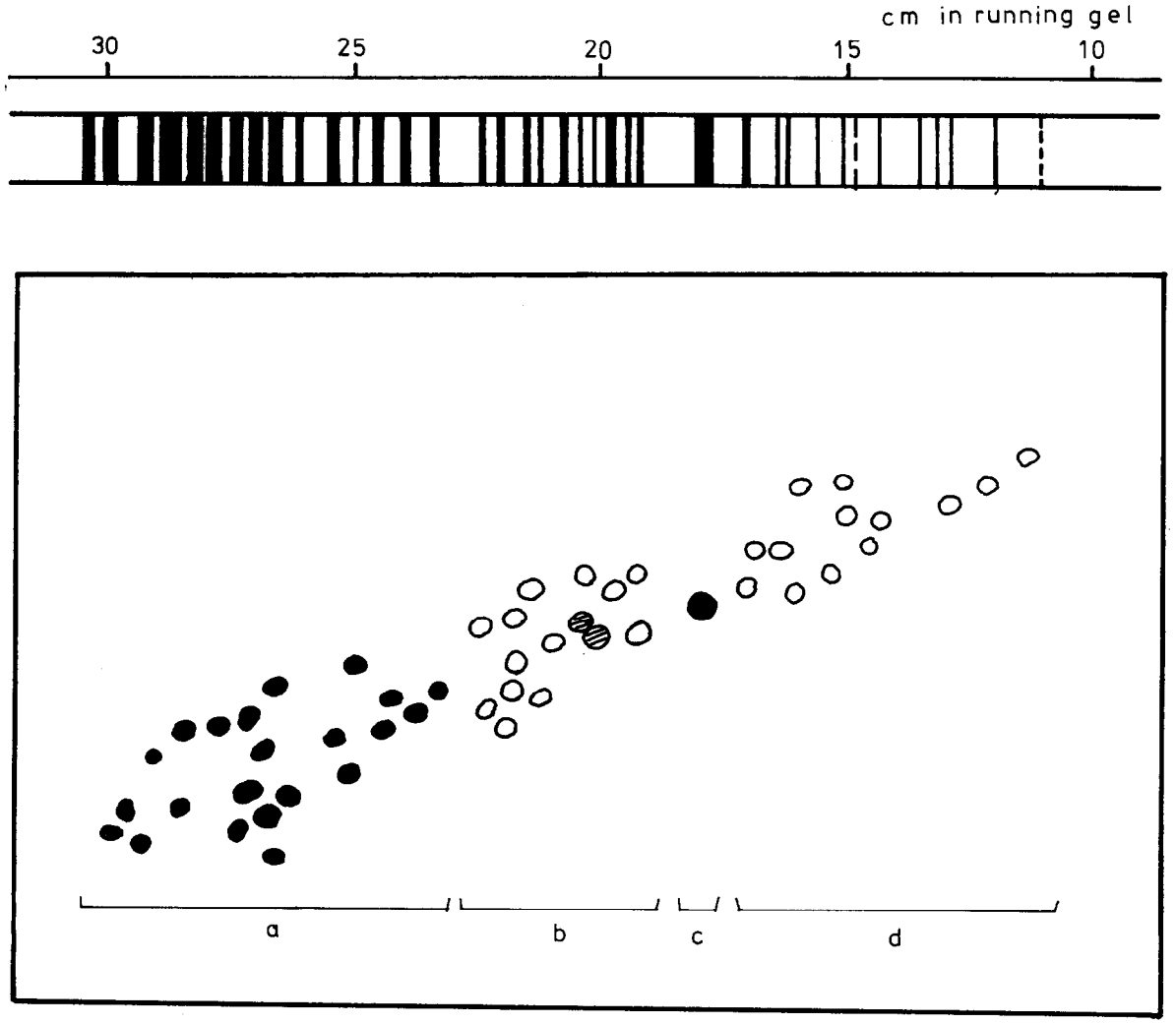


Fig. 2. Electrophoresis of [^{32}P] pulse labeled low mol. wt RNA from yeast in two dimensions. Growing conditions and labeling of yeast strain *S. cerevisiae* C 836 with [^{32}P] phosphate and the isolation of RNA are described in [7]. Conditions for electrophoresis see legend to fig. 1. a = most intensive spots from tRNA, b = smaller tRNA precursors, c = 5S RNA, d = larger tRNA precursors. The intensities of spots in group b was approx. 20% compared with those from tRNAs, that of group d approx. 1%. In one labeling experiment conditions were such as to produce the hatched spots with an intensity comparable to that of the tRNA spots.

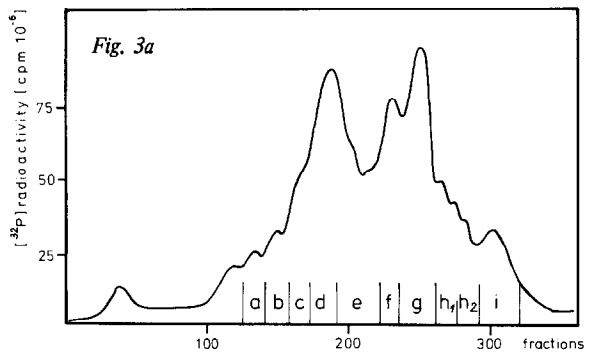
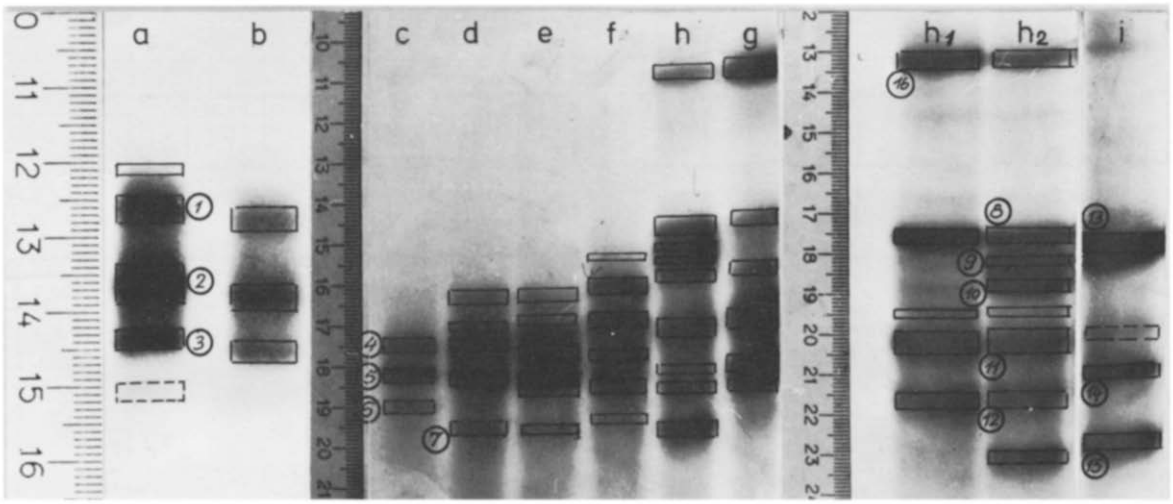


Fig. 3. Purification of yeast [^{32}P] tRNAs. a) Chromatography on benzoylated DEAE cellulose in 7 M urea, pH 3.0, The procedure described in [17] was followed. b) Electrophoresis of tRNA from the fractions indicated in fig. 3a on 10% gels. The numbers refer to bands that represent single species of tRNA, as seen by fingerprinting and electrophoresis in the second dimension. 3 = $tRNA^{Trp}$(?), 8, 9, 10 = $tRNA^{Leu}$ species, 13 = $tRNA^{Ser}_{2(1)}$; 14 = $tRNA^{Pro}$; 15 = $tRNA^{Ala}_{1}$, 16 = 5 S RNA.

Fig. 3b



tRNA hydroxy acid esters were reported to be greater by a factor of about 100 in comparison with the corresponding aminoacyl tRNAs. Even under the conditions of the electrophoresis, enough of the label was retained with the cognate tRNA (s). The gels were analyzed after staining by combustion of slices in a sample oxidizer. The mobility of modified charged tRNA and uncharged tRNA were the same, and the label released during electrophoresis passed through the gel. As can be seen from fig. 2, also the tRNA precursors could be separated into a variety of subspecies by two-dimensional electrophoresis. The group of larger precursors was resolved into about 15 spots, the group of the smaller precursors into about 12 spots.

A number of further applications of the systems described are possible and have been made to some extent. Once individual tRNAs from an organism have been mapped, it is possible to determine the amount of specific tRNAs within a tRNA population, using uniformly labeled RNA. By electrophoresis of modified charged tRNAs it is also possible to determine the multiplicity of isoaccepting tRNAs, as demonstrated here for some tRNA species from yeast.

In another context [16], we have employed the mapping of tRNAs as a screening procedure to see the extent of in vitro remethylation of mutant yeast tRNAs that are undermethylated in vivo.

Furthermore, one column chromatography of yeast [^{32}P]tRNA on benzoylated DEAE-cellulose [17], with subsequent electrophoresis of tRNA fractions from this column on the 10% gel has yielded several pure tRNA species (fig. 3). This could be demonstrated by fingerprinting tRNAs from a number of bands. Upon electrophoresis in the second dimension (not shown here) some bands could be resolved into different spots, and after exposure of the gels for

appropriate periods of time, several minor tRNA components became visible.

The two-dimensional system has also proven to be helpful for the separation of oligonucleotides after partial digestions of tRNA with T1- or pancreatic RNAase.

Acknowledgements

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