ISOLATION OF A TYROSINE-tRNA–tsDNA* HYBRID

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1. Introduction

The isolation of the lactose operon [1], ribosomal RNA genes [2–6] and transfer RNA genes [7,8] demonstrate a progress in techniques for obtaining increasingly smaller units of DNA genomes.

The transducing bacteriophage $\phi 80 psu^{+}_{III}$ carries two structural genes specifying a tyrosine tRNA in which a base change in the anticodon permits the insertion of tyrosine at the amber codon UAG [9]. We would like to report the possibility of isolating the DNA sequence complementary to tyrosine tRNA from the DNA of bacteriophage $\phi 80\ psu^{+}_{III}$.

2. Methods

Escherichia coli CA274† (HfrC $lac^{-}_{125\,amber}$ trp^{-}_{amber} su^{-}) was grown on tryptone broth (tryptone 2%, NaCl 0.5%, yeast extract 1.5%) supplemented with tryptophan 20 μg/ml, to 5×10^{8} bacteria per ml and infected with bacteriophage $\phi 80 psu^{+}_{III}$ at a multiplicity of 0.1. The phage were allowed to adsorb without aeration for 20 min, the culture diluted fourfold with fresh medium and growth continued with aeration at 37° until lysis. The purification of phage, DNA extraction and preparation of tyrosine-tRNA by polyacrylamide gel electrophoresis have been previously described [10, 11]. *Neurospora crassa* endonuclease was isolated from conidia as reported in the literature [12, 13] with a slight modification [14]. The enzyme purified by phosphocellulose chromatography had an activity of 180 units per mg protein and an absolute specificity for denatured DNA in the assay used [15]. The procedures for hybridization, gel filtration, caesium sulphate density gradient centrifugation and determination of melting profiles were as reported previously [7].

3. Results and discussion

The experimental model is presented in fig. 1.

* tsDNA: DNA sequence complementary to tyrosine tRNA

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† *Abbreviations*
lac : lactose
trp : tryptophan

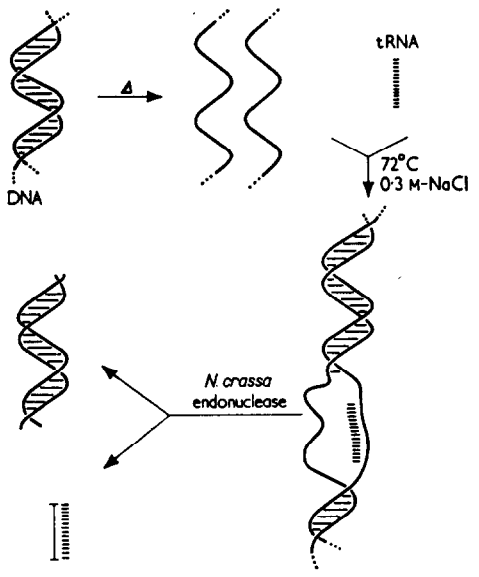

Fig. 1. Experimental model for the release of the tyrosine-tRNA–tsDNA hybrid. Denatured ϕ80 psu^{+}_{III} DNA is hybridized with tyrosine tRNA. During hybridization partial reannealing of DNA occurs. Treatment of the hybrid with *N. crassa* endonuclease, an enzyme specific for single-stranded nucleic acids, releases regions of reannealed DNA and hybrid molecules composed of tyrosine-tRNA and its complementary DNA sequence (tsDNA).

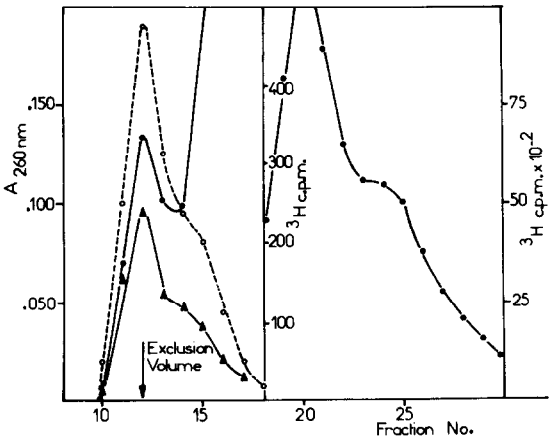

Fig. 2. Separation of a ϕ80psu^{+}_{III} DNA–tyrosine-tRNA hybrid from unhybridized tyrosine tRNA by gel filtration. Denatured ϕ80psu^{+}_{III} DNA, 250 μg, was hybridized with 1μg of ^{3}H-labelled tyrosine-tRNA (400,000 cpm/μg) in 5 ml of 2 X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) for 2 hr at 72°. Following slow cooling the mixture was chromatographed on a Sephadex G100 column, 2.5 cm X 37 cm, equilibrated with 2 X SSC, at room temperature. Fractions of 5 ml were collected every 30 min and DNA in each fraction measured by absorbance at 260 nm (o- - -o). Aliquots of 2 ml were precipitated with 5% trichloroacetic acid (•——•) or tested for DNA–tRNA hybrid formation by filtering directly through a nitrocellular filter (Millipore HA, 0.45 μm), washing with 2 X SSC, and digestion of the filter with pancreatic RNase, 20 μg/ml, in 2 X SSC for 60 min, at room temperature (▲——▲).

Tyrosine-tRNA hybridized to DNA of phage ϕ80psu^{+}_{III} was separated from excess unhybridized tyrosine tRNA by gel filtration on Sephadex G100 (fig. 2).

The ϕ80psu^{+}_{III} DNA–tyrosine-tRNA hybrid (fractions 11–14 from fig. 2) was digested with *N. crassa* endonuclease and rechromatographed on Sephadex G100. Following enzymatic digestion hybridized tyrosine-tRNA is displaced from a position coincident with that of DNA (fig. 2) to a position intermediate between the excluded DNA peak (MW $\geqslant$ 100,000) and that of tRNA (MW = 30,000) (fig. 3). This position is that expected of a hybrid molecule composed of a tyrosine tRNA and its complementary DNA sequence, having a M.W. of 60,000.

To rule out the possibility that the displaced tyrosine-tRNA was part of a tRNA dimer [16] or aggregate formed during digestion with *N. crassa* endonuclease, the digest was centrifuged in a caesium sulphate density gradient. The displaced tyrosine-tRNA sediments in a position intermediate between DNA and tRNA, consistant with the buoyant density of molecule composed of DNA and RNA in equimolar quantities (fig. 4).

The melting curve (fig. 5) shows that the product of digestion is resistant to thermal denaturation until 60° and has a T_m of 75°.

Although protein–tRNA complex formation was observed when free tRNA was incubated with *N. crassa* endonuclease, control experiments in which a ϕ80psu^{+}_{III} DNA–^{32}P-tyrosine-tRNA hybrid was digested with *N. crassa* endonuclease in the presence of marker ^{3}H-tyrosine-tRNA ruled out the possibility that ^{32}P-tyrosine-tRNA displaced from the hybrid during enzymatic digestion was part of an enzyme–tRNA complex. In addition phenol extraction of the

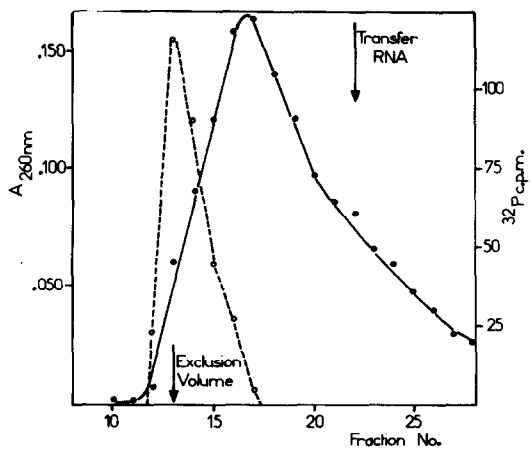

Fig. 3. Chromatography of a $\phi 80psu^{+}_{III}$ DNA–tyrosine-tRNA hybrid on Sephadex G100 following digestion with *N. crassa* endonuclease. A $\phi 80psu^{+}_{III}$ DNA–^{32}P-tyrosine-tRNA (100,000 cpm/μg) hybrid (fractions 11–14 from fig. 2) was digested in 5 ml of 0.1 M NaCl, 0.01 M $MgCl_2$, 0.1 M tris-HCl buffer, pH 7.5 with 50 units *N. crassa* endonuclease at 30° for 8 hr. The digest was chromatographed on a Sephadex G100 column, 2.5 cm × 40 cm, as described in fig. 2. Fractions were analyzed for presence of DNA by absorbance at 260 nm (o- - -o) and tyrosine-tRNA by acid-precipitable cpm (•——•). The arrow represents the elution position of tRNA alone in a parallel chromatography.

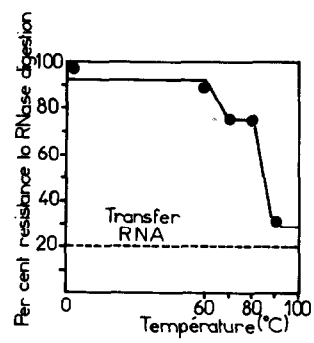

Fig. 5. Melting profile of $\phi 80psu^{+}_{III}$ DNA–tyrosine-tRNA hybrid after digestion with *N. crassa* endonuclease. A $\phi 80psu^{+}_{III}$ DNA–^{3}H-tyrosine-tRNA (400,000 cpm/μg) hybrid (fractions 11–14 from fig. 2) was digested with *N. crassa* endonuclease as described in fig. 3. Aliquots of the digest were heated for 3 min at the indicated temperatures, quick-cooled, incubated for 10 min with pancreatic RNase (20 μg/ml) at room temperature, and precipitated with 5% trichloroacetic acid. Percent resistance to RNase digestion (•——•) was calculated from acid-precipitable cpm. The dotted line represents the melting profile of tRNA alone.

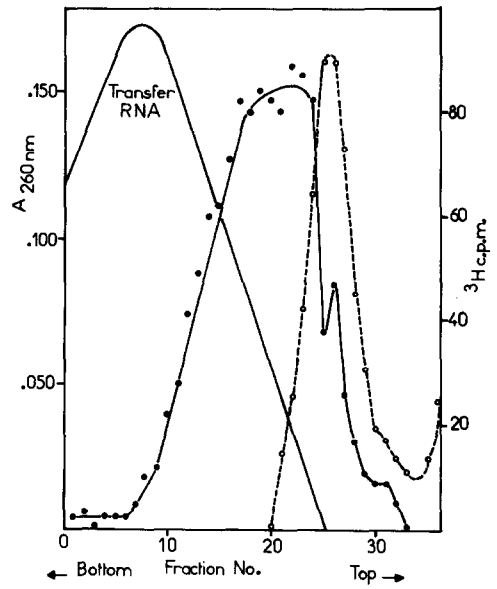

Fig. 4. Centrifugation of a $\phi 80psu^{+}_{III}$ DNA–tyrosine-tRNA hybrid in a cesium sulphate density gradient following digestion with *N. crassa* endonuclease. A $\phi 80psu^{+}_{III}$ DNA–^{3}H-tyrosine-tRNA (400,000 cpm/μg) hybrid (fractions 11–14 from fig. 2) was digested with *N. crassa* endonuclease as described in fig. 3. The digest was adjusted to a final density of 1.540 by addition of cesium sulphate and centrifuged for 72 hr at 33,000 rpm in a Spinco SW 39 rotor at 20°. Ten-drop fractions were collected from the bottom of the tube. Each fraction was diluted with 1 ml H_2O and analyzed for the presence of DNA by absorbance at 260 nm (o- - -o) and tyrosine-tRNA by acid-precipitable cpm (•——•). The solid line represents the sedimentation of tRNA alone in a parallel gradient.

digest following incubation with *N. crassa* endonuclease did not alter the profile shown in fig. 3.

Thus following digestion of a $\phi 80psu^{+}_{III}$ DNA–tyrosine-tRNA hybrid with *N. crassa* endonuclease, the elution profile of the digest from Sephadex G100, the sedimentation behaviour during cesium sulphate density gradient centrifugation, and the melting profile, all provide evidence for the release of a hybrid molecule composed of a tyrosine-tRNA and its complementary DNA sequence which can be separated from unhybridized DNA. The specificity of the method was confirmed in preliminary experiments using ^{3}H-tyrosine-aminoacyl-tRNA.

The width and asymmetry of the peaks obtained during gel filtration and density-gradient centrifugation and the biphasic melting curve suggest a hetero-

geneity of the hybrid molecules and a contamination of some hybrids with DNA tails.

We are attempting to purify sufficient quantities of the tyrosine-tRNA–tsDNA hybrid to confirm these results by sequence analysis and transcription studies.

Acknowledgements

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