THE GENE FOR THE 26 S rRNA IN *PHYSARUM* CONTAINS TWO INSERTIONS

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1. Introduction

In *Physarum*, most if not all ribosomal RNA (rRNA) genes (19 S, 5.8 S, 26 S) reside on linear, extrachromosomal and palindromic ribosomal DNA (rDNA) molecules of 57.5 kilobases length [1–4]. As shown by hybridization of rRNA to restriction fragments, one gene copy for 19 S, 5.8 S and 26 S rRNA is located symmetrically towards each end of the rDNA molecule ([2–4], see also fig.3). The 5′-ends of the two primary transcripts are directed to the center of the rDNA, the order of transcription being 19 S–5.8 S–26 S [5]. In an attempt to map these genes by electron microscopy as reported for other organisms [6,7], it was found that the 26 S sequence is not contiguous. Two insertions of 0.5 and 1.0 kilobases length split this gene into three parts. The present communication describes the positioning of these insertions on the rDNA.

2. Materials and methods

rDNA was purified from *Physarum polycephalum* plasmodia (strain M_3C VIII) as in [8] and kindly donated by T. Seebeck. 26 S rRNA was purified from a postnuclear supernatant fraction by repeated phenol extractions and sucrose-gradient centrifugations [1,3]. The length of the isolated 26 S rRNA was measured in the electron microscope together with *Xenopus* 28 S rRNA as internal standard as in [9]. Since under these conditions, the 26 S rRNA fails to exhibit the characteristic secondary structure of rRNA from higher eukaryotes [9], it can be readily distinguished from the *Xenopus* rRNA. R-loop reactions were performed according to [6]. Formation of rRNA/rDNA-hybrids was as in [7]. In brief, rDNA and rRNA were incubated at 18 μg/ml and 80 μg/ml, respectively, in 5 μl total vol. R-loop incubations with intact rDNA were carried out in 100 mM Tris–HCl (pH 7.5), 100 mM NaCl, 10 mM EDTA, 70% formamide at 52°C for 1–2 h in flame-sealed glass capillaries. Hybrids between *Eco*RI-restricted rDNA [1] and rRNA were formed in the same buffer by heating the RNA–DNA mixture first to 70°C for 10 min and then incubating for 2.5 h at 55°C [7]. Electron microscopy and analysis of the samples were as in [10,11].

3. Results

Intact rDNA from *Physarum polycephalum* strain M_3C VIII was subjected to the R-loop reaction and spread for electron microscopy. The loops due to hybridized 26 S rRNA could be clearly discerned; in addition, they proved to be interrupted twice (fig.1). Thirty molecules bearing the two inserts were measured. The total length of hybrid amounts to 90–95% (3700 nucleotides) of the expected value (3900 nucleotides, see [12]). Intactness of the rRNA used as starting material was established in parallel by spreading it under fully denaturing conditions together with *Xenopus* 28 S rRNA as internal standard [9]. Length measurements allowed the positioning of the two inserts: insert 1, 0.5 kilobases length, is situated at position 0.64 of the 26 S rRNA; insert 2, 1.0 kilobases length, at position 0.84. Positions are expressed relative to the 5′-end of the 26 S rRNA [5],

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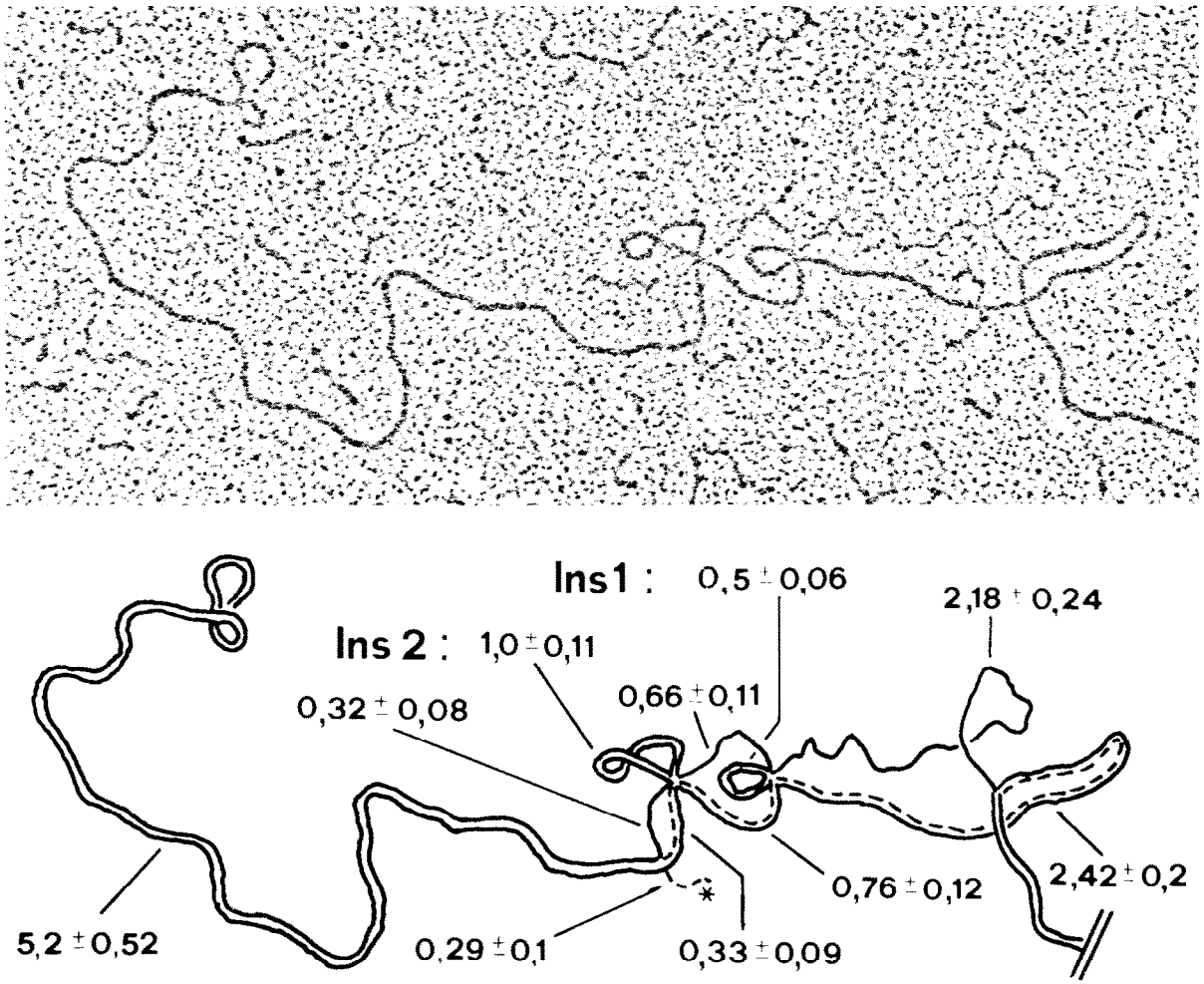

Fig.1. Gene region of an rDNA molecule with an R-loop formed by 26 S rRNA. Lengths were calculated as in [10,11] and are given in kilobases ± SD. $N = 30$. Since intact rDNA is quite large (57.5 kilobases), molecules were photographed in two halves to allow a higher magnification. The asterisk marks an unhybridized RNA tail. Length measurements are based on $N = 16$ for this structure. The reason for this apparently incomplete hybridization at the 3′-end of the RNA is at present not known. (- - -) RNA; (——) DNA.

by defining the 5′-end as position zero and the 3′-end as position one of the RNA.

Comparison of these data with the restriction maps from [1,2,4] suggested that both inserts should be part of the 2.71 kilobases *Eco*RI fragment. Therefore, an analysis of hybrid molecules between 26 S rRNA and the 2.71 kilobases fragment was undertaken. A typical molecule is shown in fig.2. Quantitative analysis of such hybrid molecules confirmed the R-loop data: positions of inserts 1 and 2 are 0.62 and 0.83, respectively, as defined above. Since the average DNA-length in structures as shown in fig.2 (2.67 kilobases) fitted the expected value for the restriction fragment (2.71 kilobases) very well, the positioning of the inserts is accurate also relative to the *Eco*RI cutting sites. Combination of these data with the ones already available in [2,4] leads to the schematic rDNA-map as shown in fig.3.

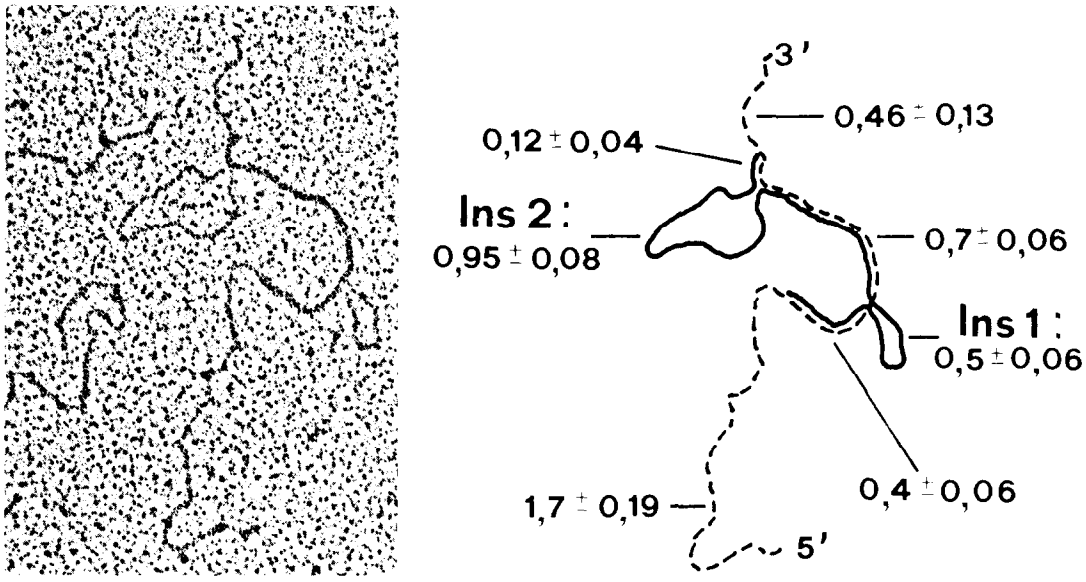

Fig.2. Hybrid between 26 S rRNA and the 2.71 kilobases *Eco*Ri fragment. $N = 49$. Lengths are given in kilobases ± SD, calculated as for fig.1. (- - -) RNA; (——) DNA.

4. Discussion

Cleavage of rDNA with several restriction enzymes gives fragments of uniform size [1,2,4]. The end fragments are a special case insofar as they do vary in length, suggesting the presence of frayed ends. For the internal restriction fragments however, there is no indication of heterogeneity. Here we show that the two inserts in the 26 S sequence are part of the internal, homogeneous 2.71 kilobases *Eco*RI fragment. All these findings support the notion that the inserts are part of the functional rDNA molecules and are likely to be transcribed into pre-rRNA molecules. To be sure of this point, it remains to be shown that the presumed immediate precursor to 26 S rRNA of ~5.6 kilobases length ([13] and our own unpublished data) does hybridize to the intervening sequences.

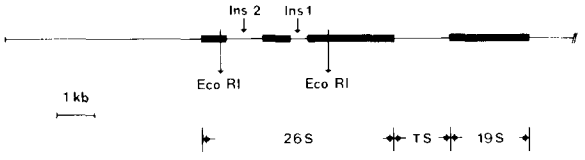

Fig.3. Map of the gene region of rDNA. Positioning of 19 S, 5.8 S rRNA and of the transcribed spacer are taken from [4]. The insertions (Ins 1 and 2) in the 26 S sequence are indicated.

With respect to inserts, the organization of rDNA in *Physarum* is similar to the one found in macronuclei of *Tetrahymena* [14], yeast mitochondria [15] and *Chlamydomonas* chloroplasts [16]: in these three cases, the single insert in the large rRNA sequence is also part of a functional gene. The size of the three intervening sequences is comparable, ranging from ~0.4–1.0 kilobases. *Physarum* however shows two inserts. No other rDNA has so far been shown to contain two or more inserts. The function or evolutionary significance of transcribed insertions is unknown in *Physarum* as it is in all other organisms so far.

Addendum

On completing this work, we learned from Campbell et al. [17] that they came to the same basic conclusions.

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