SEQUENCE SIMILARITY BETWEEN HETEROGENEOUS NUCLEAR RNA AND POLYSOMAL MESSENGER RNA IN HIGHER PLANTS

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1. Introduction

The existence of heterogeneous nuclear RNA (HnRNA) in animal cells is now well documented [1–3]. Recent reports [4] present evidence for the occurrence of these molecular species in higher plants as well. It is firmly established [5] that the majority of these RNAs has a high turnover and never leaves the nucleus. Moreover, the possible precursor relationship of HnRNA to polysomal messenger RNA (mRNA) has been the focus of considerable interest [6, 7] in recent years. The possible sequence similarity of these molecular species has been investigated mainly by the use of molecular DNA–RNA hybridization [7, 8]. However, firm conclusions cannot be drawn from these studies because of the existence of reiterated sequences in the DNA of eukaryotic cells [9].

The aim of the present paper is thus to perform molecular hybridization experiments on plant DNA having no reiterated sequences in view of establishing a possible sequence similarity between HnRNA and polysomal mRNAs.

2. Methods

Heterogeneous nuclear RNAs were obtained from lentil roots (*Lens culinaris* var Ronde Blonde Vilmorin) following a technique previously described [4]. Only fractions having a sedimentation constant higher than 35 S were used in this study. They were obtained after density gradient centrifugation [8]. ^{32}P-labelled polysomal mRNAs were isolated using the technique of Miassod et al. [4]. DNA was extracted from the roots using the technique of Smith and Halvorson [10] and purified by hydroxylapatite chromatography [11]. The purified DNA is 100% resistant to alkaline hydrolysis. Its hyperchromicity is 34% (in 0.1 × SSC*) and its modal sedimentation constant is 20.5 S. This value corresponds to an estimated molecular weight [12] of 7.5×10^6 daltons. On analytical centrifugation, two components are detected. One is the "bulk" DNA (buoyant density 1.698), the other the "satellite" DNA (buoyant density 1.720). Melting profiles of the lentil DNA give a T_m of 81.4°, which allows the calculation of a guanine plus cytosine content equal to 45%. Breakage of the purified DNA is effected in a pressure cell at 45,000 psi. After denaturation at 100° and incubation at 60° for various intervals of time, the annealed sequences are isolated by hydroxylapatite chromatography from the non-reassociated DNA [13] (see fig. 1).

Molecular DNA–RNA hybridizations are effected following a technique modified from Gillespie and Spiegelman [14]. Single-stranded non-reiterated DNA, with a *Cot*** of 680 and a mean molecular weight of 15×10^4 daltons, is loaded on nitrocellulose membranes (SM 11307 Sartorius filters with a 0.2 μ porosity). The use of high salt concentrations (6 × SSC) and filtration under reduced pressure at low temperature keep the loss of DNA from the membrane lower than 5%. Either ^{32}P-labelled or unlabelled HnRNAs and

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*SCC: saline sodium citrate.

** A "*Cot*" equal to 1 results from incubating DNA 1 hr at a conc. of 83 μg/ml [13].

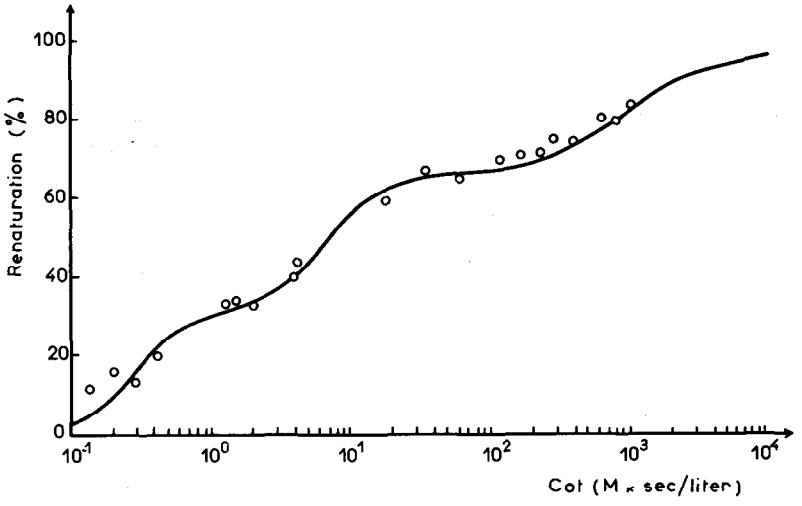

Fig. 1. Reannealing profile for denatured lentil root DNA. Data are obtained by measurement of binding to hydroxylapatite columns. The DNA, in 0.1 × SSC, was sheared at 45,000 psi, concentrated by lyophilization, and transferred to 0.12 M phosphate pH 6.8 by passing through Sephadex G-25 equilibrated in the same buffer. The samples were adjusted to suitable concentrations (10, 100, 222 and 900 μg/ml), sealed in ampoules, boiled for 15 min and incubated for various intervals of time at 60°. Each sample of DNA (hyperchromicity on melting 34%) was loaded on hydroxylapatite columns (1.5 × 3 cm) equilibrated in 0.12 M phosphate at 60°. Double-stranded DNA is eluted by increasing molarity to 0.4 M. The extent of reassociation is expressed as the percentage of double-stranded DNA recovered from the columns. The points are experimental, and the curve corresponds to the equation:

$$\% \text{ DNA reassociated} = \frac{106.5\,Cot}{1 + 3.33\,Cot} + \frac{5.3\,Cot}{1 + 0.166\,Cot} + \frac{0.0324\,Cot}{1 + 0.00091\,Cot}$$

This curve is characteristic of a DNA having three families of sequences (32%, 32% and 36% half reassociated at respective *Cot* values of 0.3, 6, and 1100).

polysomal mRNAs are denatured at 90° in 0.1 × SSC, 0.1% SDS buffer prior to their use in hybridization experiments. Both molecular species are then "pre-hybridized" with lentil reiterated DNA (*Cot* lower than 45) using the technique of Pagoulatos and Darnell [15]. The unhybridized RNA fractions are then used for hybridization experiments with non-reiterated DNA. These hybridizations are allowed to proceed for either 24 hr or 240 hr at 38.5° in a 5 × SSC, 0.1% SDS buffer containing 44% formamide (v/v). The final volume of the medium is 1 ml. Various assays with urea, perchlorate, and formamide have shown that the latter chemical is the most efficient in lowering the T_m of lentil DNA, and permits the use of a low temperature (38.5°) for hybridization studies.

3. Results

Studied with the Britten and Kohne technique [9, 13], the kinetics of DNA renaturation exhibits three components (fig. 1). The same situation has been described in animal and plant DNAs [17, 9, 13, 16]. The results of hybridization experiments of the "slow" component of lentil DNA with the polysomal ^{32}P-mRNAs are presented in fig. 2A, B. These results again show the occurrence of three components in polysomal mRNAs: i) a "fast' component, with sequences saturating the homologous DNA sites during short hybridization times, and at low RNA concentrations; ii) an "intermediate" component, with sequences saturating the homologous DNA sites during short hybridization times, and at high RNA concentrations; iii) a "slow" component, with sequences

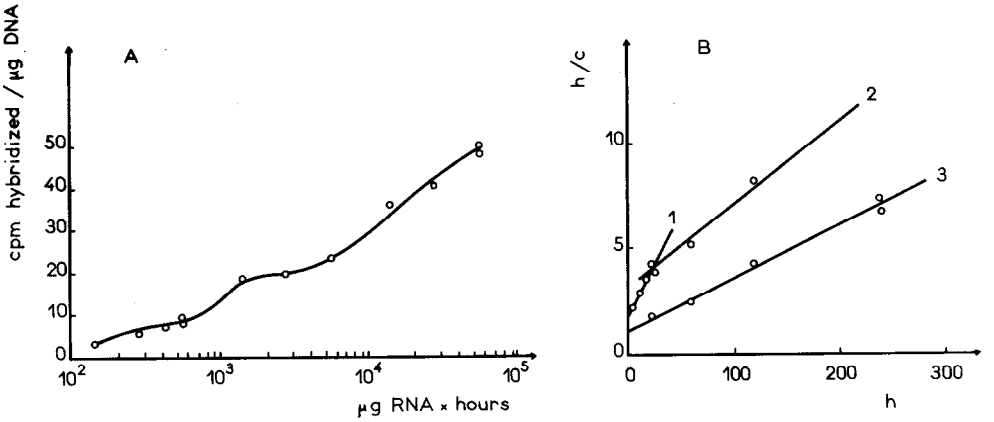

Fig. 2. Hybridization of non-reiterated DNA fraction of lentil roots with ^{32}P-polysomal mRNAs. A) Occurrence of the three components ("fast", "intermediate" and "slow") in polysomal mRNAs. This curve clearly shows the occurrence of reiterated sequences even in the so-called non-reiterated DNA. The last portion of the curve (for a product concentration × time higher than 10^4) corresponds to the hybridization of non-reiterated sequences of both DNA and mRNAs. B) Occurrence of the three components of polysomal mRNAs in the representation of Bishop et al. [18]. *h* is the concentration of polysomal mRNAs (µg/ml), *c* the number of cpm hybridized per µg of DNA. Annealing was carried out for either 24 hr (plots 1 and 2), or 240 hr (plot 3). Plot 3 corresponds to the hybridization of non-repetitive sequences of both DNA and mRNAs. In all cases, the Sartorius filters were loaded with 10 µg of non-repetitive DNA (non-reassociated at *Cot* 680).

saturating the homologous DNA sites during long hybridization times, and at high RNA concentrations.

Competition hybridization experiments between polysomal mRNAs and HnRNAs have been performed. Some of the results are presented in fig. 3. In the linear representation of Bishop et al. [18], the reciprocal of the slope corresponds to the proportion of sequences *p* in labelled RNA that would be competed against by an infinite concentration of the unlabelled RNA. If polysomal ^{32}P-mRNAs are hybridized with DNA, and if various concentrations of the same unlabelled polysomal mRNAs are introduced in the hybridization mixture, the theoretical value of *p* would obviously be 1. This value is nearly obtained (0.97) from the results of fig. 3.

Unlabelled HnRNA also strongly competes against the three components of polysomal ^{32}P-mRNAs. In this case, however, the value of *p* is slightly lower (0.84).

4. Discussion

The question of a possible precursor relationship between the heterogeneous nuclear and polysomal messenger RNAs is an appealing challenge. Recent experiments by Penman et al. [19], have shown that the syntheses of these molecular species are affected in different ways by the inhibitor cordycepin. This was taken as an argument that mRNAs and HnRNAs are transcribed from different genes.

Most hybridization experiments performed with eukaryotic DNAs and mRNAs are indicative only of interactions between the highly reiterated sequences of both nucleic acid molecules. However, after a partial isolation and purification of the non-repetitive component of DNA it then becomes possible to detect the hybridization of non-reiterated sequences of both macromolecules.

In addition, the strong competition between polysomal mRNAs and HnRNA for the same non-reiterated sequences of DNA, implies a close similarity between the sequences of these two types of ribonucleic acids. This result cannot be taken as a proof for a precursor relationship of heterogeneous nuclear RNA to polysomal messenger RNAs in plants, but is compatible with this interpretation.

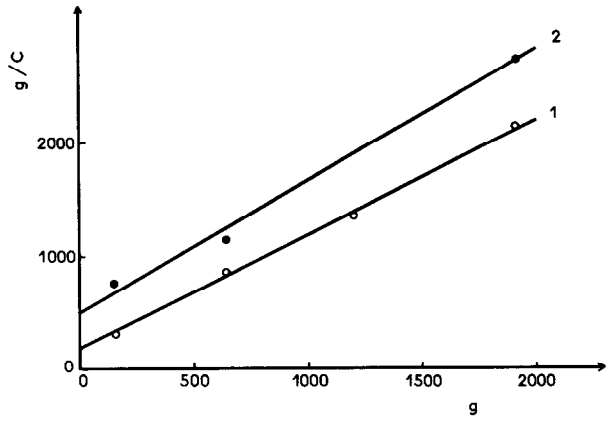

Fig. 3. Hybridization competition between ^{32}P-labelled polysomal mRNAs and either the same unlabelled polysomal mRNAs, or unlabelled HnRNAs (Bishop et al. [18] plots). If c_0 and c are the numbers of cpm hybridized/μg of DNA, either in the absence (c_0) or in the presence (c) of unlabelled RNA, the "competition" is defined as $C = c_0 - c/c_0$. g is the concentration of unlabelled RNA (μg/ml). Plot 1) competition between ^{32}P-labelled polysomal mRNA and the same unlabelled polysomal mRNAs. Plot 2) competition between ^{32}P-labelled polysomal mRNAs and the unlabelled HnRNAs. The DNA used corresponds to the non-reiterated fraction with a *Cot* of 680. Each example contains 240 μg/ml of labelled RNA (c_0 = 54 cpm/μg of DNA). Hybridization is run during 240 hr.

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References

[1] G.P. Georgiev, Prog. Nucl. Acid Res. Mol. Biol. 6 (1967) 259.
[2] K. Scherrer and L. Marcaud, J. Cell. Physiol. 72 (suppl. 1) (1968) 181.
[3] J.E. Darnell, Bact. Rev. 32 (1968) 262.
[4] R. Miassod, P. Penon, M. Teissere, J. Ricard and J.P. Cecchini, Biochim. Biophys. Acta 224 (1970) 423.
[5] R. Soeiro, H.C. Birnboim and J.E. Darnell, J. Mol. Biol. 19 (1966) 362.
[6] J.E. Darnell, G.N. Pagoulatos, U. Lindberg and R. Balint, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 555.
[7] K. Scherrer, G. Spohr, N. Granboulan, C. Morel, J. Grosclaude and C. Chezzi, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 539.
[8] R. Soeiro and J.E. Darnell, J. Cell. Biol. 44 (1970) 467.
[9] R.J. Britten and D.E. Kohne, Science 161 (1968) 529.
[10] D. Smith and H.O. Halvorson, in: Methods in Enzymology, Vol XII part A, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York and London, 1967) p.538.
[11] G. Bernardi, Biochim. Biophys. Acta 174 (1969) 423.
[12] J. Eigner and P. Doty, J. Mol. 12 (1965) 549.
[13] R.J. Britten and D.E. Kohn, Carnegie Inst. Wash. Year Book 65 (1966) 78.
[14] D. Gillespie and S. Spiegelman, J. Mol. Biol. 12 (1965) 829.
[15] G.N. Pagoulatos and J.E. Darnell, J. Mol. Biol. 54 (1970) 517.
[16] Y.M. Sivolap and J. Bonner, Proc. Natl. Acad. Sci. U.S. 68 (1971) 387.
[17] M. Melli and J.O. Bishop, Biochem. J. 120 (1970) 225.
[18] J.O. Bishop, F.W. Robertson, J.A. Burns and M. Melli, Biochem. J. 115 (1969) 361.
[19] S. Penman, M. Rosbash and M. Penman, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1878.