LOCALIZATION OF MESSENGER RNA NEAR THE 3'-END OF THE dRNA PRECURSOR MOLECULE

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1. Introduction

Recently a hypothesis on operon structure in eukaryotic cells has been described by one of us [1]. According to this model the structural cistrons are localized promoter-distal. The promoter-proximal part of the operon mainly consists of acceptor sites, which do not carry any structural information, but interact with regulatory proteins. One of the predictions from the model is that only a part of the newly-formed giant dRNA molecule is transferred into the cytoplasm and this part is localized near the 3'-end of the molecule, the 5'-part being degraded inside the cell nucleus. To check this suggestion giant dRNA randomly labeled with ^{14}C was additionally labeled by ^{3}H in 3'-end nucleoside. This double-labeled dRNA was studied in hybridization-competition experiments. It was found that the 3'-ends of giant dRNA are enriched in sequences transferred from the nucleus into the cytoplasm.

2. Material and method

Rat livers and Ehrlich ascites carcinoma cells randomly labeled with ^{14}C-orotic acid (which was injected three times during the 40 hr period of labeling) were used. Nuclear dRNA was isolated by hot phenol fractionation in the temperature intervals 55–75° and 75–85° [2–4]. After centrifugation in SDS–sucrose gradients the heavy (40–70 S) and light (12–20 S) dRNA fractions were collected. The RNAs obtained were labeled in the 3'-end position by oxidation with $NaIO_4$ followed by reduction with NaB^3H_4 (activity 10 mCi/mg) [5]. The RNA was then purified by treatment with DNase and pronase, deproteinization by phenol and chloroform, reprecipitation with 2.5 M NaCl in the cold, gel filtration through Biogel P-100, reprecipitation with 5% TCA followed by washing with 0.2 M sodium acetate in 70% ethanol, and ethanol reprecipitation.

The RNA obtained was hybridized with homologous DNA using DNA gels cross-linked by UV-radiation [6]. Hybridized and non-hybridized RNAs were collected quantitatively and hydrolysed with 0.5 N KOH for 18 hr. $HClO_4$ was added, $KClO_4$ and acid-insoluble material were removed, and the solution neutralized and passed through a Dowex-1-formate column, to separate nucleotide and nucleoside fractions. Nucleosides passed through the column. Nucleotides were retained on the column, eluted with 2 N HCl, dried, and counted. The nucleoside fraction was dried and chromatographed on Whatman 3 MM paper in a *tert.*-butanol:ethyl methyl ketone:H_2O:conc. HCOOH mixture (44:44:19:0.26) [7]. Four non-labeled nucleosides oxidized with $NaIO_4$ and then reduced with $NaBH_4$ were added as markers. The spots corresponding to the nucleosides and nucleotides were eluted and counted in an Intertechnique scintillation counter. RNA isolated from cytoplasmic polysomes [8] was used as a competitor in the hybridization experiments. The polysomal preparations obtained by this methods do not contain free informosomes (M.I.Lerman, personal communication).

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3. Results

It was found that in hybridized as well as in non-hybridized material the 3′-ends are mainly adenosine (70–75%). About 20% of ends are uridine and about 5–10% are guanosine and cytidine. Such a distribution of 3′-ends is typical of light as well as of heavy dRNA. The similarity in the nature of 3′-ends in giant and in low-molecular-weight dRNAs may indicate conservation of the 3′-ends of dRNA during the processing.

Hybridization experiments (table 1) demonstrated that the hybridizability of the 3′-ends is much higher than that of total dRNA. In the presence of excess of DNA (DNA/dRNA = 50:1), the percentage of ^{3}H hybridized was 2–3 times higher than that of ^{14}C. It is known that in the usual conditions of hybridization experiments only those RNA sequences which are synthesized on highly repetitive DNA base sequences may be bound [9, 10]. Thus the 3′-ends in many different dRNAs are made up of identical or very similar sequences.

It was found that polysomal RNA competes much more effectively with 3′-end sequences (^{3}H) of giant precursor molecules, than with the total chains (^{14}C). This means that the 3′-end sequences of giant dRNA molecules are predominantly transferred into the cytoplasm and incorporated into the polysomes. In other words the sequences encoding the formation of mRNAs are localized near the ends of the operons.

The strong inhibition of 3′-end hybridization is accompanied by only a very slight decrease in the amount of total RNA bound. Thus only a small part of all repetitive base sequences in DNA corresponds to the ends of operons. On the other hand competition of polysomal RNA with 3′-end sequences and with total RNA in the case of light dRNA is of the same order. This may indicate that the significant part of the repetitive base sequences in the light dRNA belongs to the 3′-ends.

4. Discussion and conclusion

The results obtained in this paper give experimental support to one of the main predictions from the above-mentioned hypothesis [1], according to which mRNA is localized near the 3′-end of giant dRNA precursor molecules.

On the other hand it was shown that the sequences common for a number of different operons are localized at the ends of the operons. Their function remains unclear. One can speculate that they correspond to

Table 1
The results of a typical experiment on the competitive hybridization of DNA and dRNA labeled randomly with ^{14}C and with ^{3}H in the 3′-end position.

Material		Conditions of hybridization				$^{14}C/^{3}H$		% total radioactivity in hybrid		% of competition	
Source	RNA fraction	DNA (mg)	dRNA (mg)	Polysomal RNA (mg)	Volume (ml)	in hybrid	in non-hybridized RNA	^{14}C	3H*	^{14}C	^{3}H
Ehrlich ascites carcinoma cells	Heavy (≥35 S)	13	0.25	–	2.7	0.44	1.8	5.2	18.3	–	–
		13	0.25	18	2.7	1.25	1.8	4.7	5.7	−10	−69
	Light (∿18 S)	10	0.20	–	2.2	0.08	0.56	3.2	19.2	–	–
		10	0.20	15	2.2	0.14	0.56	2.5	8.8	−22	−54
Rat liver	Heavy (≥35 S)	8	0.16	–	2.7	21.0	52	12.7	26.5	–	–
		8	0.16	8	2.7	32.6	52	12.8	17.2	0	−35
	Light (∿18 S)	5	0.09	–	2.2	2.8	6.6	11.3	22.9	–	–
		5	0.09	5	2.2	3.1	6.6	7.2	13.3	−37	−42

* The absolute ^{3}H counts in hybrids were more than 150 cpm in all cases. Only 3′-end ^{3}H recovered in the form of a nucleoside derivative on the paper chromatograpm was considered.

terminators of transcription or translation or to other service sequences. These end sequences are transferred into polysomes and their presence in cytoplasmic mRNA may explain the fact that the latter hybridizes to some extent with DNA.

During the processing of dRNA, the precursor 3′-end of the latter is conserved.

Asymmetrical degradation of giant dRNA indicates that the same precursor molecules contain the sequences of both the mRNA and the rapidly-metabolized nuclear dRNA. This is also in agreement with the above mentioned model [1].

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