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Strand-specific transcription of the simple sequences poly[(dG-dT):(dC-dA)] and poly[(dG-dA):(dC-dT)] in *D. melanogaster* embryos revealed by S_1 nuclease treatment

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The transcription of different strands of the simple sequences poly[(dG-dT):(dC-dA)] and poly[(dG-dA):(dT-dC)] in *Drosophila melanogaster* embryos was studied. After selective labelling of one strand, the polymers were hybridized with immobilized RNA and hybrids were treated on filters with S_1 nuclease. Appropriate control has shown that this treatment destroys sandwich-like complexes and leaves only true hybrids. Transcription of poly[(dG-dA):(dT-dC)] proved to be nearly symmetric, while poly(dG-dT) is transcribed 3-times more intensively than poly(dA-dC). Our approach allowed us to estimate the relative content of simple sequences in RNA.

Simple nucleotide sequence; Nuclease S_1; Transcription; (*Drosophila melanogaster*)

1. INTRODUCTION

The simple sequences GT/CA (GT/CA, poly-[(dG-dT):(dC-dA)]), GA/TC (GA/TC, poly-[(dG-dA):(dT-dC)]) and AA/TT (AA/TT, poly-[(dA):(dT)]) are more or less abundant in all eukaryotic genomes including *Drosophila melanogaster* [1,2]. Their transcripts were found in many different RNAs by Northern hybridization with labelled double-stranded polymers [3,4]. However, it is well known that labelled DNA forms sandwich-like structures upon hybridization and therefore it is impossible to estimate the transcription of its strands separately. The occasional determination of simple sequences in cloned cDNAs does not give any consistent pattern. Here, we aim to estimate the transcription of GT/CA and GA/TC strands separately through treatment of hybrids on filters with S_1 nuclease.

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2. MATERIALS AND METHODS

RNA was isolated from 0-16 h embryos of *D. melanogaster* line Oregon RC as described in [5]. GT/CA, GA/TC and AA/TT as well as nuclease S_1 were purchased from PL-Pharmacia.

We used a cloned DNA fragment containing 42 bp of GT/CA out of 329 bp inserted in the *Sal*I site of pBR322 (sequence of the fragment not shown) in control experiments. The fragment was cut out from the vector by *Sal*I, its strands separated by electrophoresis polyacrylamide, transferred by electroblotting to Hybond membranes (Amersham) and hybridized with nick-translated polymers.

Nick-translation, labelling of RNA with [γ-^{32}P]ATP in the presence of polynucleotide kinase, and all hybridizations were carried out as in [2,4]. The specific activity of all nick-translated polymers was carefully adjusted to a standard value of 3×10^7 cpm/μg by dilution. This is important for qualitative assessment and comparison of the transcription of different strands and polymers.

S_1 nuclease treatment of the filters was carried out in 50 mM NaCl, 50 mM sodium acetate (pH 4.1), 1 mM $ZnCl_2$ at room temperature and a nuclease S_1 concentration of 1000 U/ml.

3. RESULTS AND DISCUSSION

Although active transcription of simple sequences in eukaryotes including *Drosophila* has

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long been known [4,5], there are no data as yet on the transcription of their chains separately. The labelling of only one chain of a polymer does not allow one to solve the problem, because the labelled chain is capable of binding with RNA on filters through an intermediate unlabelled chain. We propose here the use of treatment of hybrids on filters with S_1 nuclease to disrupt all single-stranded regions and leave only true hybrids.

To monitor the reliability of the method we used a DNA fragment containing a GT/CA sequence of length 42 bp (fig.1, lanes 1,2). Its strands were separated by electrophoresis, transferred to the Hybond membrane and hybridized with GT/CA using only a CA chain labelled by nick-translation in the presence of [α-^{32}P]dCTP and unlabelled dATP. The label first binds with both chains of the insert (fig.1, lane 3), but after S_1 nuclease treatment only the upper chain remains (fig.1, lane 4). If the GT strand is labelled similarly, only the lower strand is stained (not shown). The intensity of the remaining signal is in both cases somewhat weaker after S_1 nuclease treatment due to partial hydrolysis of the sandwich complexes.

Another control experiment addressed the question of the susceptibility of filter-bound RNA to the action of S_1 nuclease. RNA was kinased, applied to the nitrocellulose sheets as dots, counted, treated with S_1 nuclease and counted again. Despite the well-known RNase activity of S_1 nuclease [7], more than 80% of the label survived. This can be explained by the protection of RNA upon binding with the membrane. At any rate, there is no reason to suggest that degradation of any specific RNA sequences occurs.

We applied aliquots of total RNA to the nitrocellulose sheets to estimate the amount of simple sequences in the transcripts. Filters were hybridized with different probes, treated with S_1 nuclease and counted. The results are presented in table 1. The transcription of GT/CA is clearly asymmetric: GT is transcribed 3-times more intensively than CA. This means that the transcription of these sequences or the processing of their transcripts (or both) is strand-specific. This fact suggests that GT/CA sequences are not merely primitive transposons [8], but at least in some cases take on an unknown function.

The transcription of GA/TC is nearly symmetric and is about 3-times weaker than that of GT/CA.

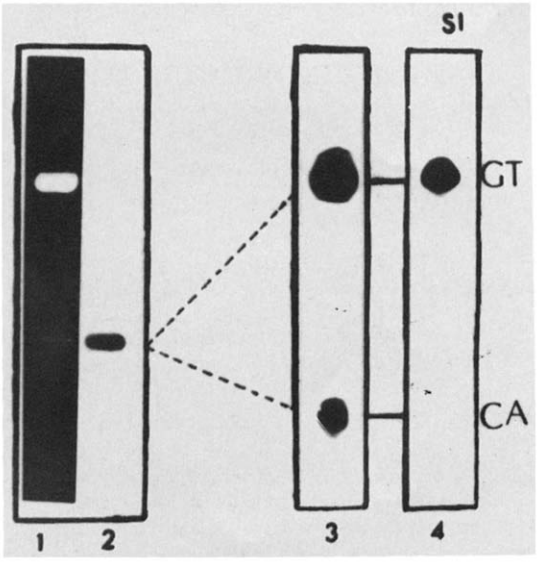

Fig.1. Strand-specific hybridization of ^{32}P-labelled GT/CA with GT/CA-containing clone. The clone (see text) was treated with *Sal*I, electrophoresed in 1% agarose gel and stained with ethidium bromide (lane 1). Fragments were transferred to Hybond and hybridized with [^{32}P]GT/CA (CA chain labelled) (lane 2). In the other case, the fragment was denatured, the two strands separated by electrophoresis in 5% polyacrylamide gels, transferred to Hybond and hybridized with the same probe (lane 3). The filter was then treated with S_1 nuclease and again exposed to the same conditions (lane 4).

However, bearing in mind that GA/CT sequences are about 100-times less abundant in the *Drosophila* genome [2], we conclude that their transcription is relatively more efficient.

The data obtained provided the possibility of

Table 1

Transcription of simple sequences in *Drosophila* embryos

Simple sequence[a]	Amount of hybridized radioactivity (cpm)	Amount of radioactivity after S_1 treatment (cpm)	Relative amount of transcripts (%)[b]
GT/CA*	927	187	2.5
G*T/CA	2547	647	8.7
GA/CT*	576	153	2.0
GA*/TC	427	129	1.7
AA*/TT	1120	175	2.4
AA/TT*	31218	7433	100

[a] Asterisks mark the labelled nucleotide

[b] Content of poly(A) in RNA taken as 100%. Obviously, it refers mainly to post-transcriptional poly(A)

estimating the relative amount of simple sequences in RNA. The content of poly(A) estimated by the hybridization with [^{32}P]poly(T) was taken as 100%. The data for all simple sequences studied are listed in table 1. The only estimate known from published reports was made for poly(U)$^+$-RNA [9]: in mammalian cells, it amounted to 10% of poly(A)$^+$-RNA.

Finally, the interesting question arises as to the distribution of simple sequences among different classes of mRNA. For example, do poly (GU)-containing molecules form a specific subset of mRNA, as does poly(U)$^+$-RNA [10]? We are currently addressing this problem.

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