COUPLING OF POLYOMA DNA AND RNA SYNTHESIS

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SUMMARY: Polyoma virus RNA from infected mouse embryo cells was examined by gel electrophoresis and in nucleic acid hybridisation experiments. The extent of representation of the polyoma genome in RNA sequences when cytosine arabinoside is present throughout infection is 30 to 40% of that at late times in infection. When viral DNA synthesis is inhibited during the period in which it is rising to its maximum, the resulting cytoplasmic RNA resembles 'early' RNA both in size and by its behaviour in competition hybridisation experiments.

Polyoma virus RNA has many of the properties of eukaryotic messenger RNAs: In the nucleus it is heterogeneous in size and undergoes rapid turnover (1, 2) ; poly A sequences are added and species of more uniform size move into the cytoplasm (1). According to Hudson et al., transcription of the polyoma genome may be divided into early and late phases (3). The present experiments examine differences between early and late RNA isolated from nuclear and cytoplasmic cell fractions. In addition the dependance of late RNA synthesis upon DNA synthesis is examined under conditions in which DNA synthesis is inhibited late during infection whilst it is rising to its maximum.

METHODS: Mouse embryo secondary cells were grown in 80 oz. roller bottles in Eagle's medium with 10% calf serum or on 90 mm. dishes in low serum as described by Fried and Pitts (4). The small plaque strain of polyoma virus was used (5).

Viral DNA was isolated by the method of Hirt (6). Component I DNA was purified by isopycnic centrifugation in cesium chloride/ethidium bromide gradients (1). ^{14}C thymidine labelled DNA was prepared by adding ^{14}C thymidine (50 μCi/μmol) at 5 μCi/ml. at 24 hours post infection and harvesting at 36 hours p.i.

Cells were fractionated by the method of Penman (7). The cytoplasmic cytoplasmic fractions and the DNase treated nuclear fractions were added to an equal volume of predigested pronase (1mg./ml) in 0.05M NaCl, 0.01M disodium EDTA, 0.1M tris/HCl, polyvinyl sulphate 4μg/ml 1% SDS, pH 7.5 and incubated at 37°C for two hours. The nucleic acids were precipitated withtwo volumes of ethanol. The precipitate

was pelleted and dissolved in 0.01M sodium acetate, 0.01M $MgCl_2$, 20μg/ml DNase and incubated at 37°C for 45 minutes. The mixture was then subjected to a phenol extraction and sephadex G100 chromatography

Polyacrylamide gels (2.7% acrylamide, 0.27% ethylene diacrylate) were prepared and fractionated as described by Knowland (9).

Schleicher and Schuell filters were loaded with DNA for hybridisation as described by Gillespie and Spiegelman (10). The DNA to be loaded was component I DNA that had been nicked by X irradiation(42 rads/ hour for 30 minutes) and alkali denatured. The optimum temperature for the initial rate of polyoma DNA/RNA hybridisation in 50% formamide 1 x SSC was 45°C. However some thermal degradation of RNA occured so the reactions were performed at 39°C. Hybridisation with 24 mm. filters was carried out with 200μl RNA solution in scintillation vials. Following hybridisation (15 to 20 hours) the filters were washed a)twice, for 30 minute periods, with 50ml hybridisation buffer/24mm filter at 39°C. b)twice at room temperature with hybridisation buffer c)twice at room temperature with 50ml 2 x SSC, d) twice with 20ml ice cold distilled water for two to three minutes. 80% of the RNA could be eluted from the filters in two incubations with 0.2ml 50% DMSO: 50% H_2O for 5 minutes at 42°C.

For hybridisation competition experiments, unlabelled competitor RNA was reacted in an initial incubation and labelled RNA in a second incubation. The RNA had been partially degraded by heating at 50°C for 24 hours in 2 xSSC. The hybridisation reactions were carried out for 24 hours in a volume of 20μl using 4mm diameter filters.

RESULTS AND DISCUSSION: The size distribution of 'late' cytoplasmic polyoma RNA, eluted from DNA/RNA hybrids on filters, is shown in Fig. 1. The RNA migrates as a broad band corresponding to RNA of mean molecular weight about 0.9 to 1.3 x 10^6. This result together with the heterogeneous distribution of nuclear RNA (not shown) some of which is larger than expected for one complete transcript of the genome, is in agreement with the results of Acheson et al. (1). The slight heterogeneity of the cytoplasmic polyoma RNA could result in part from some contamination with nuclear RNA, as a small amount of the 32 S rRNA precursor was detected in total cytoplasmic RNA. Some degradation of RNA during this isolation procedure is also to be expected.

As the onset of DNA synthesis in infected cells is asynchronous, 'early' RNA was labelled in the presence of the DNA synthesis inhibitor

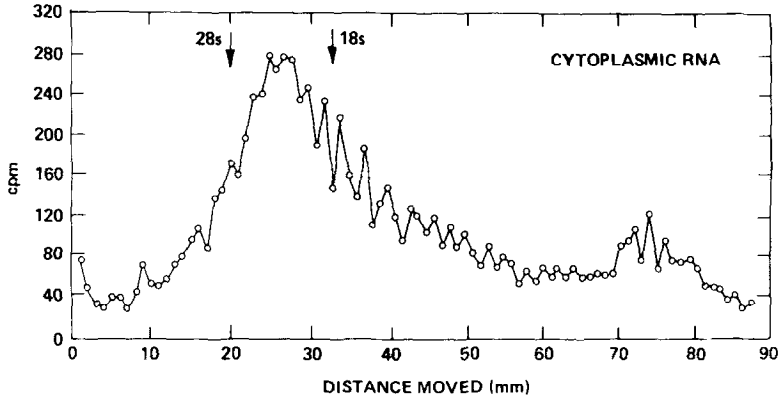

Fig. 1:Infected mouse embryo cell cultures (m.o.i. 20 p.f.u./cell, about 3 x 10^7 cells) were pulse labelled at 28 hours post infection for 30 minutes with 400μCi/ml ^{3}H uridine (0.5 ml/90 mm dish) and chased for 90 minutes with 100μg/ml cold uridine. The cells were harvested and RNA prepared from nuclear and cytoplasmic fractions. The electropherogram of cytoplasmic polyoma RNA selected by preparative nucleic acid hybridisation is shown. When RNA was selected from the cytoplasmic fraction of uninfected cells by hybridisation to polyoma DNA, and analysed on gels, a heterogeneous profile was seen not rising above 30 c.p.m./gel fraction.

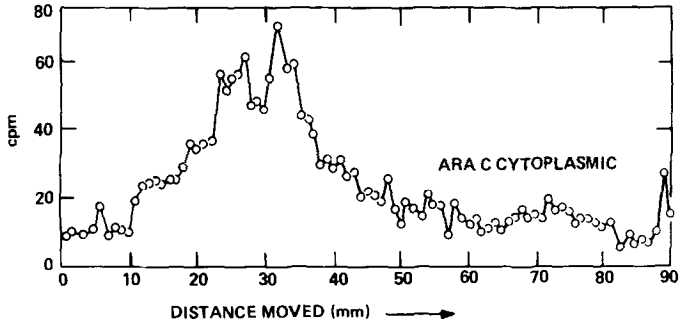

Fig.2: Three additions of medium containing 40 μCi/ml ^{3}H uridine and 10 μg/ml cytosine arabinoside were made at 8 hour intervals to infected low serum cell cultures. Nuclear and cytoplasmic RNA was extracted from the cells 24 hours post infection. The electropherogram shown is of cytoplasmic polyoma RNA selected by nucleic acid hybridisation. 28 S and 18 S marker ribosomal RNA ran at fractions 20 and 32 respectively.

cytosine arabinoside. Again the nuclear polyoma RNA contained much RNA larger than one transcript. The gel electropherograms of cytoplasmic RNA (Fig.2) suggest two broad bands with molecular weights of about 0.9 and 0.7 x 10^6. Such species were never found to predominate 'late' in infection.

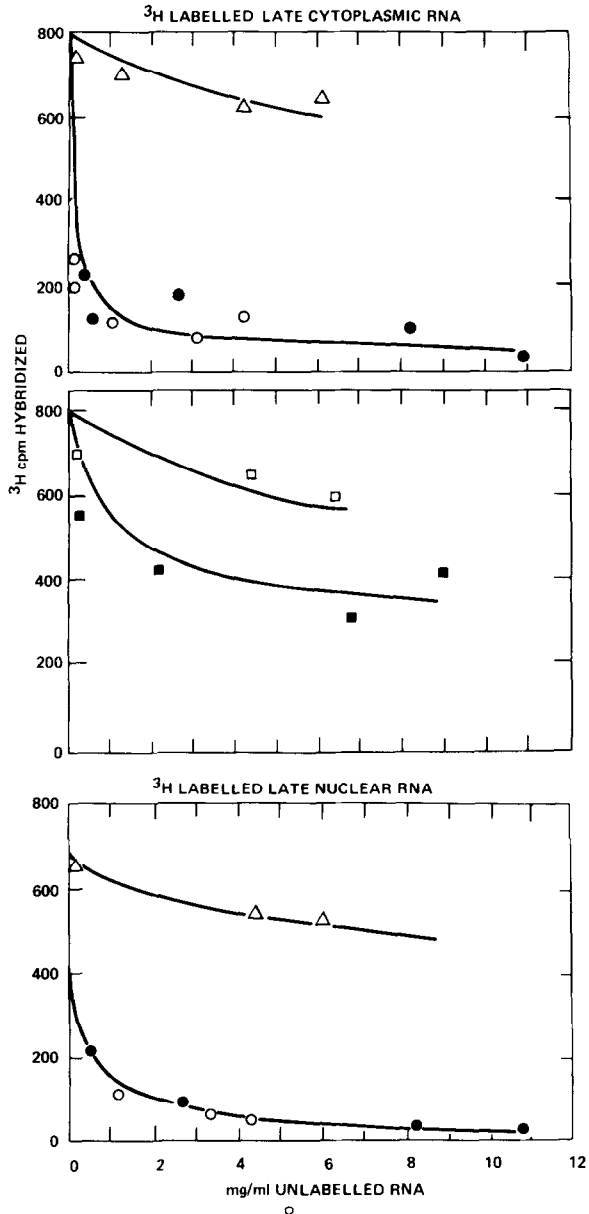

Fig. 3: Approximately 1.5 x 10^8 mouse embryo cells in 0.5% serum were pulse labelled for 30 minutes at 30 hours post infection with 1mCi/ml ^{3}H uridine (40 Ci/mmol), 0.5 ml/dish, and chased for 90 minutes with 100 μg/ml cold uridine. The nuclear and cytoplasmic RNA was extracted. Hybridisation was with 0.01 μg ^{14}C polyoma DNA(20,000 cpm/μg) per filter. In all cases saturating levels of ^{3}H cytoplasmic RNA were used, and 90% saturating levels of ^{3}H nuclear RNA were used. Unlabelled 'late RNA' was extracted from 10^9 infected cells grown in roller bottles 32 hours post infection. (late nuclear RNA —●—, late cytoplasmic RNA —○—). Unlabelled 'ara C RNA' was prepared from 10^9 cells grown in the presence of 10μg/ml cytosine arabinoside for 24 hours after infection (nuclear 'ara C RNA' —■—, cytoplasmic 'ara C RNA' —□—). (—△— RNA from uninfected cells) Fig. 3a, top panel; 3b, middle panel; 3c, bottom panel.

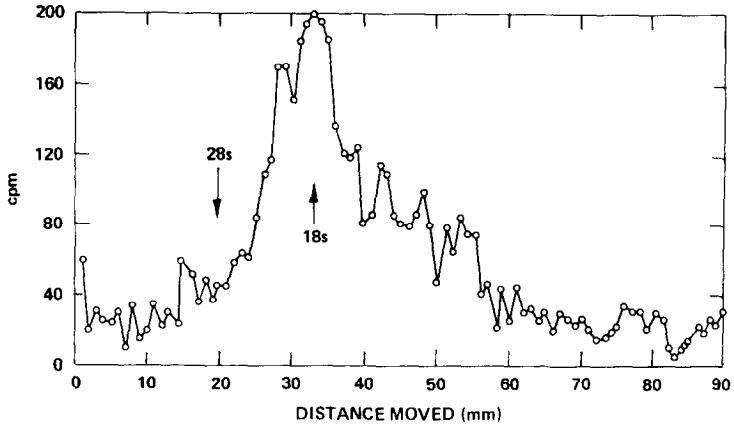

Fig. 4: Infected cells were treated with 20 μg/ml cytosine arabinoside at 24 hours p.i. for 4 hours. The cultures were incubated with 400 μCi/ml ^{3}H uridine at 26 hours p.i. for 30 minutes followed by 100 μg/ml cold uridine for 90 minutes all in the presence of 20 μg/ml cytosine arabinoside. Under these conditions the incorp-oration of ^{3}H thymidine into polyoma DNA was reduced by 98% and ^{3}H uridine into polyoma RNA by 50 to 60%. The electropherogram shown is polyoma RNA selected by hybridisation from the cytoplasmic RNA fraction. A similar profile was obtained when this procedure was follwed starting at 28 hours post infection.

Competition hybridisation experiments between 'early' and 'late' RNA are shown in Fig. 3. The following conclusions may be drawn:
1) Unlabelled 32 hour nuclear (late nuclear) and cytoplasmic (late cytoplasmic) RNA completely compete with labelled cytoplasmic RNA for hybridisation to polyoma DNA (Fig. 3a). The reciprocal experiment was carried out with 90% saturating labelled nuclear RNA (Fig. 3c). Complete competition in these experiments shows that the same sequences are present in both nuclear and cytoplasmic RNA late after infection.
2) Unlabelled nuclear RNA made in the presence of cytosine arabinoside (ara C nuclear) reduces the hybridisation of late labelled sequences from the cytoplasm by 30 to 40% (Fig. 3b). This result indicates that 30 to 40% of the sequences found in the cytoplasm at late times after infection are also transcribed in the absence of DNA synthesis.

It has not proved possible to carry out satisfactory competition experiments with 'ara C cytoplasmic' RNA because of the very low concentration of polyoma RNA in the cytoplasm of cells in the continued presence of cytosine arabinoside. The reciprocal competition experiment against labelled RNA made in the presence of cytosine arabinoside was not possible as such RNA preparations were not of high enough specific activity for accurate quantitative hybridisation to the low amounts of DNA used in these experiments.

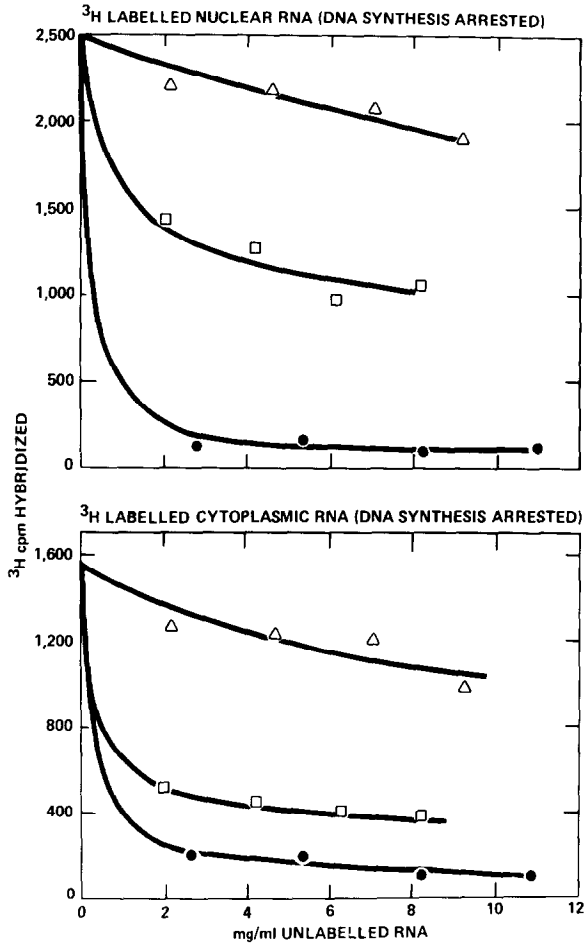

Fig. 5: DNA synthesis was inhibited by the addition of 20 μg/ml cytosine arabinoside to 1.5 x 10^8 infected cells at 26 hours post infection. At 28 hours post infection 500 μCi/ml ^{3}H uridine was added (1ml/dish) together with 20 μg?ml'ara C ' for an additional 2 hours. RNA was extracted from the nuclear and cytoplasmic fractions at 30 hours p.i. Saturating levels of ^{3}H RNA were used in these experiments. Unlabelled late RNA —●—, unlabelled ara C RNA —□—, unlabelled uninfected cell RNA —△—.

The size of cytoplasmic RNA was also examined when DNA synthesis was inhibited whilst it was rising to its maximum, late in infection. (Fig. 4) The cytoplasmic RNA resembles 'early' RNA in size. The nuclear RNA was again high molecular weight and heterogeneous. Competition experiments were performed to see whether indeed 'early' RNA was preferentially produced under these conditions. The results are shown in Fig. 5:

1) Unlabelled "late" cytoplasmic RNA (synthesised in the absence of cytosine arabinoside) competes fully with both labelled nuclear

and cytoplasmic RNA (synthesised in the presence of cytosine arabinoside) indicating that all sequences present in RNA made after the arrest of DNA synthesis are also present in RNA made late in the normal infection cycle.

2) Unlabelled RNA from infected cells, which have had cytosine arabinoside present throughout infection (ara C RNA) competes for 50% of the hybridisation of nuclear RNA from cells arrested in DNA synthesis The data in Fig.3 suggests that ara C RNA represents 30 to 40% of the genome. The competition plateau of ara C RNA in Fig.5 therefore defines 30 to 40% of the genome, and so complete competition in this experiment represents 60 to 80% of the genome. The experiment therefore indicates a 30 to 50% reduction in the transcription of "late" sequences.

The extent of inhibition of the hybridisation of cytoplasmic RNA made after DNA synthesis arrest using unlabelled 'ara C RNA ' as competitor is 75%. If this plateau is equivalent to 30 to40% of the genome, then complete competition in this experiment represents 40 to 50% of the genome . This is equivalent to an 80 to 85% reduction of the 'late' sequences in the cytoplasm following the arrest of DNA synthesis.

This data indicates that 'late'RNA synthesis is coupled to DNA synthesis This coupling may involve the processing of nuclear RNA as the reduction of 'late' RNA sequences in the nucleus is less than that observed in cyto--plasmic RNA. Aloni has reported that the processing of viral RNA does occur late in the infection of monkey cells with SV 40.(11)

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