SOME EVIDENCE FOR REPLICATION-TRANSCRIPTION COUPLING IN
PHYSARUM POLYCEPHALUM

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SUMMARY
Hydroxyurea, at concentrations of 40–60 mM, selectively and effectively blocked incorporation of thymidine into DNA. Inhibition occurred within 5–10 min of application of the agent when DNA synthesis was in progress, while the onset of replication at the beginning of S-phase and DNA synthesis in G₂ phase were not affected.

Uridine incorporation into TCA-precipitable material, in the presence of hydroxyurea, was significantly (up to 70%) inhibited in early S-phase of the cell cycle. Selective inhibition of RNA synthesis was confirmed for RNA separated into rRNA-rich and poly-(A)-rich RNA fractions and analysed by the 2 kinds of DNA-RNA hybridization reactions. Uridine incorporation into poly (A) RNA was also inhibited under conditions where cycloheximide prevented maturation of nascent DNA molecules in early S-phase.

We assume that chromatin which is replicating early DNA sequences may be a more competent template for transcription.

INTRODUCTION

In eucaryotic cells, doubling of the nuclear DNA content is a prerequisite for mitosis (Mitchison, 1971). Moreover, it could be argued that the sequence of DNA replication might contain a programme responsible for certain transition points of the cell cycle. Such a programme could be spelled out by the coupling of transcription of RNA with the temporal sequence of DNA replication. The synchronous cell cycle of Physarum (Rusch, 1970) lends itself to an investigation into the possibility of a coordinated replication and transcription, since there is good evidence for sequential replication during S-phase and some indication of differences in RNA during the cell cycle.

A temporal sequence of replication of Physarum DNA exists as demonstrated by density shift experiments (Braun, Mittermayer & Rusch, 1965). Ten steps, or rounds of replication, each dependent on the synthesis of a short-lived protein, have been concluded from experiments with cycloheximide (Muldoon, Evans, ...
Inhibition of DNA synthesis by cycloheximide might be due to blocking maturation of nascent DNA chains into large chromosomal DNA molecules (Brewer, 1972). Nuclear DNA from Physarum contains a significant amount of redundant base sequences and none of these seems to be replicated early in S-phase (Fouquet, Bierweiler & Sauer, 1974a). Therefore, qualitatively different DNA segments might be replicated at various points of the S-phase, starting perhaps with euchromatic portions of the chromatin.

Differences in the RNA composition during the cell cycle have previously been claimed after studying various parameters: the rate of uridine incorporation (Mittermayer, Braun & Rusch, 1964), base composition (Cummins, Weisfeld & Rusch, 1966) and nearest neighbour frequency distribution (Cummins, Rusch & Evans, 1967), DNA–RNA hybridization at high Cot values (Fouquet & Braun, 1974), the labelling pattern of poly (A) RNA (Fouquet et al. 1974a) and the residual RNA polymerase activities of isolated nuclei (Grant, 1972). Experiments with fluorodeoxyuridine (FUDR), an inhibitor of DNA synthesis affecting TMP synthesis, have led to the conclusion that in Physarum, the completion of the S-phase is an important step among the events that lead to mitosis (Sachsenmaier & Rusch, 1964). At the same time, FUDR has a direct effect on RNA synthesis, possibly after a conversion into FU, which can be counteracted by carefully adjusting the level of uridine in the growth medium (Sachsenmaier & Rusch, 1964).

In a first attempt to demonstrate a dependence of RNA synthesis on DNA synthesis, FUDR has again been used to block DNA synthesis. The results clearly indicate that in early S-phase, incorporation of deoxycytidine into DNA and RNA was significantly lower than in controls, even when enough uridine was added to overcome the direct effect of FUDR on RNA synthesis (Rao & Gontcharoff, 1969). We have begun to analyse further this possible correlation of DNA and RNA synthesis. We have used hydroxyurea and cycloheximide to inhibit DNA synthesis and have tried to determine which kinds of RNA become inhibited.

**MATERIALS AND METHODS**

*Preparation and labelling of cultures*

Mitotically synchronous macroplasmodia of Physarum polycephalum were set up as described elsewhere (Mittermayer, Braun & Rusch, 1965). Intermitotic time was 10 h. Experiments were performed between the second and third postfusion mitosis. The S-phase lasted 25–3 h and began in telophase. All times were expressed relative to metaphase, which lasted 5 min.

Labelling of macromolecules was done by adding tritiated precursors (uridine, adenosine, thymidine or leucine) with or without adding the agents to be tested (hydroxyurea, cycloheximide) to the growth medium. Incorporation of radioactive material was determined as we have previously described (Sauer, Babcock & Rusch, 1970).

*Preparation and analysis of nucleic acids*

Nucleic acids were prepared from frozen plasmodia with a phenol method suitable for Physarum (Fouquet et al. 1974a). Gel electrophoresis was performed on these preparations without further steps of purification to minimize degradation of RNA molecules and to visualize the conversion of some uridine into DNA label.

Electrophoresis of 40 μg nucleic acids was carried out on 2.2% polyacrylamide gels at
Replication-transcription coupling in Physarum

8 mA/gel for 2 h at 14 °C (Bishop, Claybrook & Spiegelman, 1967). O.D. was continuously measured over the whole gel and radioactivity was determined in hydrolysed slices of 2 mm thickness by scintillation counting. Radioactivity patterns were normalized, excluding material that remained on the gel start.

Further purification of Physarum RNA from DNA and slime was achieved by DNase treatment and chromatography on hydroxyapatite, as reported earlier (Fouquet et al. 1974a).

Chromatographic separation on Sigma Cell 38 and oligo-(dT) cellulose of RNA into fractions of peak I (rich in ribosomal RNA) and peak II (rich in poly (A) RNA) was done as previously described in detail (Fouquet et al. 1974a).

We applied 2 DNA–RNA hybridization procedures. In the first experiments the amount of DNA was limiting in the hybridization mixture. DNA–RNA hybridization was performed in a liquid system (Nygard & Hall, 1964). Conditions for the hybridization reaction were such that most of the single copy DNA would have reacted during the 2 weeks of incubation as described previously for Physarum (Fouquet & Braun, 1974). In one experiment 0.1 μg of DNA and equal amounts of radioactivity (about 25-50 μg) of RNA of peak I were added to the reaction mixture. (This RNA contained mainly ribosomal RNA and an unknown proportion of non-ribosomal RNA of 3 possible kinds: never to be polyadenylated, not yet polyadenylated or broken 5’ ends of poly (A) RNA).

In the other experiment approximately equal amounts of radioactivity of peak II RNA were present in each hybridization mixture together with 0.1 μg of denatured DNA. This RNA was prepared from 50-100 μg of total RNA and had no measurable optical density.

In the second kind of RNA–DNA hybridization reaction radioactive RNA was limiting (Melli et al. 1971). We used poly-(A)-rich RNA prepared by oligo (dT) cellulose chromatography from controls and plasmodia treated with hydroxyurea in S-phase or G2 phase. Denatured DNA was present in an approximately 10^2-10^4-fold excess and DNA was allowed to renature over a wide range of Cot values to gain an estimate of which DNA segments might have coded for the poly (A) RNA present in the reaction mixture. RNA hybrids were measured after RNase digestion. Percent hybridization was computed from control values (100 %) obtained for each point of the graph (see Fig. 4).

CHEMICALS

[3H]Uridine (26 Ci/mmol), [3H]Adenosine (0.1 Ci/mol), [3H]Thymidine (28 Ci/mmol) and [3H]Leucine (47 Ci/mmol) were purchased from Amersham Buchler. Hydroxyurea and cycloheximide were obtained from Serva and Sigma Cell 38 from Sigma. All other chemicals were of analytical grade quality.

RESULTS

Effects of hydroxyurea on DNA synthesis

When hydroxyurea (40 mM) was added to the growth medium, together with [3H]Thymidine, we noted a significant reduction of thymidine incorporation in S-phase of the cell cycle (Fig. 1A). Inhibition became effective within 10 min. When plasmodia were preincubated with hydroxyurea for 15 min before the addition of [3H]Thymidine, a more complete inhibition of thymidine incorporation was immediately observed (Fig. 1A). A mixture of deoxyribonucleosides (each at 1 mM), added together with hydroxyurea and [3H]Thymidine, resulted in no measurable inhibition of DNA synthesis (Fig. 1A).

We then studied the effect of increasing concentrations of hydroxyurea on DNA synthesis (Fig. 1B). At low concentrations (3-5 mM) of the compound we noted a significant stimulation of thymidine incorporation over that seen in controls.
Fig. 1A and B. For legend see opposite.
Inhibitions of 50 and 90% were measured at 10 and 40 mM hydroxyurea, respectively.
In the following experiments we applied this agent in appropriate concentrations (40–60 mM) to ensure blocking of DNA synthesis. At these concentrations protein synthesis, as measured by leucine incorporation (not shown), was not affected over a test period of 2 h.

In the next experiment we studied the effect of hydroxyurea on the initiation of chromosomal DNA replication, which took place in telophase of mitosis. We noted that, even after a long preincubation (up to 3.5 h in 30 or 60 mM hydroxyurea),
thymidine incorporation began as in controls (Fig. 2). However, after 10 min the incorporation had ceased almost completely.

Some DNA fractions, mitochondrial and nucleolar (approx. 10% of total DNA), are synthesized also in G2 phase. Thymidine incorporation in G2-phase into DNA was not affected by hydroxyurea (60 mM) present over a period of 2 h (from 6 to 8 h post mitosis, M II).

Effects of hydroxyurea on RNA synthesis

Hydroxyurea in sufficiently high concentrations to block DNA synthesis (40 mM) had little inhibitory effect (approx. 10%) on [3H]uridine incorporation into material precipitable in 5% trichloroacetic acid (TCA), in G2-phase (Fig. 3A). However, significant inhibition of uridine incorporation was observed when the same experiment was done with a plasmodium treated with hydroxyurea in early S-phase (Fig. 3A). These experiments were repeated at many points of the cell cycle ranging from 2 h before M II until 3 h after M III. Then the percentage of inhibition

Fig. 3. Effects of hydroxyurea on uridine incorporation.
A. Plasmodia were incubated in [3H]uridine (2 μCi/ml medium), and incorporation into TCA-precipitable material (cpm/μg protein) was measured. ○, control; △, hydroxyurea (40 mM) in G2 phase (start was at 5 h post mitosis); ●, hydroxyurea (40 mM) in S-phase (start was 25 min post metaphase).
B. At several points of the cell cycle, ranging from 2 h before M II until 3 h after M III, the influence of hydroxyurea on uridine incorporation was determined as in Fig. 3A. The percentage of inhibition was computed from the incorporation values after 60 min.
of incorporation was computed from curves like the ones seen in Fig. 3A. Controls and experimental samples for each curve were taken from one macroplasmodium.

On comparing the effect of hydroxyurea at various stages of the cell cycle (Fig. 3B) we noted some inhibition during \( G_2 \) phase (approx. 10%), a high degree of inhibition in early \( S \)-phase (maximally 70%) and a significant inhibition in the middle part of \( S \)-phase (about 20–30%).

Next we studied the effects of hydroxyurea on nucleic acids extracted from plasmodial homogenates. Plasmodia were incubated for 30–120 min with \(^{3}H\)uridine in the presence of hydroxyurea (40 mM). Inhibition of incorporation ranged from 5–20% for \( G_2 \) phase and late \( S \)-phase plasmodia and amounted to 55% in early \( S \)-phase plasmodia. Therefore, the degree of inhibition measured in homogenates (TCA-precipitable material) and in extracted nucleic acids was quite similar.

Total nucleic acids, labelled for 30 min with \(^{3}H\)uridine, were analysed on 2:2% polyacrylamide gels. In all samples we detected low-molecular-weight material (4 s RNA), 19 s and 26 s ribosomal RNA and some material near the gel start (at about 1 cm) in the optical density profile. No measurable differences in these profiles from plasmodia prepared in \( S \)-phase or \( G_2 \) phase, treated or untreated with hydroxyurea, were observed.

There were some differences in the radioactivity patterns, aside from the general decrease in labelling of samples taken from plasmodia treated with hydroxyurea, and a fraction (approx. 20%) of radioactive material which had not entered into the gel.

We detected a distinct radioactive peak corresponding to the O.D. peak close to the gel start in untreated \( S \)-phase plasmodia. This radioactive material was shown to be DNA, since it was stable in 1 M NaOH, hydrolysed by 0.5 M perchloric acid (PCA) at 70 °C, insensitive to RNase but sensitive (over 90%) to DNase. Therefore, in \( S \)-phase some radioactivity after labelling with \(^{3}H\)uridine (approx. 10–15%) was found in DNA.

In addition, minor differences were observed in normalized radioactivity patterns obtained for early \( S \)-phase and \( G_2 \) phase. Half of each plasmodium served as control while the other half had been treated with hydroxyurea. After 30 min of labelling, no mature ribosomal RNA peaks were seen in the radioactivity patterns and 14.5 or 9.1% of the radioactivity was localized in the 4–30 s region (the presumptive mRNA region) of the gels from \( S \)-phase or \( G_2 \)-phase samples, respectively. From plasmodia treated with hydroxyurea we obtained a value of 8.8% for blocked early \( S \)-phase and 9% for \( G_2 \)-phase samples.

In the following experiments nucleic acids were treated with DNase. RNA was further purified by adsorption to and elution from hydroxyapatite. RNA was then separated into a fraction not absorbed at high ionic strength on a cellulose column (peak I RNA) and another fraction which eluted at low ionic strength (peak II RNA).

In Expt. 1 (Table 1) we compared the effect of hydroxyurea on uridine incorporation over 60 min into RNA of peaks I and II in the beginning of \( S \)-phase (5–65 min after mitosis, \( M \) II) with that seen in \( G_2 \) phase (5 h after mitosis; \( S \)-phase lasted 2.5–3 h). In \( G_2 \) phase we detected no inhibition of RNA synthesis in peak I or II.
However, significant inhibition was noted in early $S$-phase, 45% of peak I RNA and 51% of peak II RNA. In the next experiment (Table 1, Expt. 2) plasmodia were preincubated for 30 min with hydroxyurea and then labelled, together with controls, for 40 min with $[^3H]$uridine in early $S$-phase (15-55 min post mitosis, $M_{II}$). The same incubation procedure was repeated in $G_2$ phase (beginning 5 h after mitosis, $M_{II}$). RNA was separated in peaks I and II and aliquots of these RNA fractions were precipitated with TCA. We obtained 22% inhibition of peak I RNA in $S$-phase (Table 1) and no inhibition in $G_2$ phase. Peak II RNA was inhibited by 74% in $S$-phase and 21% in $G_2$ phase.

Table 1. Effects of hydroxyurea on purified RNA fractionated by cellulose chromatography

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Cell cycle phase</th>
<th>RNA synthesized (cpm/100 μg of total RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hydroxy-urea</td>
</tr>
<tr>
<td>1</td>
<td>$S$</td>
<td>76762</td>
</tr>
<tr>
<td>2</td>
<td>$S$</td>
<td>154360</td>
</tr>
<tr>
<td>3</td>
<td>$S$</td>
<td>207741</td>
</tr>
<tr>
<td></td>
<td>$G_2$</td>
<td>732743</td>
</tr>
<tr>
<td>1</td>
<td>$G_2$</td>
<td>167201</td>
</tr>
<tr>
<td>2</td>
<td>$G_2$</td>
<td>244539</td>
</tr>
</tbody>
</table>

For hybridization experiments, equal amounts of radioactivity (approx. 110,000 cpm for peak I RNA and 15,000 cpm for peak II RNA from each sample in $S$-phase and in $G_2$ phase treated or untreated with hydroxyurea) were incubated with denatured DNA. For both classes of RNA, hybridization values were higher (30%) in the control samples from $S$-phase (Table 1). RNA from plasmodia treated with hydroxyurea in $G_2$ phase hybridized somewhat less than controls (8 against 18%). Significantly less hybridization had occurred with RNA prepared from $S$-phase plasmodia in which DNA synthesis was blocked by hydroxyurea, by 30% for peak I RNA and 63% for peak II RNA (Table 1). In other experiments (not shown) plasmodia were labelled with $[^3H]$uridine in the second or third hour of the $S$-phase in the presence or absence of hydroxyurea. Inhibition of both classes of RNA ranged from 17 to 28% in plasmodia treated with hydroxyurea.

In some cases plasmodia were labelled with $[^3H]$adenosine. From one representative experiment (Table 1, Expt. 3), after 60 min of labelling with $[^3H]$adenosine, we observed a strong inhibition of peak II RNA, while peak I RNA was almost not affected. This result was obtained in early $S$-phase. In $G_2$ phase measurable inhibition
Replication-transcription coupling in Physarum

of peak II RNA was observed but no inhibition of peak I RNA. The higher values of peak I RNA from \( G_2 \)-phase plasmodia treated with hydroxyurea were not considered as evidence for a stimulatory effect of hydroxyurea.

For the following hybridization experiments poly (A) rich RNA (peak II RNA) was prepared by affinity chromatography from 4 macroplasmodia. Two plasmodia served as \( S \)-phase and \( G_2 \)-phase controls. The other 2 plasmodia were treated with hydroxyurea (60 mM). In the DNA-driven hybridization reaction we did not detect major differences in the kinetics of DNA-RNA hybridization when the poly (A) RNA was prepared in \( S \)-phase or \( G_2 \) phase or from a \( G_2 \)-phase plasmodium treated with hydroxyurea (Fig. 4). Only a small portion (10-20\%) of poly (A) RNA had reacted at a Cot value of 15. A substantial part of this RNA (50-60\%) had hybridized at a Cot value of 2000. Reannealing of single-copy DNA segments occurred under these conditions as was previously shown (Fouquet et al. 1974).

Poly (A) RNA from a plasmodium blocked in early \( S \)-phase by hydroxyurea behaved differently. When compared with the samples prepared from \( S \)-phase controls or \( G_2 \)-phase plasmodia (Fig. 4), this RNA had hybridized to a significant degree (40\%) already at a low Cot value of 1 and had reacted almost to completion at a Cot value of 70.

Effects of cycloheximide on RNA synthesis

Plasmodia were incubated with cycloheximide (5 \( \mu g/ml \) growth medium) for 20 or 60 min in the presence of \(^3\)H\)uridine (Table 2). Purified RNA was fractionated

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Fig. 4. Hybridization of poly (A) rich RNA with DNA in excess. Poly (A) RNA was prepared by affinity chromatography on oligo (dT) cellulose from control plasmodia in \( S \)-phase (●), in \( G_2 \) phase (○) and from plasmodia treated with hydroxyurea for 1 h in early \( S \)-phase (□) (10 min after \( M \) II) and \( G_2 \) phase (5 h post \( M \) II) (△). Plasmodia had been labelled for 50 min with 100 \( \mu Ci/ml \) of \(^3\)H\)uridine and 2·5·5 \( \times 10^4 \) cpm of RNA were incubated with 1 mg/ml of DNA. Cot values were computed from the DNA concentration and incubation time.
into peaks I and II by cellulose chromatography. We detected no significant differences in the RNA fractions analysed in $G_\text{s}$ phase (maximum inhibition was 11\%). However, inhibition of [\textsuperscript{3H}]uridine incorporation into both RNA fractions, especially into peak II RNA, was seen when the experiment was done in early $S$-phase. The extent of inhibition of labelling of both RNA species was similar to that seen with hydroxyurea.

**Table 2. Effect of cycloheximide on RNA synthesis in Physarum**

<table>
<thead>
<tr>
<th>Cell cycle phase</th>
<th>Incubation with [\textsuperscript{3H}]uridine, min</th>
<th>$%$ inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peak I RNA</td>
</tr>
<tr>
<td>$S$</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>$S$</td>
<td>60</td>
<td>32</td>
</tr>
<tr>
<td>$G_\text{s}$</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>$G_\text{s}$</td>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Hydroxyurea (above 40 mM) was shown to block almost completely DNA synthesis in *Physarum*. Our results confirmed an earlier report of a stimulation of thymidine incorporation by low concentrations of hydroxyurea, and inhibition at a higher concentration and its reversal by deoxynucleosides (Evans, Menz & Nygaard, 1968). Therefore, in *Physarum*, hydroxyurea might inactivate ribonucleotide reductases as in other systems and consequently limit the supply of DNA precursors (Adams & Lindsay, 1967; Steinert, 1969). From an analysis of pool sizes in plasmodia from *Physarum*, a DNA synthesis time ranging from 1.5 min (for dATP) to 13.8 min (for dTTP) had been computed (Bersier & Braun, 1974a, b). This calculation fitted the time span observed for a significant inhibition of thymidine incorporation (5–10 min) and was consistent with the fact that initiation of replication (at telophase of mitosis) was not prevented even after long preincubation with hydroxyurea.

In early $S$-phase of the cell cycle of *Physarum*, uridine incorporation into TCA-precipitable material was significantly reduced in the presence of hydroxyurea. This finding confirmed earlier results obtained with FUDR, another indirect inhibitor of DNA synthesis (Rao & Gontcharoff, 1969). A portion of the inhibition measured in this way (approx. 15\%) was an artifact due to labelling of DNA with uridine, possibly after conversion into cytosine (Evans, cited by Schiebel, 1973). A small fraction of low-molecular-weight RNA might also be directly involved in replication and thus also contribute to the differences in labelled RNA seen after a blockade of replication (Waquar & Huberman, 1973).

On comparing the effect of hydroxyurea on early $S$-phase plasmodia with that seen in later periods of $S$-phase and $G_\text{s}$ phase, we noted a significant reduction of 2 labelled RNA classes (peak I RNA, rich in ribosomal RNA, and peak II RNA, rich in poly (A) RNA), and a possible decrease of radioactive RNA fractions in the
Replication-transcription coupling in Physarum

mRNA region of gels. A similar degree of inhibition of the 2 RNA classes was found after a blocking of DNA replication with cycloheximide.

According to DNA-RNA hybridization experiments, both RNA classes extracted from plasmodia which had been labelled in early S-phase in the presence of hydroxyurea, hybridized to a smaller extent than controls. These results might indicate qualitative changes in the RNA composition, although the specific activities of the heterogeneous RNA preparations, which would allow an estimate of missing RNA species, were not known. The DNA-driven hybridization reaction with poly (A) RNA clearly indicated distinct differences when this RNA component was prepared from plasmodia treated with hydroxyurea in early S-phase. This RNA, contrary to controls, lacked practically all the material reacting with single copy DNA and contained mainly material which reacted with DNA of moderately redundant base sequences (Fouquet et al. 1974b).

The changes in RNA composition, both quantitative and qualitative and especially for peak II RNA (containing mRNA), observed in correlation with blockage of DNA synthesis, might constitute evidence for replication-transcription coupling.

Since replicating DNA of Physarum contained many unjoined pieces in S-phase, RNA polymerase B might preferentially initiate transcription at such open DNA structures. As a consequence, sequential replication would determine sequential transcription in S-phase. This possibility would then support a linear reading model of transcription (Tauro, Halvorson & Epstein, 1968) and might help to explain the temporal sequence of such events which are of significance in the DNA division cycle (Mitchison, 1971).

However, up to now there was no evidence for the newly replicating DNA being the template for immediate transcription. Preliminary DNA–RNA hybridization experiments with density-labelled early DNA at low Cot values were negative (Zellweger & Braun, 1971). Ribosomal DNA which was postulated to be among the early DNA segments to be replicated, according to a recent experiment with FUDR (Gontcharoff & Rao, 1972), was clearly shown to replicate at any time of the cell cycle but the early S-phase (Guttes, 1974).

Therefore, coupling of RNA synthesis with replication might also be explained by a change in chromatin structure in early S-phase, rendering some DNA segments more competent for transcription.

Since poly (A) RNA is more severely inhibited, it could be argued that the presumed replication-dependent inhibition might also be explained by a direct effect of hydroxyurea on poly (A) RNA synthesis. Then the smaller effect of hydroxyurea in G2-phase plasmodia might be explained by an assumption that, in G2 phase, there was generally less RNA polymerase B activity in plasmodia. Therefore, hydroxyurea might specifically inhibit RNA polymerase B or interfere somehow with polyadenylation.

Isolated RNA polymerases A and B from Physarum (Hildebrandt & Sauer, 1973) when assayed in vitro were almost completely inactive in the presence of 5 mM hydroxyurea. Enzyme A, however, seemed to be more sensitive than enzyme B (unpublished experiments from our laboratory). On the other hand, the inhibitory
effect of hydroxyurea on poly (A) RNA synthesis in vivo was also small in late S-phase (only 17–28%) when, according to the experiments with isolated nuclei (Grant, 1972), RNA polymerase B was shown to be at peak activity.

A direct effect of hydroxyurea on polyadenylation, responsible for some inhibition of the formation of poly (A) RNA, however, could be concluded from the relatively higher inhibition of peak II RNA in both S-phase and G2 phase, labelled with [3H]adenosine instead of [3H]uridine.

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Replication-transcription coupling in Physarum


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