Template Ability of Activated DNA From the Regenerating Lens

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SUMMARY

The template ability of DNA changes during the cell cycle. Prior to the initiation of DNA synthesis in the regenerating lens of Triturus, DNA is converted during the latter portion of the G_1 phase into a form which serves as an excellent template for heterologous DNA polymerase. This activated DNA is a much better template than DNA isolated from the G_o phase, or from the early portion of the G_1 phase.

INTRODUCTION

When the lens is removed from the eye of the newt, Triturus, a new lens will form cells of the dorsal iris [for review, see Yamada (1)]. After this transformation is initiated, profound morphological and biochemical changes occur. Among the earliest changes are: entry into the cell cycle (2), an enhancement of RNA synthesis (3), an enhancement of protein synthesis (4) and amplification of the ribosomal RNA (rRNA) cistrons (5). All of these changes occur before the first S phase. Significant events which occur later are: initiation of DNA synthesis (6,7), depigmentation of the iris cell (1) and the acquisition of lens-specific antigens (8). The earliest structural events which have been detected are an enlargement of the nucleoli and the appearance of a granular component in the nucleoli shortly after day 1 of regeneration (9).

At day 0 of regeneration, the cells of the dorsal iris are in a stationary G_o state (1). After lentectomy, they are stimulated to enter the cell cycle and by day 2-3 all are in early G_1. By day 4-5 most of the cells are in late G_1. The S or DNA-synthetic phase starts at about day 5 of lens regeneration.

After the first S phase, the relative synchrony of the cells breaks down and one finds a mixed population of cells with respect to the cell cycle. As these cells are relatively synchronized, one might be able to isolate DNA from irises in early stages of lens regeneration, and obtain essentially "G_0DNA", "G_1DNA" and "S-phase DNA". The availability of highly purified DNA from G_0 and G_1 phases of the cell cycle (10) prompted us to ask the question, "Does the template ability of DNA change before the onset of S phase?" The means whereby DNA might be converted from a chromosomal state to a pre-replicative, or activated state, is of considerable current interest. This communication represents an attempt to use a heterologous DNA-polymerizing system as a probe for the identification of biologically (i.e., in vivo) activated DNA.

MATERIALS AND METHODS

Adult newts, Triturus (Notophthalmus, Diemictylus) viridescens viridescens) were obtained from Mr. Glen Gentry of Donelson, Tennessee in November and maintained in aquaria for at least one week before use. Feeding of the newts, and the surgical procedure for lentectomy were as previously described (5).

[^{3}H]thymidine (49.2 Ci/mmole), generally labeled, was purchased from New England Nuclear, as was [^{3}H]thymidine-5'-triphosphate (50.8 Ci/mmole), methyl labeled. Electrophoretically pure pancreatic DNase and a calf thymus DNA were obtained from Worthington Biochemical Corp.

DNA was isolated from homogenized tissues essentially according to Marmur (11), as before (10). DNase-activated DNA was obtained by treating calf thymus DNA (500 μg in 5 ml) with 0.5 μg of DNase for 30 sec. This DNA was then purified by our usual procedure (10).

Nuclei were isolated from the livers of 200g Sprague-Dawley rats, 22 hr. after partial hepatectomy, by the method of Bloebel and Potter (12). DNA polymerase was isolated exactly as described by Mantsavinos and Munson (13). Our preparation corresponds to their "Fraction 2". The assay for DNA polymerase activity was that of Mantsavinos (14). Components of the assay were: [^{3}H]TTP (5 μCi) plus dTTP to

to yield a total of 15 nmoles: dATP, dGTP and dCTP, 15 nmoles each ; $MgCl_2$, 8 μmoles, mercaptoethanol, 0.5 μmoles; glycine buffer, 10 μmoles, pH 8.0; enzyme preparation, 0.1 mg; and DNA, 50 μg. The mixtures were incubated for 1 hr at 37°. Under these conditions, none of the components of the reaction are limiting (ref. 14 and J. M. Collins, unpublished results). The reaction mixtures were then precepitated according to Mantsavinos (14) and collected on Whatman No. 3 MM discs. Radioactivity was determined as previously described (10).

RESULTS

Initiation of DNA synthesis: During November dorsal irises were removed at various times after lentectomy 0 -days, 3-days, 5-days and 5 1/2 days) and incubated for 15 min in the presence of 0.4 mCi of [^{3}H]thymidine (5). DNA was isolated and purified. Specific activities (cpm/μg) obtained were: 0-day, 3.1; 3-day, 4.2; 5-day, 7.1; 5 1/2-day, 410; and 6-day, 480. Thus, DNA synthesis was initiated at about day 5 1/2. Reyer (7), and Yamada and Roesel (6), utilizing long term pulses with [^{3}H]thymidine followed by autoradiography reported that the initiation of synthesis (that is, the start of the S-phase) occurs at about day 4 of lens regeneration. The difference between our findings and theirs is most likely due to seasonal variation rather than to difference in technique, for when we tested newts during the summer (July) we found that the initiation of DNA synthesis started at about day 4 1/2.

Template ability of DNA from different stages of lens regeneration: Non-radioactive DNA was isolated from the dorsal irises of newts various times after lentectomy. The ability of these DNA preparations to serve as a template for the nuclear DNA polymerase of rat liver was examined. The results are presented in Table 1. As can be seen from experiment No. 1, the preparation of DNA polymerase exhibits good activity when native calf thymus DNA is used as a substrate. This level of activity corresponds to a value of 0.65 nmoles of dTTP incorporated per hr. which agrees surprisingly well with that reported by Mantsavinos and Munson for calf thymus DNA and this particular type of polymerase preparation (13).

Table 1. Template Ability of DNA From Various Stages of Lens Regeneration

DNA Source	Activity (cpm)
1. Calf Thymus	105,000
2. 0-day (G_0)	90,000
3. 3-day (early G_1)	100,000
4. 5-day (late G_1)	280,000
5. DNase-activated Calf Thymus	132,000
6. Minus DNA	1,400
7. 5-day[a]	1,200
8. 5-day: minus enzyme	150
9. 5-day[b]	900

DNA polymerase (fraction 2 of Mantsavinos and Munson (13)) was purified from rat liver nuclei. Components of the Assay were as described in Methods. Each value represents the average of 3 determinations.

a DNase added 2 hr before enzyme

b DNase added 1 hr after enzyme, incubated 2 hr.

This preparation of polymerase contains traces of nuclease activity. Under the conditions of the assay, 0.1 mg of polymerase released 20 c.p.m. from 50 μg of DNA released. Slight traces of nuclease activity in this type of preparation from rat liver nuclei were reported by Mantsavinos and Munson (13). In spite of the nuclease contamination, the experimental design should still be valid. That is, the polymerase preparation should be able to detect differences in the template ability of DNA isolated from various phases of the cell cycle, if such differences exist.

DNA isolated from G_0 phase (experiment No. 2) is a slightly poorer template than DNA from early G_1 (experiment No. 3), but this result may not be significant.

However, we have consistently found that early G_1 DNA contains a few nicks, or breaks in the chains, which are virtually absent from G_0 DNA (10). A significant increase in template ability was exhibited by DNA from late G_1 (experiment No. 4). This DNA is 3 times a better template than the other DNAs tested. It is perhaps significant that this DNA was isolated just prior to the onset of DNA synthesis (text).

Several controls have been included in Table I. A brief exposure of calf thymus DNA to DNase resulted in a 25% increase in template ability (experiment No. 5).

When DNA was omitted from the mixture (experiment No. 6), a low level of radioactivity was obtained. This is most likely due to slight traces of DNA in the polymerase preparation. In experiment No. 7, the DNA-containing mixture was treated with 5 μg of DNase for 2 hr at 37°, then the mixture was heated to 65° for 30 min to destroy nuclease activity, before the polymerase was added. As can be seen, about the same level of radioactivity was obtained as in experiment No. 6 (without polymerase). Again the radioactivity obtained is probably due to traces of DNA in the polymerase preparation. When the polymerase was omitted, only background levels of radioactivity were obtained (experiment No. 8). Finally, the addition of 20 μg of DNase, followed by incubation for 2 hr at 37°, resulted in the degradation of the product (experiment No. 9).

On another occasion, DNA was again isolated from 0-day, 3-day and 5-day regenerates and tested for template activity. The results (not shown) were the same as those in Table 1.

DISCUSSION

In the normal adult newt, the cells of the dorsal iris are in a G_0 state, out of the cell cycle (1,6,7). After initiation of lens regeneration by lentectomy the cells are stimulated to enter the cell cycle, and by day 3-4 all are in G_1 (1,6). Our results indicate that with the group of newts employed for this study the initiation of DNA synthesis started at day 5 1/2 of lens regeneration (text). Thus, the DNA from 5-day regenerates represents essentially "late G_1 DNA". This DNA, isolated just prior to the onset of S phase had the greatest template ability. This correlation is of considerable significance.

Preliminary characterization of the DNA preparations used in these experiments has revealed that the preparations of 0-day DNA (G_0), 3-day DNA (early G_1) and 5-day (late G_1) all had approximately the same molecular weight, with an S value of about 26 (data not shown). Elsewhere we report that the G_0 DNA contains virtually no nicks, or breaks, in the chains (10). In contrast, DNA from early G_1 contains a small number of nicks (10) DNA from late G_1 contains gaps, or runs of missing nucleotides, not found in the other DNA preparations (10). It thus appears that a DNA molecule that contains gaps is a more suitable template than one that contains only nicks. These results lead us to suggest that prior to the initiation of DNA synthesis, DNA is converted *in vivo* to an "activated DNA" (or "primer DNA") containing gaps, and, as a result, can now serve as a suitable template for DNA replication. Further characterization of this "*in vivo* primer DNA", as well as of the products formed in the polymerase reaction should furnish valuable clues as to the *in vivo* mechanisms of DNA replication in animals.

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