SELECTIVE INHIBITION OF GENETIC TRANSCRIPTION IN SEA URCHIN EMBRYOS

Incorporation of 5-Bromodeoxyuridine into Low Molecular Weight Nuclear DNA

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ABSTRACT

Experimental evidence suggests that exposure of sea urchin gastrulae to 5-bromodeoxyuridine (BUdR), an analog of thymidine, causes a reduction in the rate of synthesis of some RNA species usually transcribed at this stage of development. In pulse-chase experiments, \(^3\)H (in gastrula stage 8–15S nuclear DNA labeled with BUdR-\(^3\)H) could not be chased, with unlabeled BUdR, into 20–60S size-range DNA; in similar experiments in which gastrulae were pulsed with thymidine-\(^3\)H and then chased with unlabeled thymidine, \(^3\)H in 8–15S nuclear DNA could be extensively chased into 20–60S DNA. DNA-RNA hybridization assays indicate that gastrula stage nuclear DNA in the range of 20–60S has greater sequence homology for gastrula stage RNA-\(^3\)H than does nuclear DNA of similar size-range taken from gastrulae exposed to BUdR for 1 hr. An explanation is offered for the effect of BUdR on transcription and DNA replication in sea urchin embryos.

INTRODUCTION

It has been shown that 5-bromodeoxyuridine (BUdR), an analog of thymidine, inhibits differentiation of specific cell types and prevents normal development in embryos. Inhibition of differentiation by BUdR has been observed in chondrocytes (1), myoblasts (2), mammary epithelial cells (3), pancreas cells (4), and other specialized cells. The analog has been shown to reduce the rate of synthesis of specific proteins as well as inhibit specific functions in organisms. In rat hepatoma cells grown in culture, BUdR has been shown to reduce the rate of synthesis of tyrosine aminotransferase (an enzyme inducible by glucocorticoids) while having little effect on the rate of mitosis or on total RNA synthesis (5). BUdR inhibits the formation of casein and \(\alpha\)-lactalbumin (both induced by prolactin) in mammary epithelial cells (3). Exposure of sea urchin embryos to BUdR before the eight-cell stage results in abnormal blastulation and arrest before gastrulation as well as inhibition of echinochrome pigment production (6). Introduction of the analog at later times in cleavage stage development results in fewer observable abnormalities during blastulation and gastrulation (6).

After the onset of gastrulation in normal embryos, it appears that much of the protein being synthesized may result from immediate translation of newly made messenger RNA (7). Since the gastrula stage is also a time when morphological organization is considerable, it would seem to be a stage of development in which cell differentiation may depend to a high degree upon transcription-
level gene regulation (8). In the work to be described, we have examined the effect of BrdU on transcription and also on the size-pattern of new nuclear DNA at the gastrula stage of developing Strongylocentrotus purpuratus.

**MATERIALS AND METHODS**

**Gastrula Stage Embryos**

Washed *S. purpuratus* eggs were fertilized (9) (to \(>95\%) of the eggs) and allowed to develop (approximately \(3 \times 10^6\) embryos/ml) with gentle rotatory agitation at \(16^\circ\)C in artificial seawater (10). Penicillin G to 100 \(\mu\)g/ml and streptomycin sulfate to 50 \(\mu\)g/ml were added 10 min after fertilization.

**Isolation of Nuclei**

Nuclei were isolated from embryos by the method of Hinegardner (11) as modified by Wilt (12). Isolated nuclei were checked for cytoplasmic contamination by phase-contrast microscopy.

**Sedimentation Analysis of Nuclear DNA**

Isolated nuclei were suspended (approximately \(10^9\)/ml) in 0.15 \(M\) NaCl, 0.015 \(M\) Na citrate, 0.05 \(M\) ethylenediaminetetraacetate (EDTA), 0.05 \(M\) Tris, pH 8.4. Pronase (Calbiochem, San Diego, Calif.) was added to 1.0 \(mg/ml\) and Na lauryl sulfate to 0.2% (13) and the suspension of nuclei was incubated at \(37^\circ\)C for 16 hr. Pancreatic RNase (Worthington, Biochemical Corp., Freehold, N.J., stock solution at 2 \(mg/ml\) was added to 20 \(\mu\)g/ml, and \(\alpha\)-amylase (Worthington Biochemical Corp., 650 units/mg) was added to 50 \(\mu\)g/ml, and the nuclear DNA solution was incubated an additional hour at \(37^\circ\)C. Nuclear DNA purified by this enzymatic treatment shows sedimentation profiles on neutral sucrose gradients comparable to sedimentation profiles obtained on alkaline gradients. Phenol extraction of the enzyme-treated DNA caused a reduction in molecular weight of higher molecular weight DNA species. Therefore, sedimentation analysis and collection of size-classes of DNA were obtained by layering the enzyme-treated DNA solution directly, without further treatment, onto 5-20% (w/v) alkaline sucrose gradients made up in 0.1 \(M\) NaOH, 0.9 \(M\) NaCl, 0.001 \(M\) EDTA. Gradients were centrifuged at \(18^\circ\)C in a Spinco SW 25.1 rotor for 10.5 hr at 24,000 g. Calibration of sedimentation constant with position on a gradient was obtained by sedimentation of marker DNAs on parallel gradients. Markers used were \(\phi 80\) phage DNA (35S) \(m, w\) [14, 15] and PKC phase DNA (56.18S) \(m, w\) [16]. Radioactive fractions were precipitated along with unlabeled carrier RNA onto type HA Millipore filters by neutralization and subsequent addition of 10% trichloroacetic acid. Radioactivity was measured by liquid scintillation spectrometry.

**Preparation of Sperm DNA**

Male sea urchins were injected with 0.53 \(m\) KCl and sperm were collected directly into cold buffer (0.15 \(m\) NaCl, 0.005 \(m\) EDTA, 0.05 \(m\) Tris, pH 8.4). After three phenol extractions the sperm DNA was exhaustively dialyzed against SSC solution (SSC is 0.15 \(m\) NaCl, 0.015 \(m\) Na citrate).

**Preparation of Gastrula Stage RNA \(^{3}H\)**

Embryos were labeled and treated as described in the relevant figure legends and then were immediately washed twice with cold Ca\(^{++}\)-Mg\(^{++}\)-free seawater (17) and homogenized in a 2 vol quantity of 0.1 \(m\) sodium acetate, 4 \(\mu\)g polynucleotide sulfate/ml, pH 5.2. Sodium lauryl sulfate to 0.5% and DNase (Worthington, Biochemical Corp., electrophoretically purified) to 10 \(\mu\)g/ml were added to the homogenate. After 2 min in the cold, the preparation was extracted with phenol, the phenolated preparation then being centrifuged at 25,000 g for 10 min. The aqueous layer was set aside and the interface was extracted once with hot phenol (55°C). The aqueous layer of both the hot and cold phenol extractions were combined, 0.1 vol of 20% sodium acetate added, and the RNA was twice precipitated with a 2 vol quantity of -20°C ethanol. The RNA was resuspended in 0.1 \(m\) sodium acetate, 0.001 \(m\) MgCl\(_2\), pH 5.2, DNase was added to 10 \(\mu\)g/ml, and incubation was carried out at room temperature for 30 min. Pronase (Calbiochem, preincubated for 2 hr at 37°C in 0.1 \(m\) sodium acetate, pH 5.2) was added to 50 \(\mu\)g/ml and the RNA solution was held at room temperature for 1 hr. After being extracted twice with phenol, 0.1 vol of 20% sodium acetate was added and the preparation was alcohol-precipitated three times with 2-vo1 quantities of -20°C ethanol. The RNA was finally resuspended in 6 \(\times\) SSC at the appropriate concentration.

**Preparation of Unlabeled Egg RNA**

The same method was used in obtaining this RNA as was used in obtaining gastrula RNA, except that eggs were sonicated directly after the CA++-Mg++-free seawater wash and resuspension in the pH 5.2 buffer.

**DNA-RNA Hybridization**

Nitrocellulose filters (Millipore type HA, 25 mm) were presoaked for at least 12 hr in 6 \(\times\) SSC solution and washed before immobilization (18) of heat-denatured DNA. Hybridization reactions were carried out in 6 \(\times\) SSC at 65°C for 16 hr. A blank nitro-
cellulose filter, treated in the same way as the DNA filter, was included in each reaction vial. At the end of the annealing period, DNA and blank filters were placed in separate scintillation vials and washed three times (10 ml 6X SSC added, vortex 15 sec, aspirate). 2 ml of 6X SSC containing 10 µg/ml pancreatic RNase (Worthington Biochemical Corp., treated as previously described) was added to each vial with filter. After incubation for 1 hr at room temperature, the filters were washed three additional times and then heat dried, and the radioactivity was counted in a toluene-based scintillation fluid. The amount of radioactivity adhering to the blank filters was subtracted from the amount of radioactivity on the DNA filters.

RESULTS

In preliminary experiments it was found that the addition of BUdR to a culture of developing sea urchin embryos at gastrula stage caused a reduction in the rate of incorporation of uridine-3H into new RNA. The time course of this inhibition is shown in Fig. 1. (Control experiments have shown that 96-97% of the 3H incorporated into the nucleic acids of gastrulae during a 20 min exposure to uridine-3H is incorporated into alkali-labile (19) molecules. Thus, DNA is apparently not becoming labeled to a significant degree during the time period shown in Fig. 1.) In this experiment, uridine-3H was added to a culture of embryos at zero time. The culture was then divided into two parts and BUdR was added (to 50 µg/ml) to one of these parts at 5 min after zero time. Approximately 5 min after the addition of BUdR, the incorporation of 3H into RNA decreased compared with the control part of the culture. This inhibition by BUdR, of incorporation of uridine-3H, may reflect an actual decrease in the gross rate of transcription or, alternatively, BUdR may interfere with the utilization of uridine-3H in labeling newly made RNA.

In any case, our interest in BUdR as an inhibitor of uridine-3H incorporation was in determining whether this thymidine analog can cause a prompt change in the pattern of RNA synthesized (i.e., a qualitative change in the set of RNA species labeled with uridine-3H) in embryos. An experiment was designed to test for such an effect of BUdR on transcription. A culture of 60 hr gastrula stage embryos was split into two equal parts. One culture part was exposed to BUdR (50 µg/ml) for 20 min while the other part (control culture) did not receive the drug. The specific activity of the RNA from the BUdR-treated embryos was 15.7 cpm/µg and from the control culture 120 cpm/µg. Each DNA filter had 200 µg of sperm DNA.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Inhibition by BUdR of incorporation of uridine-3H into RNA in gastrula stage embryos. Uridine-3H was added (6.7 Ci/m mole; 2 µCi/ml) at zero time to a culture of embryos (approximately 10⁶/ml). The culture was immediately split into two parts. BUdR to 50 µg/ml was added to one part of the culture at 5 min. Aliquot portions (0.5 ml) were taken from both parts of the culture at time intervals. These aliquots were immediately mixed with several volumes of 10% trichloroacetic acid, subsequently plated onto type HA Millipore filters, and washed with additional 10% trichloroacetic acid. The filters were then heat dried and counted. •—•, BUdR; ○—○, control.

![Figure 2](https://example.com/figure2.png)

**Figure 2** DNA-RNA hybridization of increasing quantities of gastrula stage whole-cell RNA-3H. The RNA-3H was synthesized in vivo in the presence or absence of BUdR: One-half of a culture of embryos was exposed to BUdR (50 µg/ml) for 20 min; the other half of the culture did not receive the drug. During the last 13 min of the 20 min period, both halves of the culture were labeled with uridine-3H (29.2 Ci/m mole, 2.4 µCi/ml). At the end of the labeling period both halves of the culture were chilled, NaN₃ was added to 0.02 M, and the RNA was immediately extracted. The specific activity of the RNA from the BUdR-treated embryos was 15.7 cpm/µg and from the control culture 120 cpm/µg. Each DNA filter had 200 µg of sperm DNA.
FIGURE 3 Comparison by DNA-RNA competition hybridization of gastrula stage whole-cell RNAs-3H which had been synthesized in vivo in the presence or absence of BUdR. The two stock RNAs-3H used here (i.e., isolated from BUdR-treated gastrulae or from control gastrulae) are those described in the legend of Fig. 2. For each point on each competition curve, a nitrocellulose filter with 200 µg of immobilized denatured sperm DNA and a blank filter were reacted (16 hr, 65°C in 6 X SSC) with 500 µg of RNA-3H and a quantity of whole-cell unlabeled egg RNA as shown on the abscissa of the graph. The occurrence was labeled with uridine-3H during the last 13 min of development. Whole-cell RNA was extracted separately from the control culture and from the BUdR-treated culture. The ability of increasing amounts of these two labeled RNAs to hybridize to a particular quantity of denatured sperm DNA is shown in Fig. 2. The species composition of the RNA-3H from the control culture was compared with that from the RNA-3H from the BUdR-treated culture by testing separately the ability of these two types of RNA-3H to compete with increasing amounts of unlabeled egg RNA for hybridization sites on denatured sperm DNA. The resulting competition curves are shown in Fig. 3. Both the BUdR and control gastrula-RNAs-3H have produced curves which plateau. The plateau level for RNA-3H from the BUdR-treated embryos is approximately 15% below the plateau level for the control RNA-3H. This difference in plateau levels should not be taken as a quantitative measure of the difference in transcription program in the control and BUdR-treated embryos. The competition hybridization assay system as used here would compare differences in RNA species homologous only to reiterated DNA sequences (20). Also, the amount of RNA-3H per quantity of DNA is subsaturating in both competition experiments shown (compare the RNA-3H:DNA ratios used in this experiment with the results shown in Fig. 2). These conditions may decrease the sensitivity of the hybridization system in showing species differences in the two types of RNA-3H.

The occurrence of the plateaus and the relatively low percentage of hybridized gastrula-RNA-3H which can be competed under these conditions by earlier stage RNA is consistent with the previously observed extensive changes in transcription program with the occurrence of gastrulation (21–23). The implication of the result here is that the species composition of the RNA-3H from BUdR-treated embryos is reduced in certain hybridizable RNA-3H species which are present in the control RNA-3H. The possibility occurs then that BUdR inhibits within a few minutes the transcription of a portion of the species of RNA usually made by gastrula stage embryos.

An effort was made to determine whether incorporation of BUdR into DNA might act to promptly inhibit transcription of a portion of the species of RNA usually synthesized in gastrula stage embryos. We sought to test the idea that nuclear DNA which incorporates BUdR during its synthesis might also be DNA whose transcription is inhibited when BUdR is part of this DNA. Finding a portion of nuclear DNA (a) whose synthesis is inhibited in the presence of BUdR and (b) which transcribes a set of RNA molecules whose transcription is inhibited after a short exposure of embryos to BUdR would seem to constitute strong circumstantial evidence that BUdR inhibits transcription as a result of effects at the level of DNA synthesis rather than acting at the level of the transcription machinery (e.g., affecting a certain class of RNA polymerase). The experiments to be described are relevant to this point.

It was found previously (24) that labeled low molecular weight (8–15S) duplex nuclear DNA persisted through several mitotic divisions in embryos which had been pulsed with thymidine-3H for 20 min at the morula stage, washed, and then chased during further development with unlabeled thymidine. During the chase period, at the time of blastulation, the label in this low molecular weight DNA gradually became part of higher molecular weight (15–60S size-range) nuclear DNA. It was also shown (24) that in gastrulae, thymidine-3H labels 8–15S single-stranded and double-stranded DNA predominantly in a 1 or 2 min pulse. However, a labeling period of 5 min or longer resulted
in labeling of 8–60S size-range duplex DNA as well as DNA (on an alkaline gradient) of > 200S. The results from the pulse-chase experiments and these latter findings suggested that 8–15S DNA may link together to form 15–60S DNA (24).

The possibility that 8–15S DNA is a precursor of 15–60S size-range DNA is strengthened by the following pulse-chase experiments (Fig. 4). Gastrulae were exposed to thymidine-3H for 1 min before quickly washing the embryos once and resuspending them in seawater containing unlabeled thymidine (50 µg/ml). Aliquot portions of embryos were removed during the chase period at 3, 5, 10, and 20 min after the start of the pulse period. Nuclear DNA was obtained from the isolated nuclei of each aliquot portion. The different nuclear DNAs were then sedimented independently on alkaline-sucrose gradients. The radioactivity of the sedimentation profiles is shown in Fig. 4 A. These results show that 8–15S DNA is initially labeled. Not until the 5th min (during the chase period) does 3H appear in DNA of > 15S. By the 10th and 20th min, most of the 3H is in the 15–60S size-range of DNA. It can be argued that the unlabeled thymidine chase is effective here since 8–15S DNA which is normally rapidly labeled (24) shows a decrease in 3H during the chase period. During the chase period there is an increase in 3H in the 15–60S range, suggesting that newly made 8–15S nuclear DNA is an immediate precursor for 15–60S nuclear DNA in gastrula stage nuclei.

Fig. 4 B shows results of experiments similar to the experiments shown in Fig. 4 A, except that gastrulae were labeled with BUdR-3H for 1 min and then chased with unlabeled BUdR (50 µg/ml). In these results, 3H counts initially seen in 8–15S DNA (3rd min) are not chased by BUdR into 15–60S DNA by the end of the 20th min. One interpretation of these results is that exposure of gastrulae to BUdR (rather than thymidine) prevents new 8–15S DNA from becoming part of higher molecular weight DNA. An alternative, that 15–60S DNA is synthesized directly and is not made from previously synthesized 8–15S pieces and that BUdR-3H discriminantly does not label 15–60S DNA, seems less likely. The latter case would require that 15–60S DNA is synthesized by a mechanism which does not allow incorporation of BUdR-3H but does allow incorporation of thymidine-3H.

The results shown in Fig. 4 suggested that exposure of gastrulae to BUdR resulted in a change in the species composition of new RNA. The possibility occurs then that such a change in transcription program by BUdR may be reflected in the differences in sedimentation pattern, during the chase period, of radioactive nuclear DNA pulse-labeled with BUdR-3H compared with the pattern obtained by pulse-labeling with thymidine-3H (Fig. 4). The inference here is that a portion of 15–60S size-range nuclear DNA from non-BUdR-treated gastrula stage embryos may be template DNA for a set of RNA species usually transcribed at this stage.

![Figure 4](image-url)
Embryos exposed to BUdR for a period during gastrulation may lack a portion of the set of 15–60S range nuclear DNAs normally present and, therefore, have a reduced number of species of RNA transcribed at this stage. An experiment was designed to test this point. Nuclear DNA from (a) embryos treated with BUdR (50 µg/ml) for 1 hr during gastrulation or (b) control embryos not exposed to BUdR was sedimented on alkaline sucrose gradients. Gradient fractions were pooled for the 8–15S range and separately for the 20–60S range for both BUdR and control nuclear DNAs. The relative amount of DNA obtained from the pooled regions of the sucrose gradients was determined (25). Exposure of gastrulae for 1 hr to BUdR appears to reduce the amount of collected 20–60S size-range DNA (compared with collected 8–15S range DNA) by approximately 12%. This shift in the relative amount of 20–60S DNA obtained from BUdR-treated gastrulae is consistent with the possibility (Fig. 4 B) that BUdR prevents the making of new 15–60S DNA. The ability of pooled 8–15S range or pooled 20–60S range DNA to hybridize to whole-cell gastrula-RNA-3H is shown in Fig. 5. (The RNA-3H used in these hybridization experiments was labeled in vivo by exposing non-BUdR-treated gastrulae to uridine-3H for 20 min before terminating development.) Approximately five times as many cpm of RNA-3H anneals to a quantity of control 20–60S range DNA as anneals to the same quantity of 20–60S range DNA from the BUdR-treated embryos. Conversely, approximately two times as many counts per minute RNA-3H anneals to a quantity of 8–15S DNA from BUdR-treated embryos as anneals to the same quantity of control 8–15S DNA. These results suggest (a) that in gastrulae (not exposed to BUdR) a portion of newly made RNA is homologous to nuclear low molecular weight (<60S) DNA and may be transcribed by this DNA, (b) that 20–60S nuclear DNA from gastrulae which had been exposed to BUdR is deficient in certain species of DNA which would normally be transcribed at this stage of development. These results also strengthen the hypothesis (24) that in embryos which have not been treated with BUdR, 8–15S DNA links together to form 20–60S size-range DNA.

The results shown in Fig. 5 depend upon knowledge of the amount of DNA per hybridization filter after the hybridization reaction, RNase treatment, and filter washing. Routinely in these experiments the amount of DNA immobilized per filter and also the amount of immobilized DNA retained per filter after hybridization procedures were both monitored (25, 26). The shorter-size DNA pieces (8–15S size-range) were retained to approximately 91% of the initially immobilized DNA per filter at the end of a typical hybridization experiment. The longer-size pieces (20–60S size-range) were retained to approximately 96% of the initially immobilized DNA. The counts per minute hybridized, as shown in Fig. 5, have been normalized for 10.0 µg DNA per filter. Normalization was based simply on a linear change in cpm hybridized per amount of DNA per filter. Even without this slight correction for the amount of DNA immobilized per filter and also the amount of immobilized DNA retained per filter after hybridization procedures, these data strongly suggest that BUdR-exposed embryos have a reduced number of species of RNA transcribed at this stage of development.

Figure 5 Hybridization of low molecular weight fractions of nuclear DNAs to increasing quantities of whole-cell gastrula stage RNA-3H. Bulk nuclear DNA was obtained either from embryos treated with BUdR (50 µg/ml) for 1 hr during gastrulation or from control gastrula stage embryos not exposed to BUdR. These two kinds of DNA were fractionated on alkaline sucrose gradients. From each gradient, fractions were pooled separately for the 8–15S range and for the 20–60S range. These DNA fractions were denatured and immobilized on nitrocellulose filters at 10 µg DNA per filter (±0.2 µg DNA per filter, depending upon the particular sample of DNA utilized) and hybridized with RNA-3H. This RNA (specific activity 212 cpn/µg) had been labeled by exposing non-BUdR-treated gastrulae to uridine-3H (99.2 Ci/mole, 3.3 µCi/ml) for 30 min before termination of development. The cpm hybridized for each of the four derived types of DNA has been normalized for hybridization to 10.0 µg DNA per filter. Normalization factor in any case was small (<9%) and was determined by the amount of DNA per filter after the hybridization reaction, RNase treatment, and washing procedure. △—△, 8–15S DNA, control; ▲—▲, 20–60S DNA, control; ○—○, 8–15S DNA, BUdR; ●—●, 20–60S DNA, BUdR.
DNA per filter, the raw results of the hybridization experiments are essentially as shown in Fig. 5. Similar hybridization experiments were also performed in which the two different size-ranges of DNA were sonicated before immobilization of the DNA. Under these conditions the portion of starting DNA which became immobilized during preparation of the DNA filters (approximately 96%) and also the portion of initially immobilized DNA which came loose from the filters during the hybridization procedures (approximately 9%) was the same (+1%) for either type of starting DNA (i.e., 8-15S or 15-60S, before sonication). The results of hybridization experiments utilizing these sonicated DNAs were qualitatively similar to those shown in Fig. 5.

**DISCUSSION**

The experiments presented here suggest that BUdR causes a noncoordinate decrease in the set of RNA species transcribed by gastrula stage sea urchin embryos. Exposure of gastrulae to BUdR may prevent the making of some 15-60S nuclear DNA, a size-range of DNA which is normally (24) rapidly synthesized. Comparison of bulk 20-60S size-range nuclear DNA from control embryos with similar DNA from embryos exposed to BUdR for 1 hr indicates that there is a decrease in DNA species homologous to RNA being synthesized in normal gastrulae. The possibility therefore exists that BUdR turns off some transcription by preventing the synthesis of certain low molecular weight classes of nuclear DNA.

A mechanism by which BUdR exerts its effect on this differentiating system can be suggested. If rapidly synthesized short pieces of double-stranded DNA normally link together in vivo as has been hypothesized (24), perhaps incorporation of BUdR into end regions (e.g., “sticky ends,” (27)) prevents the short pieces from becoming covalently bound to each other. This defect, preventing the tandem linkage of the short pieces, might in effect inhibit or change the transcription ability of this DNA. The prompt action of BUdR in changing the species composition of RNA being transcribed at the gastrula stage (Fig. 2) suggests that if BUdR acts as a result of incorporation into DNA, then the DNA must (a) be synthesized just before transcription, or (b) be modified by acquisition of new regions containing BUdR. We suggest here the possibility that short pieces (on the order of 10S) of double-stranded DNA are synthesized in the nucleus and possess (or later acquire) single-stranded ends rich in polydeoxyadenylic acid (poly dA) on one end and rich in polythymidylic acid (poly dT) on the other end. Normally these separate low molecular weight DNA molecules with such sticky ends might link end to end by overlapping a single-stranded poly dT region on the left end of one molecule with a single-stranded poly dA region on the right end of another molecule followed by gap closure and covalent linkage via a ligase enzyme. However, if BUdR is incorporated into the poly dT end, such linkage may be prevented. Prevention of linkage could conceivably result from (a) insecure hydrogen bonding of poly BUdR single-stranded sticky ends with poly dA sticky ends on other molecules, (b) inability of the terminal BUdR of a sticky end to allow ligase to attach this BUdR to the adjacent nucleotide of a hydrogen bonded molecule, or (c) the presence of a gap between the terminal BUdR of a sticky end and the beginning of a double-stranded region of a tandem-linked molecule. In the latter case the terminal BUdR may not allow the start of gap closure by a DNA polymerase. Information on any of these latter points or on the validity of the proposed mechanism for the action of BUdR on transcription is not indicated by the set of experiments presented here.

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