EFFECT OF 5-BROMODEOXYURIDINE ON NUCLEAR RNA SIZE IN FROG EMBRYOS

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1. Introduction

Although frog embryo nuclear RNA is much smaller than that of mammals and birds [1], recent work has shown that the average size of nuclear RNA molecules increases during early development of the frog embryo [2]. During the same period the quantitative level of turnover of nuclear RNA also increases [2]. The importance of post-transcriptional nuclear regulation in development is illustrated by the qualitative similarity of nuclear RNA molecules transcribed from unique DNA sequences at the early neurula and larval stages of developing frog embryos [3]. It is known that the thymidine analogue 5-bromodeoxyuridine (BrdU) can suppress differentiation, or causes dedifferentiation, in a number of cell types [4]. The effect of BrdU upon nuclear RNA size and accumulation of labeled cytoplasmic and nuclear RNA was examined in this study.

2. Materials and methods

The procedures for labeling the frog embryos, isolation of nuclei and nuclear RNA, sucrose density gradient centrifugation under denaturing conditions and estimation of turnover of nuclear RNA have been published [2]. Several hundred stage 14 neurula embryos [5] were cut into neural plate–dorsal mesoderm and belly regions and cultured in Niu-Twitty saline [6] or Niu-Twitty saline containing BrdU (250 μg/ml) for 1 day. 5-[^{3}H]Uridine (50 μc/ml; specific activity 25 Ci/mM) was then added for 0.5 h at the end of the culture period. Neurulation progressed normally in both the control and BrdU-treated embryos, indicating that BrdU at this concentration was not inhibiting development. Nuclear RNA was prepared [2] and treated with dimethylsulfoxide to insure denaturing conditions [7]. Macaloid (0.5 mg/ml) and polyvinyl sulfate (40 μg/ml) were present during isolation of the nuclei and the RNA in order to prevent degradation by RNase. The denatured RNA was sedimented through an isokinetic 5–26.8% sucrose gradient (in 0.05 M NaCl, 0.01 M EDTA, 0.01 M Tris–HCl (pH 7.4) and 0.2% SDS) [8] at 44 000 rev./min at 20°C for 3.4 h in a Beckman SW 50.1 rotor. The fractions were collected from the bottoms of the tubes and aliquots of each fraction counted. Processing intermediates and small nuclear RNA species are present in the gradient profiles since a gel filtration step was not performed [2].

For the investigation of the effect of BrdU upon accumulation of labeled cytoplasmic and nuclear RNA 40 stage 10 gastrulae, stage 14 neurulae or stage 18 tailbuds [5] were cut into dorsal ectoderm–mesoderm and belly endoderm regions and incubated in Niu-Twitty saline containing BrdU at various concentrations and Niu-Twitty saline alone for 1 day for gastrulae or 2 days for neurulae and tailbuds. 5-[^{3}H]-Uridine (2.5 μCi/ml; spec. act. 25 Ci/mmol) was then added for 3 h. Nuclear fractions were obtained by homogenizing the embryos at 4°C in 0.25 M sucrose–0.003 M $CaCl_2$ containing 1% citric acid, centrifuging at 600 × *g* and washing the nuclear pellet 3–4 times with the sucrose medium, followed by 3–4 washes in cold 5% trichloroacetic acid (TCA). The cytoplasmic fraction was centrifuged at 10 000 × *g* to pellet pigment granules and TCA was added to 10% in the supernatant fraction. The precipitate then was washed

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3–4 times with cold 10% TCA. Both TCA precipitates were dissolved in 0.3 N KOH and the RNA was hydrolysed at 37°C for 18 h. The macromolecules were precipitated with cold TCA and centrifugal supernatants were collected and counted.

3. Results and discussion

The data show that the size range of the nuclear RNA molecules isolated from the embryos cultured with BrdU (250 μg/ml) is similar to that of the hnRNA obtained from the control embryos (fig.1). However, there are more copies of the larger hnRNA molecules in the BrdU-treated embryos. Similar results were obtained in four separate experiments. The inclusion of a 32 S denatured RNA marker (yeast killer factor RNA labeled with ^{32}P) in a tailbud nuclear RNA preparation did not alter its sedimentation rate. This indicates minimal degradation of the nuclear RNA during the isolation procedure. Furthermore, extractions of nuclear RNA from a mixture of labeled and unlabeled embryos of different stages show that differential degradation does not account for the size differences observed in the nuclear RNA at the two stages [2].

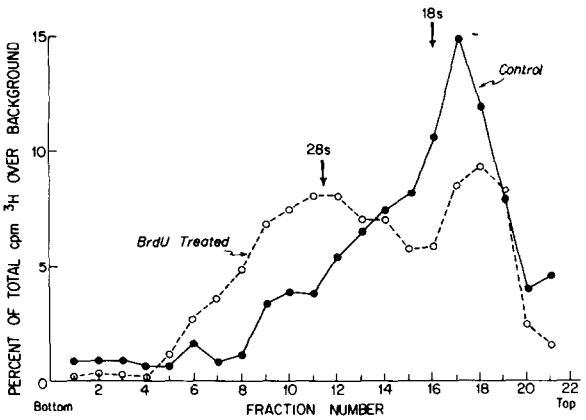

Fig.1. Effect of BrdU upon hnRNA size. Neural plate and belly regions of 200 stage 14 neurulae were cultured in Niu-Twitty saline or Niu-Twitty saline containing BrdU (250 μg/ml) for 1 day. At the end of the culture period the explants were labeled for 0.5 h with 50 μCi/ml 5-[^{3}H]uridine (spec. act. 25 Ci/mM). Nuclear RNA was prepared, denatured with dimethylsulfoxide and sedimented at 20°C in an isokinetic 5–26.8% sucrose gradient for 3.4 h at 44 000 rev./min in a Beckman SW 50.1 rotor. The arrows indicate the positions of [^{3}H]rRNA from *Drosophila* run on a parallel gradient.

One interpretation of the centrifugation data is that in the BrdU-treated embryos there is a decreased turnover of hnRNA, thus causing more copies of hnRNA to accumulate in the rapidly sedimenting fractions. In support of this idea there is a greater reduction of labeled cytoplasmic RNA than of labeled nuclear RNA in embryos that had been cultured in BrdU (table 1). This could result from an increased turnover or decreased processing of the larger hnRNA molecules.

The mechanism(s) which BrdU substitution for thymidine caused certain types of cells to loose their differentiated characteristics [4] is presently unknown. However, the following data suggest one possible mechanism. DNA sequences containing BrdU bind H1 histone more strongly [9,10] and have a

Table 1
Effect of 5-bromodeoxyuridine on accumulation of labeled nuclear and cytoplasmic RNA

Embryonic stage	cpm cytoplasmic RNA / cpm nuclear RNA	
Gastrulae (stage 10)		
Controls	$\frac{318}{216} = 1.47$	$\frac{317}{225} = 1.41$
BrdU 125 μg/ml	$\frac{198}{160} = 1.24$	$\frac{177}{217} = 0.82$
Neurulae (stage 14)		
Controls	$\frac{3823}{2748} = 1.39$	
BrdU 500 (μg/ml)	$\frac{780}{810} = 0.96$	
Tailbuds (stage 18)		
Controls	$\frac{1851}{3374} = 0.549$	$\frac{4885}{7264} = 0.672$
BrdU 50 μg/ml	$\frac{563}{1394} = 0.404$	
500 μg/ml		$\frac{861}{1714} = 0.502$

In each experiment 80 embryos were cut into dorsal ectodermmesoderm and endoderm regions, half were incubated in Niu-Twitty saline and half in Niu-Twitty saline containing BrdU for 1 day (gastrulae) or 2 days (neurulae and tailbuds). 5-[^{3}H]Uridine (2.5 μCi/ml; spec. act. 25 Ci/mM) was added for 3 h, nuclear and cytoplasmic fractions obtained and the RNA was hydrolyzed and counted

higher T_m [11]. Omission of H1 histone in chromatin reconstitution experiments allows formation of more initiation sites for transcription in vitro [12], suggesting that H1 histone normally blocks initiation sites for transcription in vivo. If incorporation of BrdU reduces the number of initiation sites for transcription, one result might be transcription of an increased number of larger hnRNA molecules. This hypothesis presumes that RNA polymerase will 'read through' such blocked sites, thus increasing the number of larger hnRNA molecules. Since the mRNA size range is fairly constant during early development in amphibian embryos [13], this implies that a greater turnover of the sequences in the larger hnRNAs would occur in order to account for the reduction of size to that of the mRNA molecules. The data of table 1 do show decreased processing of nuclear RNA in embryo explants that did incorporate BrdU into DNA, as compared to the controls, and support this possibility. However, the data do not obviate the turnover of entire hnRNA molecules as well.

Much lower concentrations of BrdU (10–25 μg/ml) cause an increase in replicon size in cultured minnow and rat hepatoma cells [14]. This suggests an effect upon the initiation sites for replication and lends support to the speculation that transcription initiation sites are affected in the present work. Higher levels of BrdU are used in the present work since this is necessary to insure penetration of the compound into the blocks of cells which are partially covered by a surface coat of low permeability. There are other data which fit the idea that BrdU affects initiation sites for transcription. At 10^{-7} M BrdU is preferentially incorporated into intermediate repetitive DNA sequences [15] and hybridizations with RNA labeled with [γ-^{32}P]ATP and [^{3}H]uridine suggest that transcription initiation sites are rich in repetitive DNA sequences [16]. Taken together, this may mean that BrdU is preferentially entering transcription initiation sites.

The present study shows that BrdU increases the number of larger hnRNA molecules and decreases processing of nuclear RNA. Previous work indicated an increase in average hnRNA size during early development of the frog embryo and decreased processing of nuclear RNA at later stages of development. Together these data indicate that post-transcriptional processing may play a key role in the accumulation of mRNA during embryonic development.

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