A DIFFERENTIAL EFFECT OF THE INCUBATION TEMPERATURE ON THE INHIBITION OF RNA SYNTHESIS BY DRB IN CELLS OF *DROSOPHILA HYDEI*

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1. Introduction

The nucleoside analogue DRB (5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole) has been shown to be a specific inhibitor of initiation of chromosomal RNA synthesis in *Chironomus tentans* [1,2]. A compound with such properties would be of considerable importance and usefullness in our studies of RNA metabolism after experimental puff induction in cells of *Drosophila hydei*. We have therefore determined the effect of DRB on RNA synthesis in both (polytene) salivary gland cells of *Drosophila hydei* and in (diploid) embryonic cells, before and after experimental gene activation by a heat-shock, i.e. a change in incubation temperature of the cells from 25°C to 37°C. Such a treatment induces four major puffs in salivary glands of *D. hydei* (for review, see [3]) and a concomitant change in the protein synthetic pattern [4]. An identical change in the protein synthetic pattern is found in heat shocked embryonic cells (unpublished observations).

2. Materials and methods

2.1. *Labeling of and RNA extraction from embryonic cells*

Cells were prepared from 12-h-old *Drosophila hydei* embryos essentially as described by Shields and Sang [5], except that the cells were filtered through nylon gauze with a 80 μm mesh to remove large debris. The cells were incubated for 1 h in complete Poels medium [6], washed with incomplete Poels medium and resuspended in incomplete Poels medium (i.e. Poels medium modified to remove exogenous sources of uridine) at 50 mg (wet weight) cells/ml. After appropriate preincubation, as denoted in the figure legends, cells were labeled with 20 μCi [^{3}H] uridine (40–50 Ci/mM)/ml.

Total [^{3}H]uridine incorporation was determined by precipitating 50 μl aliquots of the cell suspension with 1 ml 10% ice-cold TCA containing 0.2% yeast hydrolysate. The precipitate was collected on Whatman GF/A filters, the filter was washed three times with 10% ice-cold acetic acid, then with ethanol and dried. Filters were counted in a toluene based scintillation fluid. After labeling, the cells were collected by centrifugation for 5 min at 2000 rpm, and the cell pellet was treated with pronase–SDS as described by Rosbach and Ford [6]. After pronase digestion, nucleic acids were precipitated with ethanol, the precipitate was collected by centrifugation, washed twice with ethanol and dried. The pellet was redissolved in column buffer and chromatographed over a poly(U)–Sepharose column as described by Lindberg and Persson [8]. The eluted fractions were concentrated by alcohol precipitation and analyzed by gel electrophoresis according to Bishop et al. [9]. Gels were sliced, dissolved in Soluene and counted in a Philips scintillation counter.

2.2. *Labeling and autoradiography of Salivary glands*

Salivary glands were hand isolated from late third instar larvae of a mass culture of *D. hydei*. After appropriate preincubation, as detailed in figure legends, in incomplete Poels medium, they were labeled in 5 μCi [^{3}H]uridine in 25 μl incomplete Poels medium, and prepared for autoradiography as described [10]. Autoradiographs were exposed for 5 days.

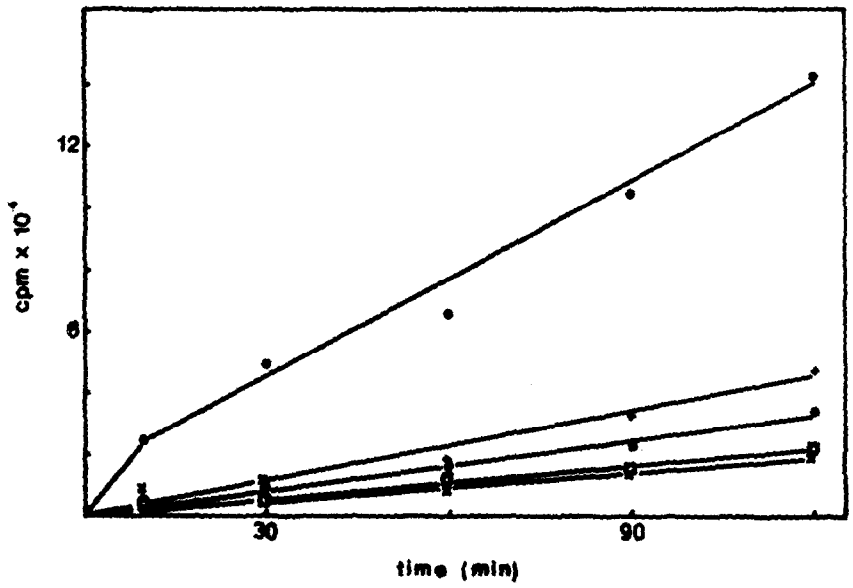

Fig.1. Effect of different concentrations of DRB on total RNA synthesis in embryonic cells during incubation at 25°C. DRB was added at the beginning of the incubation at the following concentrations: (· – ·) no DRB; (+ – +) 10 μg/ml; (○ – ○) 25 μg/ml; (□ – □) 50 μg/ml; (× – ×) 100 μg/ml.

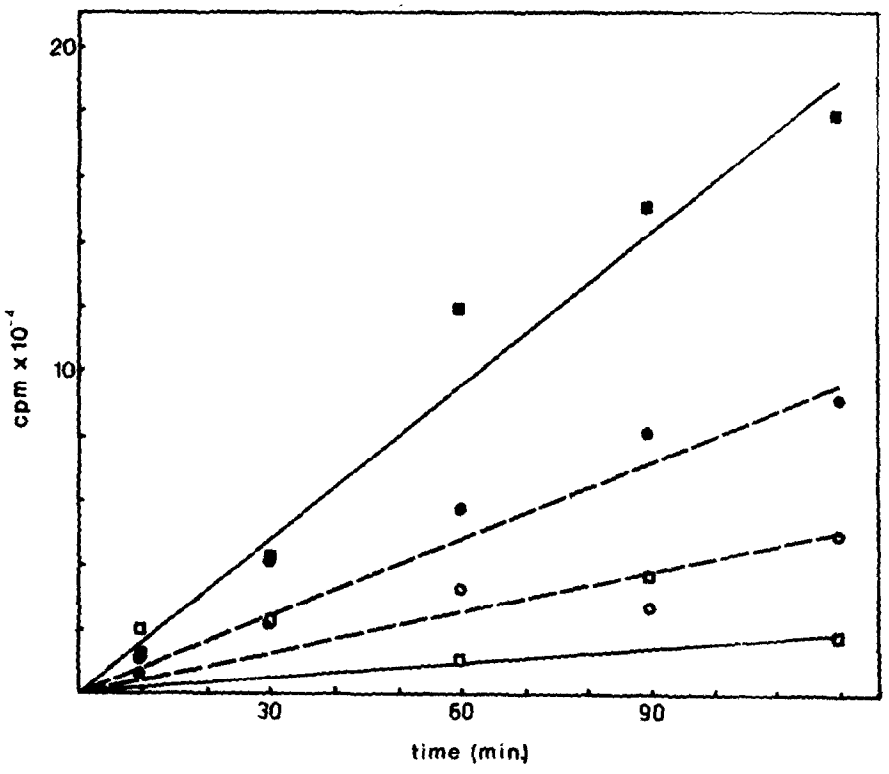

Fig.2. Effect of incubation temperature on the inhibition of total RNA synthesis by DRB (50 μg/ml): (■ – ■) no DRB, 25°C; (□ – □) with DRB, 25°C; (● – ●) no DRB, 37°C; (○ — ○) with DRB, 37°C. DRB was added at the beginning of the incubation.

3. Results

The specificity of inhibition of RNA synthesis by DRB in *Chironomus tentans* has been reported to be concentration dependent. Initially, we therefore determined the effect of the concentration of DRB used in studies with *Chironomus tentans*, namely 20 μg/ml, on chromosomal RNA synthesis in salivary glands. Such qualitative analyses showed that 20 μg/ml DRB was not effective in inhibiting chromosomal RNA synthesis, but 50 μg/ml DRB was. For a more quantitative analysis, the effect of different concentrations of DRB on total RNA synthesis in embryonic cells incubated at 25°C was determined, as shown in fig.1. Maximal inhibition of RNA synthesis was obtained with 50 μg of DRB and this concentration was used in further experiments. At this concentration, total RNA synthesis was inhibited by about 85% at 25°C (fig.2). When the effect of DRB was tested on cells incubated at 37°C, the inhibition of DRB of total RNA synthesis was much less, namely about 50% (fig.2).

As a first approximation to a determination of the site of inhibition of DRB in these cells, RNA, extracted from cells labeled at 25°C with [^{3}H]uridine in the presence of DRB, was separated into RNA with and without poly(A) stretches by poly(U)–Sepharose chromatography. Although some poly(A) containing RNA is found in the mitochondria, most poly(A) containing RNA originates from chromosomal loci (for review see [11]), while at least part of the non-poly(A) containing RNA should be nucleolar in origin. As expected from the data published about the effect of DRB on *Chironomus tentans* [1,2], a virtual complete inhibition of synthesis of poly(A) containing RNA was observed at 25°C (fig.3A). DRB also markedly inhibited the synthesis of non-poly(A) containing RNA at 25°C (fig.3B). During incubation of the cells at 37°C, relatively little poly(A) contain-

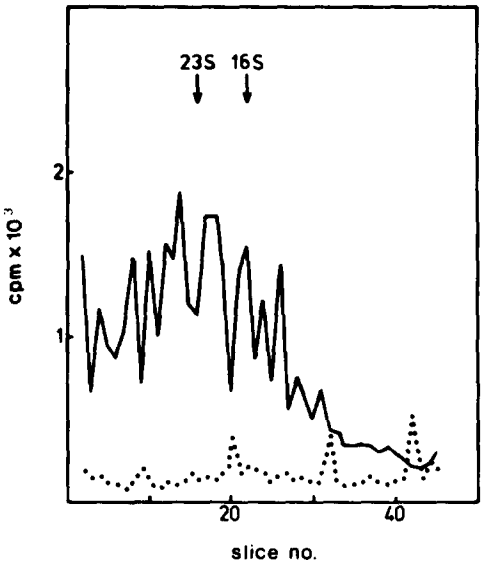

Fig.3A

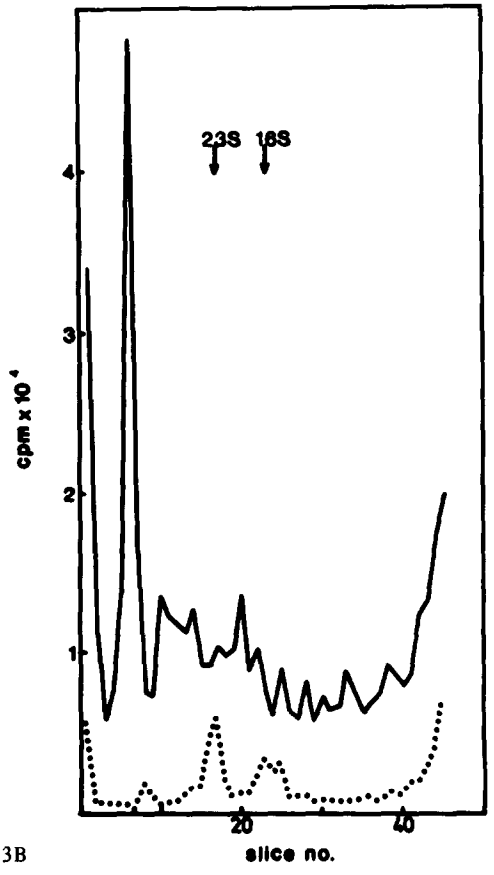

Fig.3B

Fig.3. Electropherogram of RNA synthesized during incubation at 25°C with or without added DRB (50 μg/ml). The position of *E. coli* rRNA, used as mol. wt markers, is indicated. DRB was added 30 min before the [^{3}H]uridine. Labeling time was 2 h. A, Poly(A) containing RNA; (——) without DRB; (. . . . .) with DRB. B, Non-poly(A) containing RNA; (——) without DRB; (. . . .) with DRB.

ing RNA (as compared to cells incubated at 25°C) appears to be synthesized. The synthesis of this RNA was incompletely inhibited by DRB (fig.4A). No inhibition by DRB of the synthesis of non-poly(A) containing RNA was observed at 37°C (fig.4B). Most of the non-poly(A) containing RNA synthesized at 37°C does not migrate with the mobility expected of rRNA or its precursors [12] and is unlikely to be of nucleolar origin. This conclusion was substantiated by examining autoradiographs of salivary glands labeled before and after exposure to DRB: at 25°C a marked reduction of grains over the chromosomal regions was observed in the presence of DRB, while the number of grains over the nucleolar regions also decreased somewhat but to a lesser extent (fig.5A,B). A qualitative effect on nucleolar RNA synthesis might be indicated by the confinement of the grains over the pars fibrosa in the presence of DRB. In contrast, in control squashes, the grains are spread over the whole nucleolar area. At 37°C, however, no reduction of grains was observed over either nucleolar or chromosomal regions. Furthermore, the (heat shock induced) puffed areas were strongly labeled in the presence of DRB (fig.5C,D).

4. Discussion

The data presented here show that DRB is an effective inhibitor of RNA synthesis at 25°C but not at 37°C in *Drosophila hydei*. The failure of DRB to inhibit RNA synthesis at 37°C cannot be due to impermeability of the cells to DRB at 37°C since cells were preincubated with DRB at 25°C for 30 min before shifting to 37°C and, as shown in fig.1, at 25°C the effect of DRB on RNA synthesis is immediate.

The experiments with salivary glands show that at 25°C chromosomal RNA synthesis is affected much

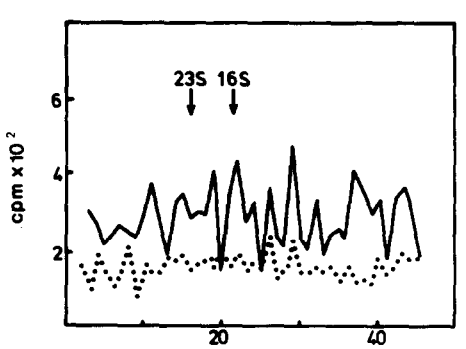

Fig.4A

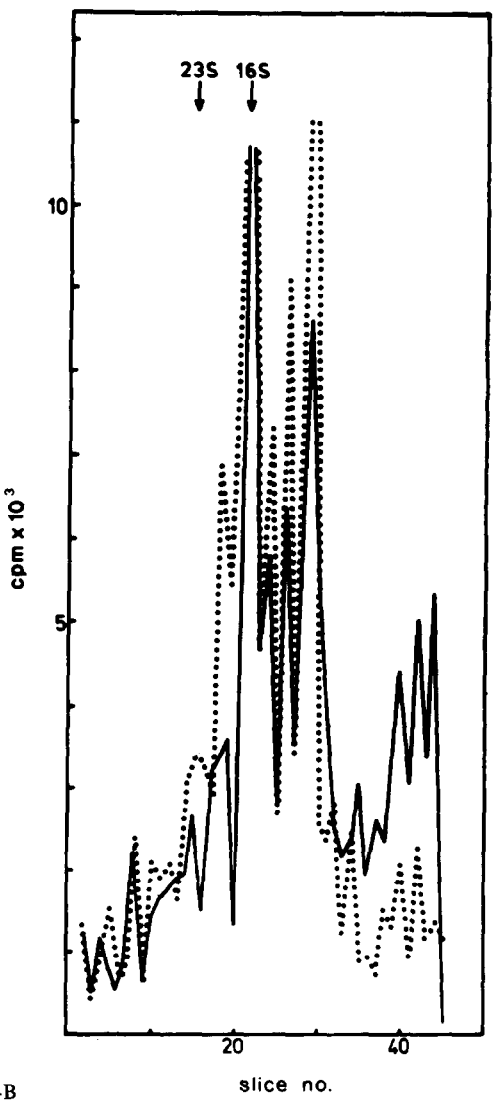

Fig.4B

Fig.4. Electropherogram of RNA synthesized during incubation at 37°C with or without added DRB (50 μg/ml). Cells were preincubated for 30 min at 25°C with or without DRB, then shifted to 37°C and incubated for another 15 min before label was added. Labeling time was 2 h. A, Poly(A) containing RNA; (——) without DRB; (. . . .) with DRB. B, Non-poly(A) containing RNA; (——) without DRB; (. . . .) with DRB. The position of *E. coli* rRNA, used as mol. wt markers, is indicated.

more than nucleolar RNA synthesis by DRB, as has been reported for *Chironomus tentans* [1]. Nevertheless, nucleolar synthesis is affected. This is shown not only by the decreased number of grains over the nucleolar area in salivary gland squashes, but also by the RNA profiles obtained from embryonic cells: after treatment with DRB, only a small peak with a mobility of 38 S (the size of the rRNA precursors [12]) is seen and no RNA species corresponding to the 28 S and 18 S rRNA are found. It is possible that

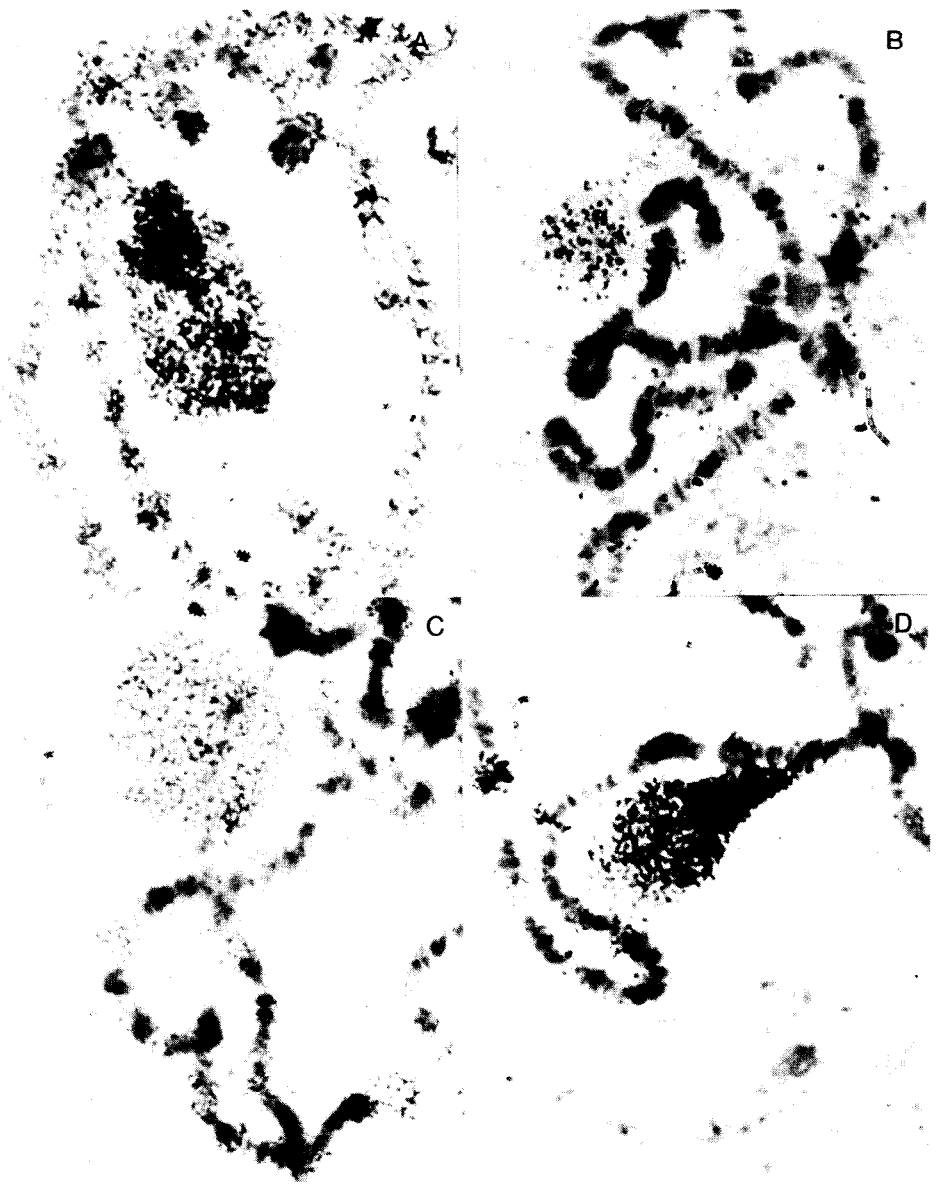

Fig.5. Autoradiograms of salivary gland squashes. Glands were preincubated for 30 min with or without DRB (50 μg/ml), some of the glands were then labeled and prepared for autoradiography directly, while the rest was further incubated at 37°C for 30 min before analysis. A. 25°C, no DRB. B. 25°C, with DRB. C. 37°C, no DRB. D 37°C, with DRB.

DRB acts like other nucleoside analogs (such as 5-azaguanidine) and might allow some synthesis of the ribosomal RNA precursor but not its processing [13]. At 37°C, neither with, nor without DRB, are strongly labeled RNA species with the mobility of rRNA observed: most RNA species migrated with mobilities around 16 S. They are similar in size to those found in *D. melanogaster* cells after heat shock [14, 15], although in that case the RNA did contain poly(A), and may represent transcription products from the loci activated by heat shock.

The mechanism by which DRB inhibits initiation of RNA synthesis is unknown, but apparently the inhibition is strongly temperature dependent. The lack of inhibition is not dependent upon puff induction, since RNA synthesis at these sites is sensitive to DRB when these puffs are induced at 25°C with vitamin B_6 (unpublished observations). Even at 25°C, we have found that *Drosophila hydei* cells are less sensitive to DRB than *Chironomus tentans* cells. In the latter case, a lower concentration yielded a more specific inhibition of RNA synthesis, since nucleolar synthesis was not affected [1]. The lesser effect of DRB on *D. hydei* may be explained not only by a species difference but also by the lower incubation temperature used for *Chironomus tentans* salivary glands, namely 18°C.

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