EFFECT OF 5'-METHYLTHIOADENOSINE ON GENE ACTION DURING HEAT SHOCK IN *DROSOPHILA MELANOGASTER*

Ronald E. LAW, Ralph M. SINIBALDI, Adolph J. FERRO and Michael R. CUMMINGS
Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680, USA

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1. Introduction

The pattern of puffing in dipteran salivary gland chromosomes undergoes stage-specific alterations during larval development which are interpreted as the morphological manifestation of differential gene expression [1,2]. In addition, a discrete set of puffs can be experimentally induced by heat shock of whole larvae [3] or explanted salivary glands [4].

During heat shock in *Drosophila melanogaster*, 9 new puffs are induced which are regions of intense [^{3}H]uridine incorporation [5]. A dramatic change in the translational pattern of salivary glands accompanies the appearance of the temperature-induced puffs, resulting in the predominant synthesis of ~7 polypeptides [5,6]. In heat shocked *D. melanogaster* cells in culture, changes in polyribosome metabolism [7] and the induction of a novel class of polyadenylated polysomal RNA which hybridizes in situ to heat shock loci have been observed [7–9].

The heat shock response of polytene salivary glands of *Drosophila*, therefore, provides an excellent system for cytological, autoradiographic, and biochemical studies of gene expression. We reported [10] that 5'-methylthioadenosine (MTA), a product of polyamine biosynthesis, inhibits the synthesis of α-amanitin-sensitive RNA in explanted salivary glands of *D. melanogaster*. We report here on the effect of MTA, a naturally occurring nucleoside, on both the specific transcriptional changes accompanying heat shock as visualized by autoradiography and the kinetics of puff induction at several heat shock loci. A study of the effect of MTA on the heat shock response in *Drosophila* may provide insight concerning the physiological significance of this nucleoside, and in addition may contribute to the elucidation of its action at the molecular level.

2. Materials and methods

Stocks of Oregon R wild-type *Drosophila melanogaster* were maintained at 25°C on agar–cornmeal medium supplemented with yeast. 5'-Methylthioadenosine was prepared by the method in [11]. [5,6-^{3}H]Uridine was purchased from Schwarz-Mann.

The incorporation of [^{3}H]uridine into RNA in explanted salivary glands of synchronized late third-instar larvae was determined as in [10] except that glands were incubated in Schneider's *Drosophila* medium (GIBCO) instead of 0.6% NaCl.

For autoradiography, salivary glands were preincubated in Schneider's medium in the presence or absence of 1 mM MTA for 20 min at 37°C at which time [^{3}H]uridine was added to 50 μCi/ml final conc. Incubation was continued for an additional 20 min at 37°C, glands were removed from the labeling medium and immersed in 3:1 ethanol–acetic acid for 5 min. Glands were then rinsed in 100% and 95% ethanol before transferring them to a drop of 45% acetic acid on a microscope slide, and allowed to soften for 10–15 min before squashing with a siliconized coverslip. After freezing the slides on dry ice, coverslips were removed with a razor blade. Slides were fixed in 3:1 ethanol–acetic acid for 2–5 min, rinsed in 100%, 95% ethanol, air dried, and coated with Ilford L-4 emulsion diluted 1:1 with distilled water. Slides were exposed for 3–5 days at 4°C, and developed with Kodak D-19.

Quantitation of puff size was by the method in [2], and expressed as the ratio of the maximum puff diameter to the diameter of an unpuffed reference band as measured with a filar ocular micrometer.

3. Results and discussion

In order to determine the effect of MTA on RNA synthesis during heat shock, the incorporation of [^{3}H]uridine into RNA was monitored in explanted salivary gland incubated at 37°C in the presence of 1 mM MTA (fig.1). In control glands, incorporation increased throughout the 60 min incubation period. Salivary glands labeled in the presence of 1 mM MTA, however, incorporated markedly less [^{3}H]uridine into RNA than glands incubated in the absence of the nucleoside. In agreement with our observation [10] on the effect of MTA on salivary gland RNA synthesis at 23°C, the nucleoside was found to be inhibitory at 0.1–2.5 mM (data not shown). For a consistent inhibition of RNA synthesis > 50%, incubation in the presence of 1 mM MTA was required and this concentration was employed in all subsequent experiments. In addition, studies with human lymphocytes (Vandenbark, A. J. F., submitted) and *Drosophila* cells in culture (Rizzo, R. E. L., unpublished observations) indicate that 1 mM MTA is not cytotoxic.

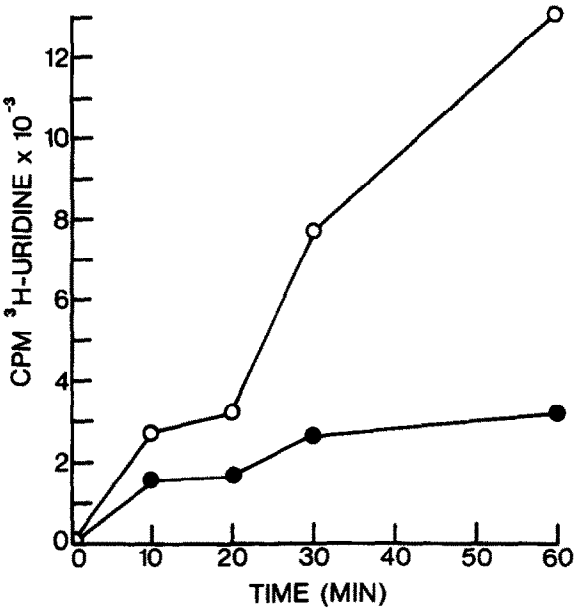


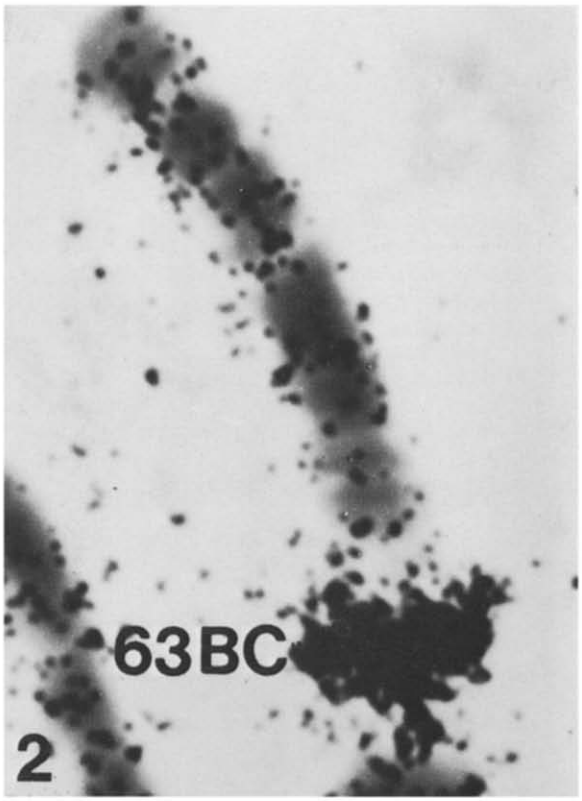
Fig.1. Effect of MTA on the incorporation of [^{3}H]uridine into RNA in heat-shocked explanted salivary glands. For each point, 5 pairs of salivary glands were incubated in the absence (o——o) or presence (•——•) of 1 mM MTA. Incubation conditions were as in section 2.

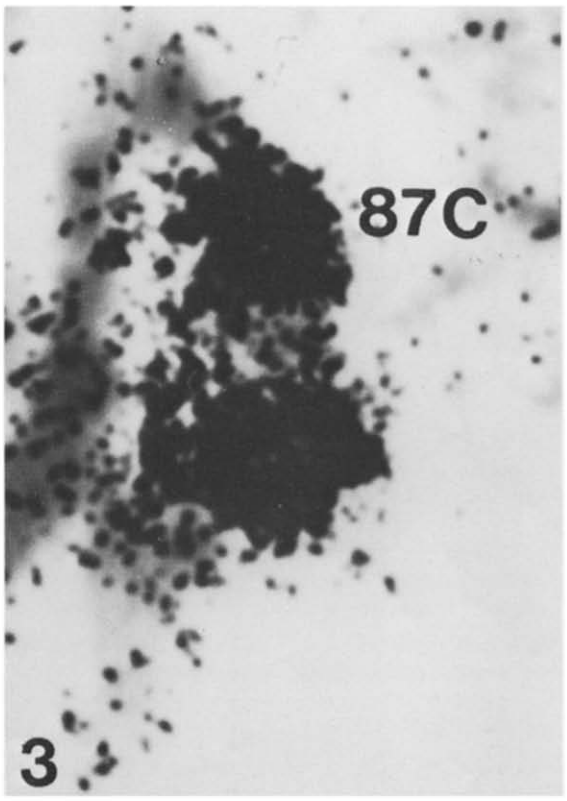
The effect of MTA on heat shock-specific RNA synthesis was determined by comparing autoradiographs of salivary glands labeled at 37°C in the presence or absence of the nucleoside. Of the 9 heat-inducible puffs in *D. melanogaster* we surveyed the heat shock loci designated 63BC, 87A, and 87Cl. Examination of the autoradiographs of MTA-treated glands reveals a reduction of silver grains over heat shock loci when compared to control glands (fig.2–5).

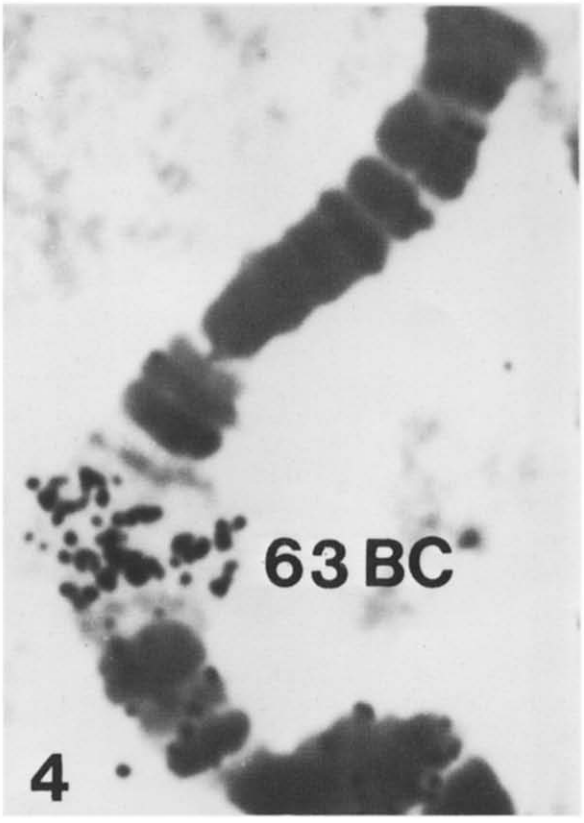
The kinetics of puff induction at 63BC and 87Cl in the presence or absence of MTA is shown in fig.6. In control glands, puffs appear very quickly after the transfer of glands from 23°C to 37°C, reach their maximum size in 20 min, then regress. Salivary glands incubated in the presence of MTA exhibit a 40–50% reduction in the maximum size attained after 20 min at 37°C, although the temporal pattern of puff induction followed by regression compares favorably to control glands.

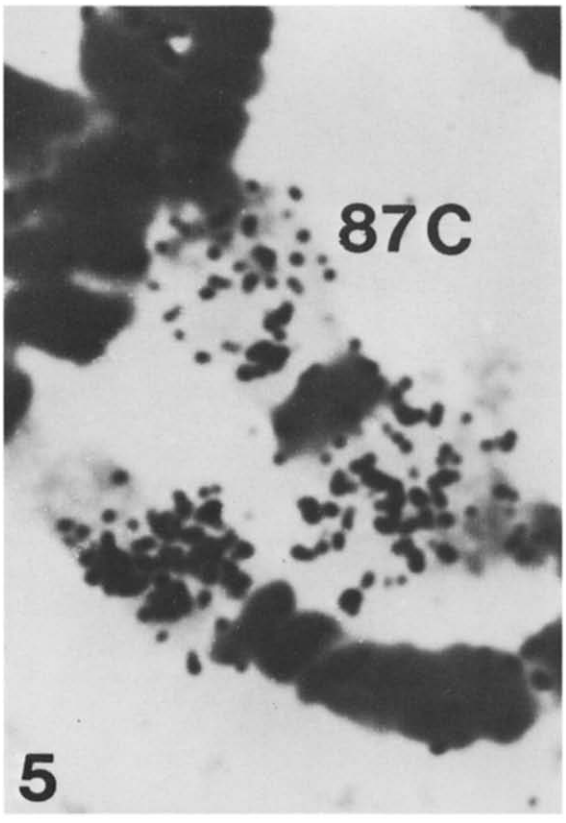
Polytene chromosomes have proven useful for the analysis of compounds that interfere with RNA metabolism [12–16]. The interest in describing the effects of MTA in this system stems from the fact that the nucleoside is naturally occurring and is of probable ubiquitous distribution in eukaryotic cells since it is a product of polyamine biosynthesis. In addition, it has been observed [17] that the polyamine spermidine is taken up by isolated *D. melanogaster* salivary glands, enters the cell nuclei, and results in a considerable enhancement in their incorporation of [^{3}H]uridine. This imparts

Fig.2–5. Effect of MTA on the incorporation of [^{3}H]uridine at heat shock loci 63BC and 87AC. Salivary glands were preincubated in Schneider's medium in the presence or absence of 1 mM MTA for 20 min at 37°C at which time [^{3}H]uridine was added to 50 μCi/ml final conc. Glands were incubated for an additional 20 min at 37°C, squashed and prepared for autoradiography. Fig.2, 63BC, no MTA; fig.3, 87AC, no MTA; fig.4, 63BC, with MTA; fig.5, 87AC, with MTA.


63BC
2


87C
3


63 BC
4


87C
5

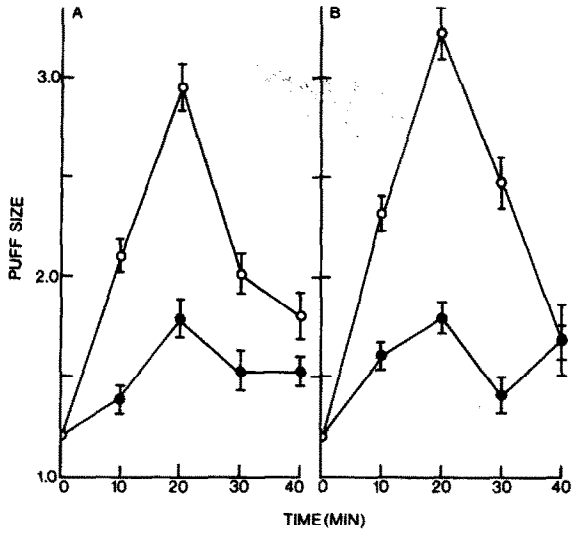


Fig.6. Effect of MTA on the kinetics of puff induction in heat-shocked salivary glands. Incubations were at 37°C in the absence (○——○) or presence (●——●) of 1 mM MTA. (A) 63BC; (B) 87Cl. Each point represents the mean ± SEM of 3 nuclei from each of 4 glands.

additional significance to the observation that MTA, the by-product of spermidine biosynthesis, inhibits salivary gland RNA synthesis.

Salivary glands and heat shock, in particular, can be employed to yield additional information on the inhibitory action of MTA. Further experiments will be required to ascertain whether MTA functions in vivo as a component in the cell's regulatory apparatus for the control of gene expression.

References

[1] Beermann, W. (1952) Chromosoma 12, 139–198.
[2] Ashburner, M. (1972) in: Developmental Studies on Giant Chromosomes. Results and Problems in Cellular Differentiation (Beermann, W. ed) vol. 4, pp. 101–151, Springer-Verlag, Berlin.
[3] Ritossa, F. (1962) Experientia 18, 571–573.
[4] Ashburner, M. (1970) Chromosoma 31, 356–376.
[5] Tissieres, A., Mitchell, H. K. and Trace, U. M. (1974) J. Mol. Biol. 84, 389–398.
[6] Lewis, M., Helmsing, P. J. and Ashburner, M. (1975) Proc. Natl. Acad. Sci. USA 73, 3604–3608.
[7] McKenzie, S. L., Henikoff, S. and Meselson, M. (1975) Proc. Natl. Acad. Sci. USA 72, 1117–1121.
[8] Spradling, A., Penman, S. and Pardue, M. L. (1975) Cell 4, 395–404.
[9] Spradling, A., Pardue, M. L. and Penman, S. (1977) J. Mol. Biol. 109, 559–587.
[10] Law, R. E., Sinibaldi, R. M., Cummings, M. R. and Ferro, A. J. (1976) Biochem. Biophys. Res. Commun. 73, 600–606.
[11] Schlenk, F., Zydek-Cwick, C. R. and Hutson, N. K. (1971) Arch. Biochem. Biophys. 142, 144–149.
[12] Beermann, W. (1971) Chromosoma 34, 152–167.
[13] Egyházi, E., Daneholt, B., Edström, J.-E., Lambert, B. and Ringborg, U. (1970) J. Cell Biol. 47, 516–520.
[14] Diez, J. L. (1973) Chromosoma 42, 345–358.
[15] Egyházi, E. (1975) Proc. Natl. Acad. Sci. USA 72, 947–950.
[16] Wiltenburg, J. and Lubsen, N. H. (1976) FEBS Lett. 70, 17–22.
[17] Dion, A. S. and Herbst, E. J. (1967) Proc. Natl. Acad. Sci. USA 58, 2367–2371.