DNA REPLICATION IN *CHIRONOMUS* POLYTENE CHROMOSOMES DURING TREATMENT WITH ETHANOL

ULF LÖNN

Department of Histology, Karolinska Institutet, S-104 01 Stockholm, Sweden

SUMMARY

DNA replication in *Chironomus* polytene chromosomes was investigated. The results show that during treatment with ethanol there is an accumulation of newly synthesized DNA with the size expected of replicons. These and previous results indicate that it is possible that in polytene chromosomes the termination of replication and merging of adjacent replicons is more sensitive to damage than the initiation and/or elongation steps.

INTRODUCTION

Polytene chromosomes in dipteran salivary glands are characterized by a specific and constant band–interband pattern. The band corresponds, at the chromatid level, to a region of highly compacted chromatin (the chromomere) followed (or preceded) by a non-compacted region (the interchromomere). On average, 95% of the DNA is located in the bands and 5% in the interbands (for references see Beerman, 1972). Moreover, recent results indicate that each band may consist of a cluster of several replication units (Lönn, 1981).

We have analysed the formation of double-stranded DNA replication intermediates in *Chironomus* polytene chromosomes previously (Lönn, 1980a). After a time-lag, the fragments are joined together to produce a high molecular weight DNA. There is no change in the formation of replication intermediates when animals of different developmental stages are analysed (Lönn, 1980b). However, when the animals are pre-treated with 5-fluorodeoxyuridine there is an accumulation of a double-stranded DNA fragment with the size expected of replication units (Lönn, 1981). This DNA fragment is formed by the joining together of the double-stranded DNA replication intermediates described earlier. Thus 5-fluorodeoxyuridine has little effect of the initiation and/or elongation steps but prevents the merging of adjacent newly synthesized replication units.

At present there is hardly any knowledge about the termination of replication and the merging of adjacent replication units in polytene chromosomes. It is possible that the band–interband structure of these chromosomes makes this step more liable to damage than the initiation and/or elongation steps. Therefore, we wished to compare the effects of treatment with 5-fluorodeoxyuridine and treatment with an unspecific agent like ethanol.
U. Lönn

Fig. 1A and B. For legend see opposite.
Fig. 1. Late fourth instar larvae of *C. tentans* were injected with 1 μl of a solution (0.67% NaCl, 0.04% KCl) containing 12% ethanol (overall body concentration about 0.4% ethanol), and then 60 min later injected a second time with 30 μCi tritiated thymidine dissolved in 1 μl of the above salt solution. After cultivation for a further 30 min (a), 6 h (b) or 48 h (c), the animals were killed and the excised salivary glands of each animal lysed. The labelled DNA was then fractionated in 0.75% agarose gels.

The controls were always cultured in parallel. The arrows indicate the position of marker *Chironomus* 28 S and 18 S ribosomal RNA localized with a Minuvue u.v. lamp. The migration of these RNA species was used to standardize the electrophoretic separation. ○—○. Ethanol-treated animals; ●—●, control animals.

**MATERIALS AND METHODS**

**Biological material and labelling conditions**

Larvae of *Chironomus tentans* were raised in the laboratory (Lönn & Edström, 1977). The average time of development from eggs to pupae is about 12 weeks. For the experiments late fourth instar larvae, 8 weeks old and weighing about 25 mg, were used.

To label the salivary gland DNA, the animals were injected into the haemolymph, with 30 μCi tritiated thymidine (24 Ci/mmol, Amersham/Searle Corp.) dissolved in 1 μl of 0.67% NaCl, 0.04% KCl. The injection was done with a de Fonbrune micromanipulator under the control of a stereomicroscope (Lönn, 1980a). To administer ethanol the animals were injected with 1 μl of the salt solution containing 12% ethanol (overall body concentration was about 0.4% ethanol).

**Extraction of DNA and agarose gel electrophoresis**

The excised salivary glands of each animal were immediately covered with 75 μl of a solution containing 0.05 M-Tris HCl (pH 7.4), 0.002 M-Na₂EDTA, 1% sodium dodecyl sulphate (SDS). After 3 h at 25°C the sample was transferred to the trough of an agarose flat-bed gel.

The electrophoretic separation of the DNA in 0.75% agarose flat-bed gels was performed as described earlier (Lönn, 1980a). Each lysate contained enough ribosomal 28 S and 18 S RNA.
to allow visualization with a Minuvis u.v. lamp. The migration of these RNA components was therefore used to standardize the electrophoretic separations. For size determinations linear DNA markers were run in a parallel lane of the same gel as described earlier (Lönn, 1980a, 1981).

RESULTS AND DISCUSSION

The existence of double-stranded DNA replication intermediates was first described in Chinese hamster ovary (CHO) cells by Taylor and co-workers (Taylor, 1973; Guy & Taylor, 1978). These DNA fragments are released from active replicons due to the action of endogenous enzymes at the moment of cell lysis. Once the cells are lysed and the DNA is in solution excision does not occur. The best way to prevent the release of the DNA fragments is to lyse the cells at 0 °C instead of 25 °C. After a time-lag the newly synthesized DNA is completely incorporated into the chromosomes and is not released from the chromosomes during cell lysis. The double-stranded DNA fragments detected in *Chironomus* polytene chromosomes show the same characteristics as those described in CHO cells (Lönn, 1980a).

To investigate the effects of ethanol on DNA replication in *Chironomus* polytene chromosomes and in order to be able to compare the results to those described earlier, the same experimental protocol was used as in the experiments with 5-fluoro-deoxyuridine published earlier (Lönn, 1981). Therefore, the animals were injected with ethanol and 60 min later injected with tritiated thymidine. Electrophoretic separation of DNA from animals injected with tritiated thymidine for 30 min showed that the labelled DNA is located heterogeneously between slices 33 and 47 (Fig. 1a). The location is the same as in the control DNA, which is run in a parallel lane of the same gel. However, in animals labelled for 6 h there is a difference between ethanol-treated and control animals; i.e. in the ethanol-treated animals, apart from the labelled DNA located at slices 33–47, there is also a peak of radioactivity located at slices 22–26, which is not present in control DNA (Fig. 1b). The control animals, on the other hand, contain labelled DNA located at slices 4–6, which is the location of stable and/or steady-state labelled high molecular weight DNA. When the animals are sacrificed after 48 h, there is a peak of radioactivity located at slices 22–26 in the ethanol-treated animals, with little or no radioactivity elsewhere in the gel (Fig. 1c). In the control the radioactivity is mainly located at slices 4–6.

The results indicate that in the ethanol-treated animals there is an accumulation of labelled DNA located at slices 22–26. This DNA is double-stranded according to CsCl-gradient centrifugation and is probably released enzymically from the chromosomes, since the release can be prevented by lysing the cells at 0 °C instead of 25 °C (results not shown). This characteristic, as well as the electrophoretic mobility of the slice 22–26 DNA, is the same as that described earlier for a DNA fragment detected during treatment with 5-fluorodeoxyuridine. The size of this fragment is very similar to the expected average size of the replicons in *Chironomus* polytene chromosomes (Lönn, 1981).

Thus, in contrast to the controls, the formation of replicon-sized DNA fragments is detectable in both cells treated with a specific agent (5-fluorodeoxyuridine) and cells
DNA replication in Chironomus chromosomes

277

treated with a non-specific agent (ethanol). In both situations this formation has the expected kinetics of low molecular weight DNA replication intermediates, indicating that the initiation and elongation steps are functioning. However, there is no merging of the newly synthesized replications to form high molecular weight DNA. Therefore, we believe that the results indicate that the termination of replication and the merging of adjacent replication units in polytene chromosomes are more sensitive to damage than the initiation and/or elongation steps.

This work was supported by grants from the Åke Wibergs Foundation, the Magnus Bergvalls Foundation and the Karolinska Institutet.

REFERENCES


(Received 28 April 1981)