A New Pathway Expressed during a Distinct Stage of Drosophila Development for the Removal of dUMP Residues in DNA*

(Received for publication, December 21, 1981)

Walter A. Deutsch$ and Andrea L. Spiering
From the Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana 70803

In view of removing lesions in DNA produced by the deamination of cytosine to uracil, uracil-DNA glycosylases were anticipated to be ubiquitous. However, an analogous activity in Drosophila melanogaster was not detected. Instead, a nuclease was identified that acts specifically upon DNA containing uracil. The cleavage of uracil-containing DNA by the nuclease generates acid-soluble oligonucleotides in a reaction which can be inhibited by pretreatment of the DNA with Escherichia coli uracil-DNA glycosylase. Uracil-containing DNA with either A:U base pairs or G:U base pairs were susceptible to cleavage by the nuclease, whereas other damaged DNA substrates were not. The nuclease activity is transient and appears only in third instar larvae, with other developmental stages of Drosophila lacking significant levels of the nuclease.

Uracil represents a nonconventional base in DNA that can arise either through the accidental misincorporation of dUMP residues instead of dTMP or by the spontaneous deamination of cytosine to uracil in DNA. The latter event is considered to be more harmful since it can lead to transition mutations. To counteract the presence of uracil in DNA, most organisms contain a uracil-DNA glycosylase, an activity which removes uracil in DNA by hydrolyzing the sugar-base glycosyl bond, forming an apyrimidinic site susceptible to base excision repair (1).

In the search for a uracil-DNA glycosylase activity in Drosophila melanogaster, we found instead a nuclease that specifically acts upon DNA containing uracil to produce acid-soluble oligonucleotides in a reaction similar to that identified for Escherichia coli endonuclease V (2). Additionally, we were able to detect the nuclease activity only in third instar larvae, as other developmental stages of Drosophila lacked significant levels of the nuclease.

EXPERIMENTAL PROCEDURES

Preparation of Third Instar Larval Extracts—Drosophila melanogaster (Oregon-R) embryos were collected 4 h subsequent to establishing a population cage. Embryos were washed with 0.01% Triton X-100, 0.7% NaCl, then repeatedly with H2O. The embryos were then transferred to a 15-ml polystyrene centrifuge tube using 70% ethanol. All subsequent operations were carried out in a sterile environment. After repeated washings in 70% ethanol, the embryos were collected on a 140-μm Nitex monofilament screen (Tetko, Inc.), divided, and introduced into sterile bottles containing sterile, dead yeast-sucrose medium (3). Incubations were at 25°C in a Forma diurnal growth chamber. Individual developmental stages were collected, identified (4), and washed as above. After the final 70% ethanol wash, the embryos were removed and replaced by 0.5 mM potassium phosphate, pH 7.5, 1 mM EDTA, and 25 μl of the protease inhibitor Aprotinin (Sigma) per ml of buffer. After removal of excess buffer, the individual developmental stages were stored at ~20°C.

RESULTS

We initially attempted, without success, to identify from crude extracts of Drosophila embryos an activity that would liberate acid-soluble material from uracil-containing T5 [3H]DNA. We then resorted to screening other developmental stages of Drosophila. Among the different developmental stages examined, the liberation of acid-soluble material from T5 [3H]DNA was detected only for crude extracts of third instar larvae. We subsequently made a more detailed analysis of different developmental stages of Drosophila to determine when the activity first appeared. We were additionally interested in determining whether the activity remained or alternatively became inactive at later developmental stages (Table I). Third instar larvae represent the first stage in the development of Drosophila in which the liberation of acid-soluble material from uracil-containing DNA can be detected. Furthermore, the activity is transient, as late pupal stages reflect little or no activity. Note that activity due to bacterial contamination would not be predicted to behave in this fashion. Also, we were able to detect activity in the absence of any divalent cation. As a result, our interpretation of those developmental stages that were indeed active was not compromised by non-specific DNases that require divalent cations, such as Mg2+, for activity.

We have additionally examined the germ-line tissue of Drosophila for the presence of an activity similar to that observed in third instar larvae. We were unable to demon-

* This work was supported by the Louisiana Agricultural Experiment Station and National Institutes of Health Grant GM 27358. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom reprint requests should be addressed.
**Drosophila Uracil Nuclease**

would liberate free uracil. The nuclease from third instar larvae was not attributable to a uracil-DNA glycosylase, 0.26 nmol of DNA nucleotide was liberated as acid-soluble material by E. coli uracil-DNA glycosylase; 0.26 nmol of DNA nucleotide was liberated as acid-soluble material in complete reaction mixtures for each developmental stage contained 2 to 3 pg of protein, except first instar incubations which had 0.8 pg of protein.

| Developmental stage | Age | Nanomoles rendered acid-soluble |
|---------------------|-----|--------------------------------|
| First instar larvae | 28  | 0.01                           |
| Second instar larvae| 54  | 0.01                           |
| Third instar larvae | 96  | 0.11                           |
| Pupae               | 192 | 0.10                           |
| Pupal/adult         | 239 | 0.02                           |
| Immature adult      | 263 | 0.02                           |
| Adult               | 335 | 0.04                           |

**DISCUSSION**

The apparent absence in *Drosophila* of a uracil-DNA glycosylase is to our knowledge unique. It is possible, however, that although the uracil-DNA glycosylase activity is easily demonstrated in both bacteria and mammalian cells, this may not be the case in *Drosophila*. Since *Drosophila* have a highly active nuclease to act on uracil-containing DNA, cleavage at uracil sites in the DNA may inhibit the DNA glycosylase from acting in vitro. Similar inhibition has been observed for purine base insertase acting on depurinated DNA subsequent to the exposure of the DNA to apurinic/apyrimidinic endonuclease (11). This does not explain the apparent absence of a uracil-DNA glycosylase activity in other developmental stages, however, where the nuclease appears to be inactive.

E. coli endonuclease V, which also shows a strong preference in vitro for uracil-containing DNA (2), provides a pre-
Drosophila Uracil Nuclease

Evidence with regard to an endonuclease degrading uracil-containing DNA to acid-soluble oligonucleotides. Endonuclease V does act on other damaged DNA substrates (e.g. apurinic/apyrimidinic sites in duplex DNA) that were not susceptible to the Drosophila activity. However, the preference of endonuclease V for uracil-containing DNA does not appear to be a major pathway in vivo (12).

Our results thus far indicate that uracil-containing DNA remains the only substrate acted on by the Drosophila nuclease and, furthermore, the nuclease acts regardless of how the dUMP was produced in the DNA. It is conceivable that dUMP residues in DNA arising from cytosine deamination are in part responsible for the presence of the nuclease activity observed in third instar larvae. One would predict that an activity directed toward this type of occurrence would remain active, particularly in adult germ-line, so that potentially mutagenic sites in DNA would not be passed on to the next generation. Alternatively, the nuclease may be produced in response to misincorporated uracil. With this in mind, it will be of interest to learn in future studies if Drosophila do incorporate a fairly significant level of dUMP into their DNA. If this is found to be the case, it may be that the incorporation of uracil into DNA is not a biological accident, but instead part of some cellular design (13) utilized by Drosophila that is facilitated by the cleavage at uracil sites in the DNA molecule.

Acknowledgments—We would like to thank Dr. James Boyd for suggesting the examination of third instar larvae, Dr. Huber Warner for his help and advice in preparing the T5 [3H]DNA, and Drs. Bruce Demple and Stuart Linn for communicating their results prior to publication. The technical assistance of Ms. Jane DeVasure and Ms. Jane Henry is gratefully acknowledged.

REFERENCES
1. Lindahl, T. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3649–3653
2. Gates, F. T., and Linn, S. (1977) J. Biol. Chem. 252, 1647–1653
3. Falk, D. R., and Nash, D. (1974) Genetics 76, 755–766
4. Ashburner, M., and Thompson, J. N. (1978) in The Genetics and Biology of Drosophila (Ashburner, M., and Wright, T. R. F., eds) Vol. 2a, pp. 2–109, Academic Press, New York
5. Spiering, A. L., and Deutsch, W. A. (1981) Mol. & Gen. Genet. 183, 171–174
6. Warner, H. R., Thompson, R. B., Mozer, T. J., and Duncan, B. K. (1979) J. Biol. Chem. 254, 7534–7539
7. Kuhnlein, U., Penhoet, E. E., and Linn, S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1169–1173
8. Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) J. Biol. Chem. 252, 3286–3294
9. Ljungquist, S. (1977) J. Biol. Chem. 252, 2808–2814
10. Lindahl, T. (1976) Nature 259, 64–66
11. Deutsch, W. A., and Linn, S. (1979) J. Biol. Chem. 254, 12099–12103
12. Tye, B. K., Chien, J., Lehman, I. R., Duncan, B. K., and Warner, H. R. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 233–237
13. Shlomai, J., and Kornberg, A. (1978) J. Biol. Chem. 253, 3305–3312

Downloaded from http://www.jbc.org/ by guest on March 20, 2020
A new pathway expressed during a distinct stage of Drosophila development for the removal of dUMP residues in DNA.

W A Deutsch and A L Spiering

J. Biol. Chem. 1982, 257:3366-3368.