Chromosomal Replication in *Drosophila virilis*

II. Organization of Active Origins in Diploid Brain Cells*

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Abstract. DNA fiber autoradiography was used to determine parameters underlying the DNA replication of the eukaryotic chromosome in *Drosophila* diploid brain cells in organ culture. The average rate of fork movement, estimated from 4 different labelling intervals, is 0.35 μm/min at 25 °C. Of the tandem arrays 93% show patterns which are compatible with bidirectional replication, 7% show unidirectional replication. The unidirectional mode of replication is interpreted as being a consequence of the experimental schedule (using hot-cold pulse labelling) combined with the occurrence of termination signals. – Some autoradiograms showed the expected two grain tracks of different densities; others showed only a high density track. The latter were most prominent in arrays of short replicons (<10 μm) which correlate with replicating satellite sequences. – The majority of replicons fall into size classes <100 μm. The frequency distribution is skewed towards larger replicon sizes; it spans 2–238 μm, has a mean of $\bar{x} = 35.6$ μm and a median of $\tilde{x} = 21.0$ μm. If the distribution is corrected for supposed satellite replicons, the median increases to $\tilde{x} = 31.0$ μm. – In experiments using warm-hot pulse labelling, arrays were scored which must have been a consequence of fixed termination signals. Furthermore, grain tracks diverging from weak labelled centers often have different lengths, indicating that these replicons contain two diverging replicating sections of unequal length.

Introduction

In eukaryotic chromosomes the DNA macromolecule is subdivided in at least two ways, the first expressing the linear arrangement of functional units, "genes", 

* Presented to Professor Dr. Wolfgang Beermann on the occasion of his 60th birthday with my best wishes
and the second, based upon the observation of multiple origins of replication, first shown by Cairns (1966) and Huberman and Riggs (1968). These authors were able to demonstrate that DNA molecules of several hundred micrometers length are subdivided into replication units, or replicons (Jacob and Brenner, 1963). The time needed for duplication of a eukaryotic chromosome is thus determined by three parameters: 1) rate and direction of the fork movement; 2) distance between replication origins and 3) timing of initiation at origins along the chromosome fiber. In general, replication proceeds bidirectionally from an origin via two diverging growing points. Two possibilities of the termination of chain growth have been discussed: 1) termination is due to fusion of the converging growing points; 2) fixed termination sites could provide stop signals for further chain elongation (reviewed in Sheinin et al., 1978).

It is believed by some authors that origins occur in clusters (reviewed in Edenberg and Huberman, 1975). In addition, some authors assume that in these clusters the origins are initiated synchronously; that is, clusters are believed to be units superior to replicons (reviewed in Hand, 1978).

In this study, DNA fiber autoradiography was used to determine replication parameters in diploid Drosophila brain cells labelled in organ culture. Two different pulse labelling protocols employing 3H-dThd were used: hot-cold and warm-hot. The first should allow determination of direction and rate of fork movement, as well as replicon length, while the second should provide further insight into the termination problem.

Materials and Methods

Dissecting and Labelling Conditions. D. virilis brains were dissected from mid 3rd instar Drosophila virilis larvae raised at 18 °C, using a light-dark cycle of 12 h each. The term "brain" refers to: both brain hemispheres usually including antenna and eye discs, the ventral ganglion or part of it, the ring gland, and variable amounts of other imaginal discs, e.g., leg discs. Brains were dissected in medium A (Steinemann, 1976). About 35 brains were transferred in a 120 μl drop of incubation medium B [60 μl 2 x medium A, 20 μl ATP (0.126 mg/ml), 20 μl Uridine (25 μg/ml), 20 μl FdU (1 μg/ml)], and were preincubated for 1 h (25 °C) in a moist chamber. The FdU preincubation was employed to exhaust the endogenous thymidine nucleotide pools but leave the brain cells unsynchronized.

Preliminary experiments with brains and salivary glands showed that 3H-Thd pulses as short as 1 min result in 3H-Thd labelling patterns in the chromosomes of both tissues. Long-term incubations of salivary glands and brains showed that 3H-dTh incorporation occurs throughout the periods tested: 8 h for brains and 24 h for salivary glands. This was assayed by TCA precipitable counts on Whatman GF 82 filters, after FdU preincubation of varying lengths followed by 3H-Thd pulse labelling. Autoradiographs of squash preparations, done in parallel (not shown) with the DNA fiber autoradiographs showed the expected labelling patterns (Steinemann, 1981).

Labelling Schedule of the D. virilis Brains. After the preincubation, labelling protocols a or b were used (Fig. 1). In protocol a, the hot pulse of 3H-dThd (54 Ci/mmol, 125 μCi/ml) was given for 5, 10, 30 or 60 min. The hot pulse was stopped in ice and the brains were transferred to the cold medium which contained unlabelled dThd at 10 times the concentration of the labelled precursor. The cold pulse length was kept constant at 45 min. FdU was left in the medium during the labelling period to increase the incorporation of exogeneous 3H-dTh or dTh respectively. The cold pulse was again stopped in ice, the brains were transferred to ice cold Ringer's solution,