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Synchrony of Replication in Sister Salivary Glands of *Drosophila kikkawai*

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Intergland synchrony of replication in the larval salivary glands of *D. kikkawai* has been studied by ^{3}H-thymidine pulse labelling and very light squashing. It is observed that there is a striking synchrony of replication between the 2 sister glands with respect to the position of the replicating nuclei and the frequency of different labelling patterns. There is also considerable synchrony within a gland where a group of neighbouring nuclei show similar kind of labelling. These observations suggest that the replication cycles of different polytene nuclei in the two sister salivary glands of a larva are developmentally determined.

CYCLIC replication in polytene cells of *Drosophila*[1-3] provides a useful system to analyse the factors regulating replication in higher organisms. At the chromosomal level, there is evidence that different units of a polytene chromosome replicate in a temporally regulated sequence[1,3,4]. However, comparatively less information is available on the regulation of polytenic replication cycles of different nuclei at the organ level[1]. In the present study, an attempt has been made to examine distribution of ^{3}H-thymidine labelled nuclei in the 2 sister salivary glands of *D. kikkawai* larvae with a view to ascertaining if the polytenic replication cycles are temporarilly and spatially regulated in a co-ordinated manner in the 2 sister glands of a larva.

Wild strain (from Brazil) of *D. kikkawai* was used for these studies. The flies and larvae were reared on standard *Drosophila* culture medium at 20° ± 0.5° C. The 3rd instar larvae in this species, initially have pigmentless (white) anterior pair of spiracles; during their growth, anterior pair of spiracles in the 3rd instar larvae changes colour first to brown and then to black and this provides a very easy marker to identify the developmental stage of a larva. The black spiracle stage lasts for about 24 hr (at 20°C) when the larvae begin to pupate. For the present study, 3rd instar larvae with black spiracles were used. Salivary glands were carefully dissected out from larvae and the paired glands were incubated in Ringer's solution containing ^{3}H-thymidine (Sp. act.—250 μCi/ml; sp. act., 10.4 Ci/m*M*; obtained from BARC, Trombay). After labelling for 10 min the glands were fixed briefly in 1 : 3 aceto-methanol and stained with aceto-carmine-orcein. The paired glands were now put on a clean slide in a drop of 50% acetic acid; the glands were placed with similar orientation so that the anterior ends of the two glands would point to same side. These were covered with a coverglass and squashed very gently so that the cells just disrupted without losing *in situ* topographical relationship with their neighbouring cells. Coverslips were removed[5] and those preparations were selected in which the majority of material of both the glands was either on the slide or on the coverslip. These were restained with Carbol-Fuchsin and coated with Ilford L4 emulsion for autoradiography. After an exposure of 9 days (at 4°—6°C), the preparations were developed with Kodak D19b developer (4 min at 18°C), fixed in acid fixer, washed, mounted and examined under microscope. Distribution of differentially labelled or unlabelled nuclei in the 2 sister glands of a larva was plotted on a paper, taking care to maintain the spatial relationship of different cells as in the preparations (Figs 1 and 2). The labelling pattern of each nucleus was identified under 100× oil immersion objective.

Autoradiographic preparations from 6 pairs of sister salivary glands have been examined. ^{3}H-thymidine labelling patterns in these nuclei have been grouped into the following categories[6] : (i) Interband type (corresponding to disperse discontinuous type of Mukherjee and Chatterjee[7]) : labelling is restricted to a few or majority of interbands and puffs; the dark bands and chromocentre are relatively unlabelled,

Figs 1 and 2 — Diagramatic representation of ^{3}H-thymidine autoradiographs of lightly squashed salivary gland pairs from 2 larvae of *D. kikkawai* to show the topographical distribution of different ^{3}H-thymidine labelling patterns in the sister glands (a and b, respectively) [Anterior end of each gland is facing upwards. Different types of labelling patterns are represented as follows : ●, interband; ⊙, 2C; ■, 3C; □, 3D; ▲, 2D, △, 1D; and ○, unlabelled]

(in the present analysis, we have not differentiated into different categories of interband type of labelling since the poor spreading of nuclei obtained with light squashing does not permit their precise subgrouping); (ii) continuous type : all bands, interbands, puffs and chromocentre are labelled; following Rodman[8], two continuous types of labelling—mid (2C) and heavy (3C)—have been recognised; and (iii) discontinuous type : labelling is restricted mainly to dark bands and chromocentre (some puff/interband sites are also labelled); again following Rodman[8], these discontinuous types are further sub-grouped into heavy (3D), mid (2D) and low (1D) categories on the basis of grain density and the number of sites labelled.

Data on the frequency of occurrence of different types of labelling patterns in the 6 pairs of sister glands are presented in Table 1. Figs 1 and 2 show diagrammatic representation of topographical distribution of differently labelled nuclei in sister glands from 2 representative larvae. It is observed that the frequency of different labelling patterns in the 2 sister glands of a larva is generally similar. It may be pointed out that the total number of nuclei seen in the 2 glands of a preparation varies to some extent; this is unavoidable since some nuclei would always be lost during the removal of coverslip and subsequent processing. This same factor can also explain the variations noted in some sister gland pairs with respect to the frequency of specific labelling patterns. An examination of the topographical distribution of differently labelled nuclei in the sister glands (Figs 1 and 2) clearly reveals that nuclei with similar labelling patterns are present in comparable anatomical locations in the 2 glands of a larva. The process of squashing would, no doubt, alter the topographical relation of different nuclei of a gland to some extent, but this is minimized by the gentle squashing technique followed here. As such the distribution of nuclei in these squash preparations would indicate the relative position occupied by these nuclei in the intact glands. It was observed in all the 6 pairs of glands that the label distribution in sister glands was similar in antero-posterior direction. The present data thus show that the sister glands replicte synchronously so that the type of labelling patterns that are seen in one gland are also seen in its counterpart and also the distribution of these labelled nuclei in the 2 glands is mirror-image like.

It may also be noted from data in Table 1 and Figs 1 and 2, that in a given pair of glands, specific type of labelling patterns are present with a greater frequency while some other types are very few or absent. Furthermore, nuclei with similar labelling occur in groups in specific regions of the glands. This may indicate a synchrony of the polytenic cycles of replications in a larva depending on its developmental stage.

In this study, an attempt has been made to ascertain if developmental or other regulatory factors determine in a co-ordinated manner the polytenic replication cycles in different nuclei of a pair of salivary glands. Several earlier studies (reviewed in Rudkin[1]) have suggested that the time of ecdysis influences the initiation of new replication cycles in salivary gland nuclei. This would introduce at least some synchrony of replication among the polytene nuclei of 3rd instar larvae. Arocos-Teran[9] reported that in the squash preparations of ^{3}H-thymidine labelled salivary glands of *D. melanogaster*, similarly labelled nuclei were often in groups—indicating some regulatory factors determining the replication cycles of adjoining cells. We confirm this observation and we further show a complete identity of replication in the 2 sister salivary glands of a larva of *D. kikkawai*.

TABLE 1 — PERCENTAGE OF DIFFERENT ^{3}H-THYMIDINE LABELLING PATTERNS IN THE SISTER SALIVARY GLANDS OF THE 6 LATE 3RD INSTAR LARVAE OF *D. kikkawai*

Larva No.	Gland No.*	Percentage of different labelling patterns						Unlabelled	Total No. of nuclei
		Interband	Continuous		Discontinuous				
			2C	3C	3D	2D	1D		
1	a	63.96	14.41	2.7	0	0	0	18.92	111
	b	73.91	12.17	1.74	0.87	0	0.87	10.44	115
2	a	61.61	9.09	2.02	1.01	0	0	26.26	99
	b	45.97	13.79	1.15	1.15	1.15	0	36.78	87
3	a	1.21	0	0	0	0	0	98.78	83
	b	5.26	0	0	0	0	0	94.73	76
4	a	9.3	2.32	0	0	0	0	88.37	43
	b	20.36	1.85	0	0	0	0	77.78	54
5	a	4.69	0	0	0	0	0	95.31	64
	b	2.38	2.38	0	0	0	0	95.24	42
6	a	34.55	3.64	1.81	0	0	0	60.00	55
	b	31.82	0	0	0	0	0	68.18	88

*Gland Nos. a and b represent the two sister glands of respective larva

Presence of similar labelling patterns in the cells of 2 glands at similar locations suggests that the polytenic replication cycles of different nuclei are developmentally determined such that groups of cells in comparable position in the sister glands respond in a like manner to the regulating factors.

An overall greater synchrony of replication in different nuclei of a pair of salivary glands is another feature of *D. kikkawai*. Partial synchrony of replication in different nuclei of salivary glands in other *Drosophila* species has been reported[1]. However, in *D. kikkawai* this synchrony seems to be much more pronounced than in other species. Thus in any one larva, depending on its age, either early, mid or late–S labelled patterns are predominantly seen. This would suggest that at specific developmental stages, polytenic cycle is initiated synchronously in larger number of adjoining cells in both the glands and that these nuclei progress through the S at comparable rates. Synchrony of adjoining cells would suggest that there may be "comparatments" of cells in a gland that have similar replicative physiology.

The information obtained in this study about the similarity of sister glands with respect to their polytenic replication cycles, would be very useful in several experimental studies, since the sister glands have similar replicative behaviour, one of the two glands can serve as an ideal control while the other gland may be exposed to any experimental treatment to probe various aspects of replication in these cells.

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