Chromosoma (Berl.) 84, 33–47 (1981)

CHROMOSOMA

DNA Replication in Polytene Chromosomes:

Similarity of Termination Patterns in Somatic and Germ-line Derived Polytene Chromosomes of *Anopheles stephensi* Liston (Diptera: Culicidae)

C.P.F. Redfern[1]

Department of Genetics, University of Cambridge, Downing Street, Cambridge, England;
[1] Present address: Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, Scotland, U.K.

Abstract. DNA replication is initiated in the ovarian follicles of the anautogenous Culicid *Anopheles stephensi* in response to a blood meal. The chronology of nurse cell polytene chromosome ^{3}H-thymidine labelling patterns has been defined and the terminal labelling patterns of the nurse cell and larval salivary gland polytene X-chromosomes have been compared and found to be essentially similar.

I. Introduction

Early studies on DNA replication in polytene chromosomes demonstrated that ^{3}H-thymidine-labelled chromosomes could be assigned to one of two classes on the basis of label distribution (Rudkin and Woods, 1959). Discontinuous labelling patterns were interpreted as evidence for autonomously replicating units (Plaut et al., 1966) and were assumed to represent the terminal phase of the replication cycle (Gabruscewycz-Garcia, 1964; Rudkin, 1972). More detailed analyses have since revealed two complementary series of discontinuous labelling patterns and polytene chromosome labelling patterns are now usually interpreted in terms of three replicative phases: an initial discontinuous labelling phase, a continuous labelling phase and a discontinuous terminal phase (Rudkin, 1972; Kalisch and Hägele, 1976).

On the basis of these observations the polytene chromosome band has been proposed to represent a replicative unit or replicon (Beermann, 1965; Pelling, 1966; and see Laird, 1980). Although estimates of replicon size have provided indirect evidence for the equivalence of replicons and bands (Blumenthal et al., 1973; Lönn, 1981), a *precise* correlation between the replication period and DNA content of bands has not been found (Hägele, 1973; Ananiev and Barsky, 1975). In view of the fact that, in general, replicon size may vary with developmental stage (Blumenthal et al., 1973), culture conditions (Ockey, 1979) and may vary between different tissues (Callan, 1972) and different classes of DNA (Zakian, 1976) it is clearly of interest to determine whether or not the polytene

0009-5915/81/0084/0033/$03.00

chromosome banding pattern determines, or results from (Laird, 1980), the pattern of chromosome replication. The polytene chromosomes in the larval salivary glands and ovarian nurse cells of *Anopheles stepehensi* have essentially the same band-interband organisation (Redfern, 1981b) and in this study the ^{3}H-thymidine labelling patterns of these chromosomes have been compared in an attempt to examine the constraints of polytene chromosome structure on replicative organisation.

II. Materials and Methods

Animal Culture. Anopheles stephensi stephensi (ST strain – Delhi, 1947) larvae and adults were cultured as previously described (Redfern, 1981a). Anautogenous females of *Anopheles stephensi* initiate ovarian maturation in response to a blood meal. Since egg production is proportional to the weight of the blood meal (Reisen and Emory, 1976) and probably also to female size (for example Service, 1977) and there is evidence that blood meal weight may influence the developmental timing of oogenesis (Gillett, 1957), additional precautions were taken in order to obtain females of consistent and optimal quality with regard to the timing and synchrony of ovarian development. Larvae were cultured at an uncrowded density (Reisen and Emory, 1976) of 0.5 ± 0.05 larvae cm^{-2} at 25° C and were "maximally fed" (Redfern, 1980) twice daily. Virgin adult females were used for all experiments. Females were starved of sucrose for 24 h before the blood meal since the presence of sucrose within the ventral diverticulum can reduce blood meal size (Reisen and Emory, 1976). Experimental animals, 5–8 days post-eclosion, were allowed to feed for 15 min on anaesthetised guinea pigs (Redfern, 1981a). Time-zero was taken as the end of the feeding period.

^{3}H-Thymidine Labelling. Ovaries or 4th larval instar salivary glands, dissected in saline solution (Becker, 1959), were labelled by incubation in a modified Graces medium (Redfern, 1981b) containing methyl-^{3}H-thymidine at a concentration of 12 μCi/ml (Amersham: 48 Ci/mmole) or 24 μCi/ml (Amersham: 40 Ci/mmole), respectively, for 12 min at 25° C.

Cytological Methods. Cultured larval salivary glands were fixed and squashed as described in Redfern (1981a). Cultured ovaries were fixed in ethanol: acetic acid (3:1) fixative (0.5–1 min), stained by transfer to 1% lacto-acetic-orcein (Redfern, 1981b) (0.5–1 min) and squashed onto subbed slides. Siliconised coverslips were removed with liquid nitrogen and the preparations dehydrated in 95% ethanol. Slides containing larval salivary gland material were stored dry whereas slides with ovarian material were stored in 100% ethanol (1–2 days), rehydrated through an alcohol series to reduce distortion and then processed for autoradiography. Slides were coated with Ilford L4 nuclear emulsion (Redfern, 1981a), exposed for 18 days at −20° C and developed in Kodak D19 developer. Slides were exposed at −20° C in order to preclude chemographic effects in the lacto-acetic-orcein stained preparations (Rogers, 1967) and unlabelled, stained preparations were included for each series to control for such effects. No chemographic effects were observed. Larval salivary gland autoradiographs were post-stained with Giemsa (Pardue and Gall, 1975). Autoradiographs were examined and photographed with a Zeiss GFL microscope (Pan X film, 50 ASA). All label was sensitive to DNase I (Sigma) digestion using the method of Bancroft (1975).

Analysis of Autoradiographs. The time-course of DNA replication in ovarian tissue was assessed by scoring follicle epithelial cell (FEC) and nurse cell (NC) nuclei in squash preparations as labelled or unlabelled. FEC nuclei with a minimum of 10 silver grains above background were scored as labelled. Due to the variable morphology of polytene nuclei, the criterion of labelling was necessarily less precise. Polytene nuclei with heavily labelled chromosome arms or with label localised to particular regions were scored as labelled. Those polytene nuclei with more diffuse labelling were classed as labelled if the label density over the chromosome arms was greater than adjacent background.

Labelled polytene nuclei were further subdivided into continuous and discontinuous labelling categories: nuclei that were either diffusely labelled or heavily labelled throughout the length of

the chromosome arms were classified as "continuously labelled" whereas those nuclei with a group or group of bands intensely labelled with respect to groups of unlabelled bands and interbands were scored as "discontinuously labelled".

FEC were scored as labelled or unlabelled from a minimum of 10 random high power ($\times 100$ objective) fields for each of 4–5 squash preparations (each from one female) per time-point. All polytene nuclei encountered during systematic scanning of squash preparations were scored.

For a detailed analysis of the polytene X-chromosome replication patterns the X-chromosome was subdivided into 23 regions (Fig. 3) according to their cytological visibility in Giemsa or orcein-stained autoradiographs. Following the protocol of Kalisch and Hägele (1973) chromosomes were scored for the presence or absence of label in each region and a region was scored as labelled if it contained a *cluster* of 3 or more silver grains.

III. Results

1. DNA Replication in the Ovarian Follicle

Each *Anopheles stephensi* ovariole, of which there may be over 100 per pair of ovaries, consists, in the adult, of a germarium, primary follicle and penultimate or secondary follicle. The follicle consists of seven nurse cells and an oocyte surrounded by a layer of follicle epithelial cells (FEC) and after eclosion the primary follicles develop, synchronously, to a resting stage at which the oocyte forms approximately half or less of the follicle volume. At the resting stage FEC nuclear volume is consistent with a 2C DNA value (Redfern, 1981a) and the nurse cell (NC) polytene chromosomes are small, tightly coiled and of poor cytological quality. Synchronous maturation of the primary follicles and concomitant development of the secondary follicles to the resting stage is initiated by blood-feeding and analysable polytene chromosomes may be prepared from approximately 12 h post-blood-meal (PBM) until approximately 36 h PBM (at 25° C); after this time the chromosomes become increasingly pycnotic. Growth of the primary follicle during blood-meal-stimulated maturation is, in the early stages, partly due to an increase in nurse cell bundle volume (Redfern, 1980 and in prep.). The increase in size and cytological quality of the NC chromosomes, together with the finding that NC and FEC nuclear volume increases during the first 24 h PBM (Redfern, 1980), strongly suggests that DNA replication is initiated in response to a blood-meal.

To confirm that DNA replication is initiated in NC and FEC in response to a blood meal, ovaries were dissected and labelled with ^{3}H-thymidine in vitro at 2 h intervals PBM, squashed and autoradiographed. The autoradiographs were analysed as described in Materials and Methods. The mean frequency of labelled FEC and NC nuclei increases rapidly after a blood meal with an approximately similar time-course for the two types of nuclei, reaching a peak after 10–12 h PBM (Fig. 1). Thereafter, the frequency of labelled NC nuclei remains at an approximately constant level with some evidence of a decline at about 34 h PBM. The time-course for the frequency of labelled FEC nuclei is more complex and at least two peaks of replicating nuclei, occurring at 10 h and 22 h PBM, are visible. Although FEC nuclear volume measurements are consistent with the occurrence of at least two replication cycles PBM (Redfern, 1980) it should be pointed out that whole ovary squashes also include FEC from the penultimate follicles, developing to the resting stage, and the

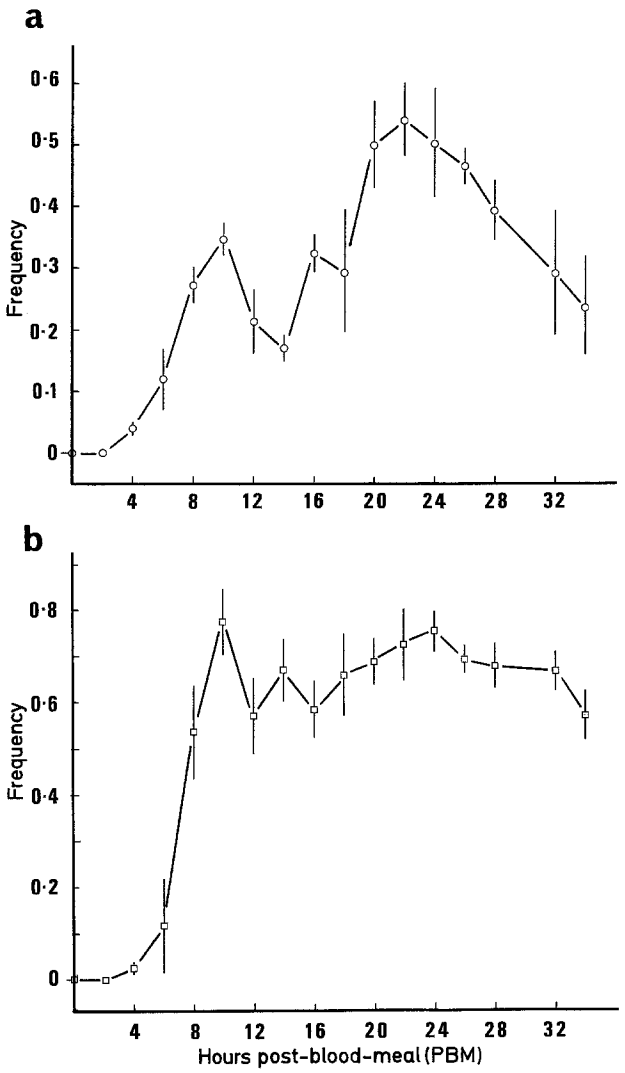

Fig. 1a and b. The mean frequency of **a** labelled follicle epithelial cell and **b** polytene nurse cell nuclei in squash preparations (4–5 females per time point). Vertical bar: ±1 S.E.M.

data consequently represent at least two populations of cells. Similar considerations apply to NC nuclei although these are small in early penultimate follicles and would not have been scored as polytene nuclei until late in the first gonotrophic cycle. These data thus demonstrate that DNA replication is initiated in the tissues of the ovarian follicle in response to a blood-meal.

2. Late Replicating Patterns in Polytene Nuclei

If it is assumed that a DNA replication cycle goes to completion once initiated, then it may be concluded from the above data that new replication cycles are initiated, from the resting stage, in response to a blood-meal. Some evidence

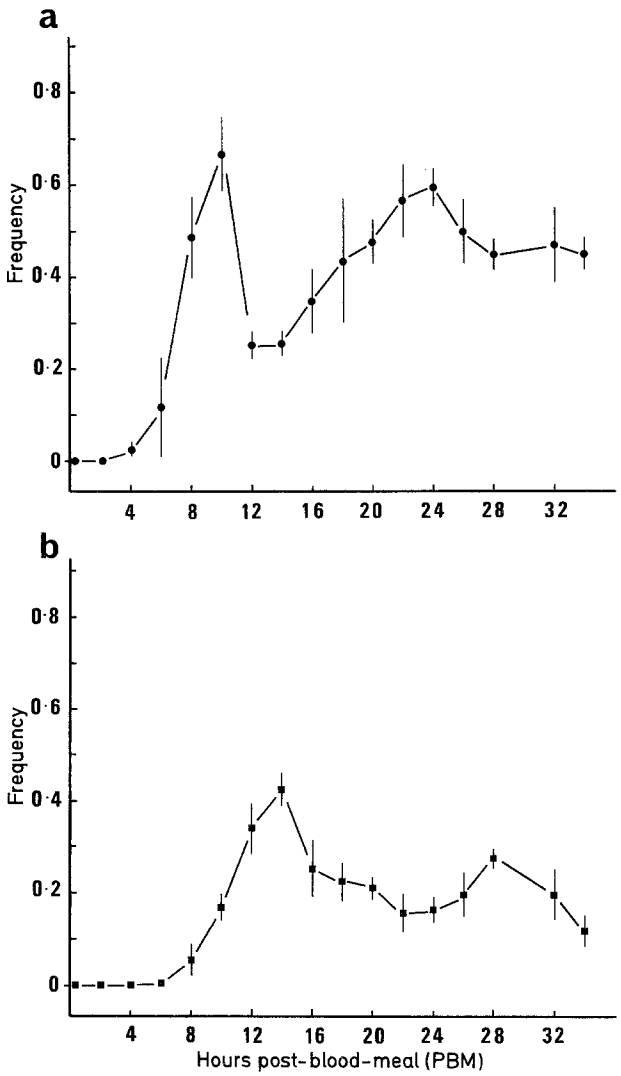

Fig. 2a and b. The mean frequency (as a proportion of the total nuclei scored) of **a** continuous and **b** discontinuous labelled polytene nuclei in squash preparations (4–5 females per time-point). Vertical bar: ±1 S.E.M.

that this assumption is reasonable is discussed by Ord (1979). Consequently, it will be possible to define the chronological sequence of DNA replication in nurse cell polytene chromosomes in terms of continuous and discontinuous labelling patterns, with the further assumption that rounds of chromosomal replication are non-overlapping.

Labelled polytene nuclei in ovary squashes were classified as continuously or discontinuously labelled according to the criteria described above. The time-course of the mean frequency of each of these classes of labelled nuclei, expressed as a proportion of the total polytene nuclei scored (per squash preparation), is shown in Figure 2. Continuously labelled nuclei are first detectable at 4–6 h

PBM and rapidly increase in frequency to a peak at 10 h PBM, before declining between 12–16 h PBM. Discontinuously labelled nuclei are detected slightly later at 8–10 h PBM and reach a peak frequency at 14 h PBM. These data indicate that discontinuously labelled nuclei, defined using the above criteria, represent nuclei in a terminal phase of the replication cycle. It should be noted that the criteria for "continuous labelling" would include diffusely labelled nuclei with label generally confined to interbands and puffs which, in other species, are reported to be characteristic of an early phase in the polytene chromosome replication cycle (Gabruscewycz-Garcia, 1964; Howard and Plaut, 1968; Kalisch and Hägele, 1976). Discontinuously labelled nuclei are first detected approximately 4 h later than continuously labelled nuclei and, with respect to the above assumptions, this indicates that the S-period of NC polytene nuclei may be of the order of 4–6 h if the discontinuous labelling phase is short relative to the continuous labelling phase (see below). This estimate is of a similar order, at least within the resolution of the method, to the value of 7.5 h estimated by Mulder et al. (1968) for the S-period of *Drosophila hydei* larval salivary gland nuclei.

3. Patterns of Labelling in the Polytene X-Chromosome

The polytene X-chromosome of *Anopheles stephensi* may be readily identified on the basis of its length (of the order of 80 μm in NC nuclei) and puffing characteristics in NC and larval salivary gland (SG) nuclei (Redfern, 1981b) and is thus amenable to a detailed analysis of polytene chromosome replication patterns. The limited resolution of light microscope autoradiography and the quality of polytene chromosome preparations after processing for autoradiography precludes, in general, analyses of the replication characteristics of single bands (for example see Kalisch and Hägele, 1976) and thus, for the purpose of this analysis the X-chromosome was divided into regions (Fig. 3) on the basis of their cytological visibility. Consequently, there will be a variable number of bands per region (estimated mean: 4 bands per region) and some regions (for example: 10, 12, 15 and 18) may contain a small number of dense bands whereas others may consist of a larger number of fine bands.

a) Nurse Cell X-Chromosome

A total of 235 NC X-chromosomes from preparations made at 20–34 h PBM were scored for the presence or absence of label in each region. All X-chromosomes encountered were scored where possible. A chromosome with all regions labelled will be defined here as continuously labelled (Fig. 4) and 155 of the NC chromosomes scored fell into this category. The remaining 80 "discontinuously labelled" chromosomes could be divided into two groups on the basis of the criteria used for analysis of the chronology of replication patterns (previous section): a group of relatively diffusely labelled chromosomes (26) in which label is predominantly over fine bands and "interbands", major bands remaining unlabelled, and a group of chromosomes (54) conforming to the

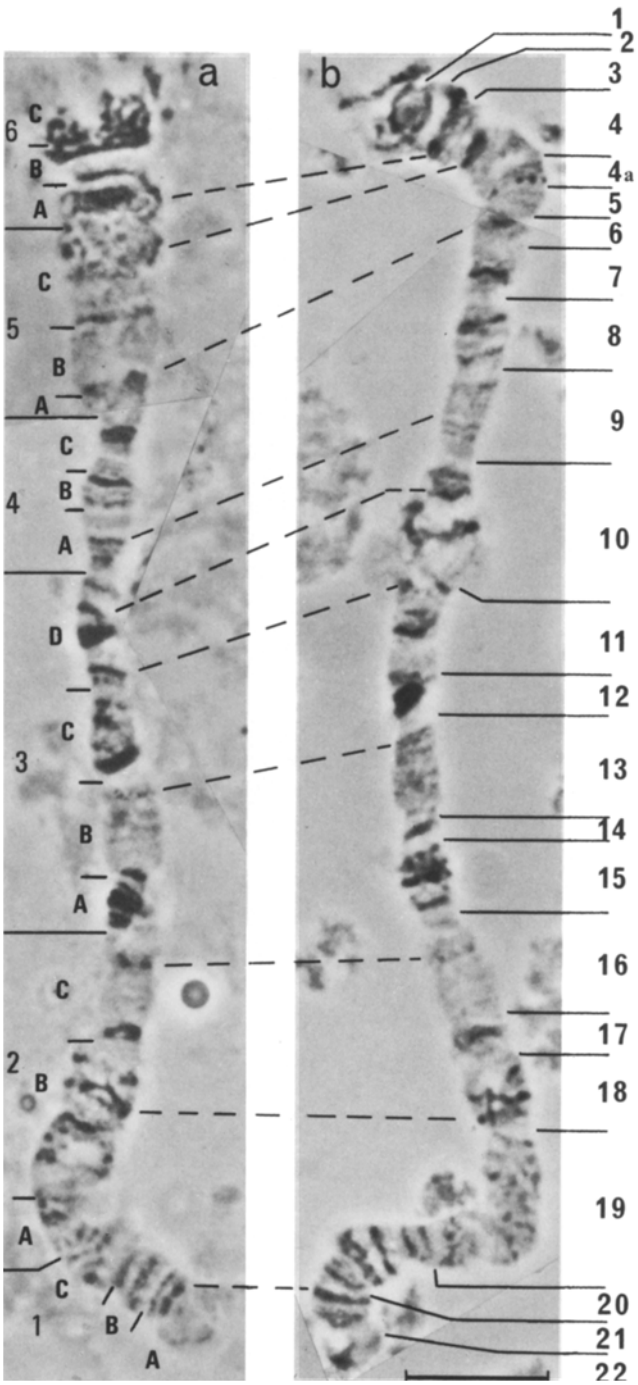

Fig. 3a and b. Polytene X-chromosomes from **a** nurse cells and **b** larval salivary gland cells (from Redfern, 1981 b) divided into 23 intervals (*left*) for an analysis of labelling patterns

definition of a "discontinuously labelled" chromosome (given in Materials and Methods) (Fig. 4). From the data of the previous section this latter group represents chromosomes in the terminal phase of replication and in this group regions 10, 12 and 15, which contain dense bands, are labelled at high frequency (Fig. 5). Conversely, the low intensity or absence of label in these three regions is characteristic of the former group of diffusely labelled chromosomes which may thus represent chromosomes in an initial phase of replication. Since the frequency of continuous and discontinuously labelled nuclei (definition of previous section) varies only slightly during the 20–34 h PBM period, the duration, as a proportion of the total S-period, of the initial, continuous (or propagative) and terminal replicative phases may be estimated from the proportion of chromosomes falling into each labelling category. These proportions are 0.11, 0.66 and 0.23 for the initial, continuous and terminal labelling patterns, respectively, and for a 4–6 h S-period this represents an initiation period of 30–40 min, an estimate comparable to the 30 min required for the "initiation" of all sites in *Chironomus* polytene chromosomes (Pelling, 1966).

The data for the labelling patterns of the 26 initial phase and 54 terminal phase chromosomes are summarised in Figure 5: chromosomes have been arranged in order of the number of sites labelled with initial phase chromosomes in ascending order and terminal phase chromosomes in descending order (method of Zhimulev and Kulichkov, 1977). As has been pointed out for this type of analysis (Rudkin, 1972; Zhimulev and Kulichkov, 1977) discontinuities in the "histograms", at least in the terminal phase chromosomes, are mainly present at the end of the replication period of particular regions and may represent variation in the timing of the termination of replication or variation due to the stochastic nature of the autoradiographic method. The data clearly show that some regions are labelled at higher frequency than others and this does not result from chance effects since there is a heterogenous frequency distribution of unlabelled scores (initial and terminal phase chromosomes combined, Index of Dispersion test: $\chi^2_{(22)}=152.3$, $p<0.001$).

The majority of regions are labelled initially at approximately the same time. Regions 2, 3, 10, 12 and 15 are labelled slightly later than other regions in the initial phase of replication and regions 1, 10, 12, 15 and 18, at least, are among the last to complete replication. In general, regions containing dense bands, for example regions 1, 4, 10, 12 and 15, have a longer replication period than other regions. The data of Sharma et al. (1978) for *Anopheles stephensi* indicate that regions 10 and 12 (equivalent to 3D and 3C of Sharma et al., 1978) have a high induced breakpoint frequency relative to the rest of the X-chromosome. "Late replication" behaviour and high induced breakpoint frequencies are characteristic of "intercalary heterochromatin" (Hannah, 1951; Ashburner, 1966; Zhimulev and Kulichkov, 1977) and the dense bands in regions 10 and 12 may thus represent intercalary heterochromatin. Other equivalent late-replicating loci in the *A. stephensi* polytene complement include the centromeric tips of 3L, 3R and 2R and one of these late-replicating loci, the chromatin block at the centromeric end of 3L, contains sequences complementary to an *A. stephensi* satellite DNA (Redfern, 1981a).

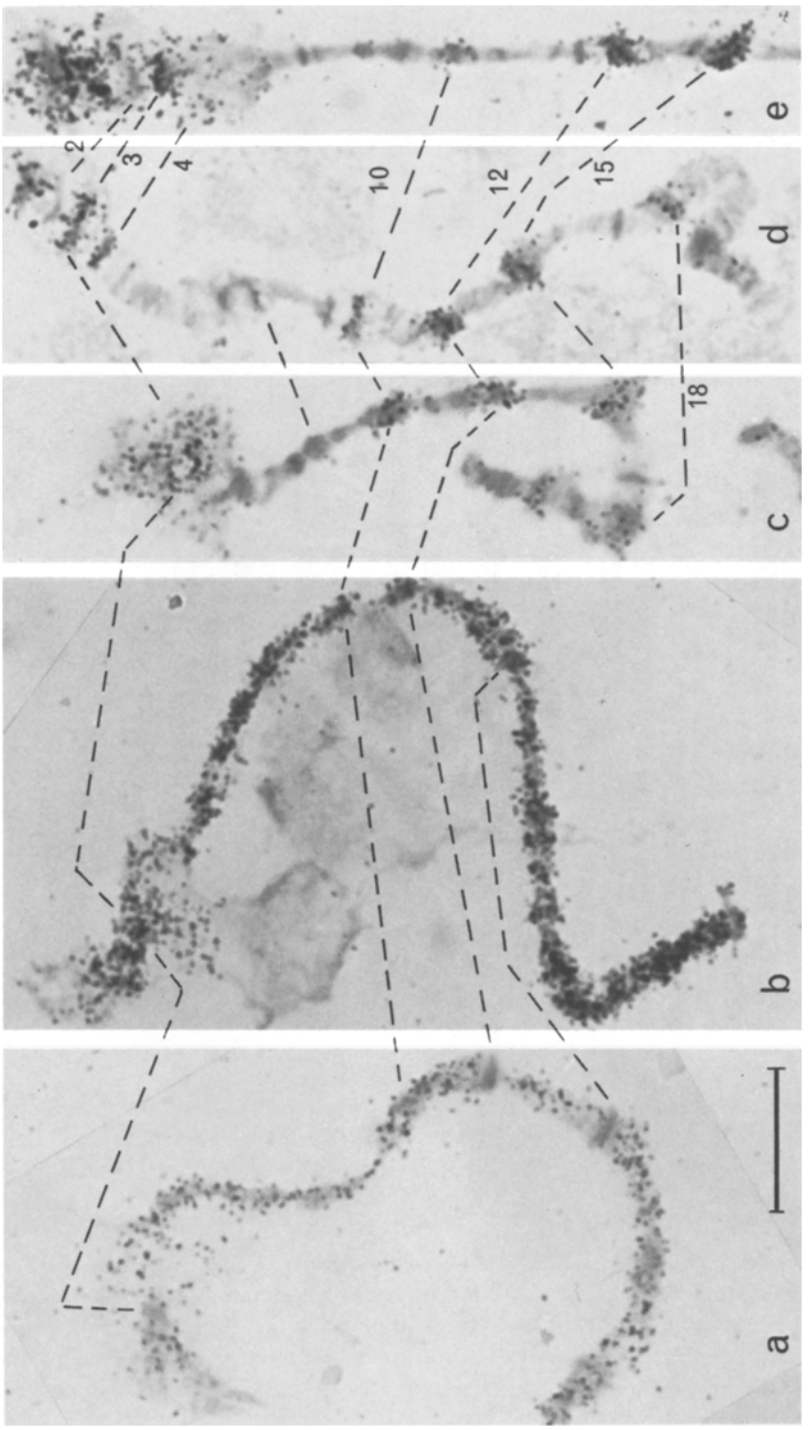

Fig. 4a–c. Giemsa-stained autoradiographs of polytene chromosomes from nurse cells (**a–c** and **e**) and a larval salivary gland cell (**d**). **a** Early pattern; **b** continuous pattern; **c** terminal pattern; **d** terminal pattern from larval salivary gland; **e** stretched terminal pattern (NC). Bar = 10 µm. Dotted lines connect homologous regions

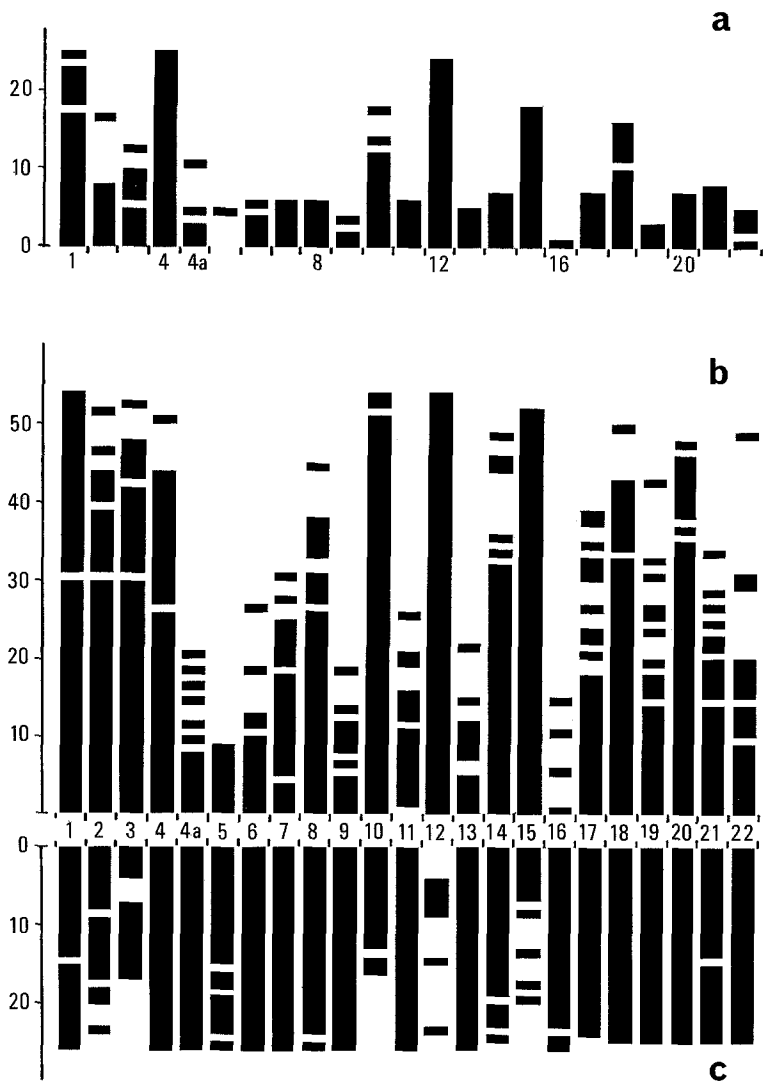

Fig. 5a–c. Labelling frequencies of X-chromosome regions: **a** larval salivary gland, **b** nurse cell, terminal phase chromosomes; **c** nurse cell, early phase chromosomes. Each chromosome analysed occupies one horizontal line on the graph and a black bar indicates the presence of label

b) Larval Salivary Gland X-Chromosome

A total of 83 ^{3}H-thymidine-labelled squash preparations of larval salivary glands were examined and, from these, 28 labelled X-chromosomes were located and scored for the presence or absence of label in each of the 23 regions. Material suitable for autoradiographic analysis could only be prepared, in general, from mid to late 4th instar larvae. Since there may be a decrease in the frequency of replicating nuclei throughout the larval instar (see Rodman, 1968; Tiepolo and Laudani, 1972), samples taken towards the end of the 4th instar may

Table 1. χ^2 analysis of late replicating patterns in nurse cell and larval salivary gland polytene chromosomes: a test of homogeneity applied to the relative frequency with which sites are labelled

Region	Nurse cell			Larval salivary gland		
	Observed	Expected	χ^2	Observed	Expected	χ^2
1	53	58.6	0.53	23	17.4	1.79
2	44	40.9	0.24	9	12.1	0.82
3	47	43.9	0.21	10	13.1	0.72
4	43	52.4	1.69	25	15.6	5.68
4a	14	14.6	0.03	5	4.4	0.1
5	9	7.7	0.22	1	2.3	0.73
6	14	14.6	0.03	5	4.4	0.1
7	26	24.7	0.07	6	7.3	0.24
8	36	32.4	0.41	6	9.6	1.36
9	12	11.6	0.02	3	3.4	0.06
10	53	51.6	0.04	14	15.3	0.12
11	17	17.7	0.03	6	5.3	0.1
12	54	60.1	0.62	24	17.9	2.1
13	12	13	0.09	5	3.9	0.31
14	37	33.9	0.28	7	10.1	0.94
15	52	53.9	0.07	18	16	0.24
16	4	3.9	0.01	1	1.1	0.02
17	28	26.9	0.04	7	8.0	0.13
18	43	44.7	0.07	15	13.3	0.22
19	24	20.8	0.49	3	6.2	1.64
20	45	40.1	0.6	7	11.9	2.03
21	25	25.4	0.01	8	7.6	0.03
22	21	19.3	0.16	4	5.7	0.52
Total	713		5.95	212		20

have frequencies of terminal phase chromosomes that are disproportionately high with respect to their proportional duration of the S-period. This is borne out by the SG X-chromosome data: of the 28 labelled X-chromosomes only two had continuous labelling patterns. The remaining 26 chromosomes consisted of 25 terminal phase chromosomes and 1 initial phase chromosome, classified according to the NC X-chromosome data (assuming that the direction of replication is the same in SG and NC chromosomes). The data for the 25 terminal phase chromosomes are summarised in Figure 5.

The pattern of late-replicating regions is similar in both NC and SG X-chromosomes (Fig. 4). For example, regions 1, 10, 12, 15 and 18 are late-replicating and have similar relative labelling frequencies in both tissues. Since terminal phase chromosomes are disproportionately represented in the SG data it is possible that, within the set of terminal phase chromosomes, the frequency of particular terminal phase patterns may also be disproportionately represented. However, despite this, the pattern of late-replicating regions in NC and SG X-chromosomes appears to be broadly similar. There is, in fact, no significant difference in the labelling frequency of the X-chromosome regions between NC and SG X-chromosomes ($\chi^2_{(22)}=25.95$, $p>0.05$, Table 1). Although region

4 has a lower labelling frequency, relative to adjacent regions, in NC than in SG chromosomes this is probably due to the fact that the major band within region 4 is puffed in NC nuclei but not SG nuclei and grain densities will consequently be lower in NC chromosomes (but see Hägele and Kalisch, 1980).

IV. Discussion

The resting stage and synchronous follicle development characteristic of ovarian maturation in the anautogenous Culicidae make the mosquito ovary an ideal system for the study of the processes, and their control, involved in insect oogenesis. While the physiological basis of the resting-stage, at a cellular level, is not well characterised, it is clear that resting-stage ovaries are inactive in DNA replication, at least on the labelling criteria used here. NC nuclei are already polytene at the resting stage and chromosome replication may be viewed in terms of preresting stage and post-resting stage phases.

The nature of the control of NC chromosome replication and whether or not developmentally specific mechanisms are involved, is at present unknown. It is interesting to note that the time-course of blood-meal stimulated DNA replication in FEC and NC nuclei is similar to that of the increase in ecdysteroid titre during this period (Redfern, 1980 and in prep.). In resting-stage females exogenous ecdysterone, while not capable of inducing vitellogenic maturation of the primary follicle, does induce development of the penultimate or secondary follicle (Redfern, 1980 and in prep.). Since the post-blood-meal growth characteristics of the primary and penultimate follicles are in fact very similar (Redfern, 1980 and in prep.) these observations implicate ecdysterone as a potential controlling factor. The relationship of ecdysterone to the initiation of DNA synthesis in polytene chromosomes is controversial (see Rodman, 1968) and while titre correlations do not necessarily imply a causal relationship, the synchronous ovarian development and ability to experimentally initiate DNA replication in NC polytene nuclei makes the *Anopheles* ovary a powerful system in which to analyse the control of the initiation of DNA replication and the ordering of polytene chromosome replication at a more detailed level.

In common with studies in other Diptera (Gabruscewycz-Garcia, 1964; Pelling, 1966; Kalisch and Hägele, 1976) the evidence presented here suggests that the three types of ^{3}H-thymidine labelling patterns in the NC X-chromosome correspond to initial, propagative and terminal phases of the polytene chromosome replication cycle. In this study, the majority of X-chromosome regions are labelled within an estimated period of 30–40 min after the initiation of replication. Chromosome regions that are apparently the last to appear labelled (e.g., regions 10, 12, 15) are also the last to complete replication (and see Kalisch and Hägele, 1976). Early labelling patterns in which groups of dense bands, such as in regions 10, 12 and 15, are unlabelled may be due to asynchrony of replicon initiation or to the existence of large replicons consisting of several bands. The existence of continuously labelled chromosomes (e.g., Fig. 4), with all bands apparently labelled, is evidence that large replicons spanning several bands, such as those proposed by Ananiev et al. (1977) for a *Drosophila* cell

line, are not found in polytene chromosomes. However, since the early labelling chromosomes in this study were generally diffusely labelled with low intensity it would be premature to equate an unlabelled score with a lack of replication. The stochastic nature of the autoradiographic method and potential differential β-particle self absorption by inter-bands, thick bands and fine bands differing in dry mass (see Laird, 1980) are factors that have to be taken into consideration.

The major conclusion from this analysis is that the termination patterns of replication are essentially similar in SG and NC polytene chromosomes. This is clearly consistent with the idea that the polytene chromosome banding pattern constrains, or results from (Laird, 1980), the pattern of DNA replication. It would, however, be premature to suggest that all parameters of replication are similar in NC and SG polytene chromosomes. The duration of the S-period may be altered by changes in the number of replicons, the rate of replication fork movement, the synchrony of replication in homologous chromatids and by changes in the synchrony of replicon initiation. While it is possible to argue against large increases in replicon size (a function of replicon number) and large changes in the synchrony of initiation (from the similarity of termination patterns) it is clear that increases in the number of replicons in NC chromosomes, relative to SG chromosomes, and changes in both the rate of replication fork movement and the synchrony of replication on homologous chromatids will be undetected in this type of analysis unless the S-period of SG polytene chromosome replication is known.

The NC and SG polytene chromosomes of *A. stephensi* are similar in some other respects: both cell types under-replicate at least one major satellite DNA (Redfern, 1981 a) and the chromosomes have essentially the same band-interband organisation (Redfern, 1981 b). This study thus confirms and extends the conclusion of previous work (Redfern, 1981 a, b) that the germ-line derived NC polytene chromosomes are differentiated from somatic SG polytene chromosomes solely with respect to differences in puffing and the pattern of transcriptional activity, differences that might be expected if polytenè chromosomes from different somatic tissues were being compared.

Acknowledgements. This research was supported by an S.R.C. studentship. I should like to thank Dr. M. Ashburner for comments on the manuscript, Brian Curtis and Jo Rennie for excellent photographic work and Betty McCready for typing the manuscript.

References

Ananiev, E.V., Barsky, V.E.: Replication rate and transcription intensity in different sections of the 1A1 – 3C8 region of the X-chromosome in Drosophila melanogaster. Molekulyarnaya Biologiya, **9**, 752–760 (1975)

Ananiev, E.V., Polukarova, L.G., Yurov, Y.B.: Replication of chromosomal DNA in diploid Drosophila melanogaster cells cultured in vitro. Chromosoma (Berl.) **59**, 259–272 (1977)

Ashburner, M.: A comparison of the distribution of puffs and intercalary heterochromatin in salivary gland chromosomes of Drosophila melanogaster. Naturwissenschaften **54**, 390–391 (1966)

Bancroft, J.D.: Histochemical techniques. London: Butterworths 1975

Becker, H.J.: Die Puffs der Speicheldrüsenchromosomen von Drosophila melanogaster. I. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, giant und lethal-giant larvae. Chromosoma (Berl.) **10**, 654–678 (1959)

Beermann, W.: Structure and function of interphase chromosomes. Genetics today **2**, 375–384 Proc. of the 11th Int. Congr. of Genet., The Hague 1963. Oxford: Pergamon Press, 1965

Blumenthal, A.B., Kriegstein, H.J., Hogness, D.S.: The units of DNA replication in Drosophila melanogaster chromosomes. Cold Spr. Harb. Symp. quant. Biol. **38**, 205–223 (1973)

Callan, H.G.: Replication of DNA in the chromosomes of eukaryotes. Proc. roy. Soc. (Lond.) B **181**, 19–41 (1972)

Gabruscewycz-Garcia, N.: Cytological and autoradiographic studies in Sciara coprophila salivary gland chromosomes. Chromosoma (Berl.) **15**, 312–344 (1964)

Gillett, J.D.: Variation in the time of release of the ovarian development hormone in Aedes aegypti. Nature (Lond.) **180**, 656–657 (1957)

Hägele, K.: Komplementäre DNA-Replikationsmuster bei Drosophila melanogaster. Chromosoma (Berl.) **41**, 231–236 (1973)

Hägele, K., Kalisch, W.-E.: DNA replication of a polytene chromosome section in Drosophila prior to and during puffing. Chromosoma (Berl.) **79**, 75–83 (1980)

Hannah, A.: Localisation and function of heterochromatin in Drosophila melanogaster. Advanc. Genet. **4**, 87–125 (1951)

Howard, E.F., Plaut, W.: Chromosomal DNA synthesis in Drosophila melanogaster. J. Cell Biol. **39**, 415–429 (1968)

Kalisch, W.-E., Hägele, K.: Different DNA replication behaviour of a tandem duplication in Drosophila melanogaster. Chromosoma (Berl.) **44**, 265, 283 (1973)

Kalisch, W.-E., Hägele, K.: Correspondence of banding patterns to ^{3}H-thymidine labelling patterns in polytene chromosomes. Chromosoma (Berl.) **57**, 19–23 (1976)

Laird, C.D.: Structural paradox of polytene chromosomes. Cell **22**, 869–874 (1980)

Lönn, U.: Double-stranded DNA with the size expected of replicons can be released from Chironomus polytene chromosomes. Chromosoma (Berl.) **81**, 641–653 (1981)

Mulder, M.P., Duiju, P. van., Gloor, H.J.: The replicative organisation of DNA in polytene chromosomes of Drosophila hydei. Genetica ('s-Gravenhage) **39**, 385–428 (1968)

Ockey, C.H.: Quantitative replicon analysis of DNA synthesis in cancer-prone conditions and the defects in Bloom's syndrome. J. Cell Sci. **40**, 125–144 (1979)

Ord, M.J.: The separate roles of nucleus and cytoplasm in the synthesis of DNA. Int. Rev. Cytol., Suppl. **9**, (J.F. Danielli and M.A. Di Berardino, eds.), pp. 222–278 (1979)

Pardue, M.L., Gall, J.G.: Nucleic acid hybridisation to the DNA of cytological preparations. Methods in cell biology (D.M. Prescott, ed.), vol. **10**, pp. 1–17 (1975)

Pelling, C.: A replicative and synthetic chromosomal unit; the modern concept of the chromomere. Proc. roy. Soc. (Lond.) B **164**, 179–289 (1966)

Plaut, W., Nash, D., Fanning, T.: Ordered replication of DNA in polytene chromosomes of Drosophila melanogaster. J. molec. Biol. **16**, 35–92 (1966)

Redfern, C.P.F.: Anopheles stephensi: polytene chromosomes and DNA replication in relation to vitellogenesis. Unpubl. Ph.D. Thesis, Cambridge University (1980)

Redfern, C.P.F.: Satellite DNA of Anopheles stephensi Liston (Diptera: Culicidae). Chromosomal location and under-replication in polytene nuclei. Chromosoma (Berl.) **82**, 561–581 (1981a)

Redfern, C.P.F.: Homologous banding patterns in the polytene chromosomes from the larval salivary glands and ovarian nurse cells of Anopheles stephensi Liston (Culicidae). Chromosoma (Berl.) (1981b)

Reisen, W.K., Emory, R.W.: Blood feeding of Anopheles stephensi. Ann. Entomol. Soc. America **69**, 193–298 (1976)

Rodman, T.C.: Relationship of developmental stage to initiation of replication in polytene nuclei. Chromosoma (Berl.) **23**, 271–287 (1968)

Rogers, A.W.: Techniques of autoradiography. Amsterdam: Elsevier, 1967

Rudkin, G.T.: Replication in polytene chromosomes. In: Developmental studies on giant chromosomes (W. Beermann, ed). Results and Problems in Cell Differentiation, vol. 4. Berlin, Heidelberg, New York: Springer, 1972

Rudkin, G.T., Woods, P.S.: Incorporation of ^{3}H-cytidine and ^{3}H-thymidine into giant chromosomes of Drosophila during puff formation. Proc. nat. Acad. Sci. (Wash.) **45**, 997–1003 (1959)

Service, M.W.: Ecological and biological studies on Aedes cantans (Meig.) (Diptera: Culicidae) in southern England. J. appl. Ecol. **14**, 159–196 (1977)

Sharma, G.P., Mittal, O.P., Dev., V., Gupta, B.D.: Gamma-rays induced visible alterations in the giant chromosomes of Anopheles stephensi Liston (Diptera: Culicidae). Caryologia (Firenze) **31**, 463–473 (1978)

Tiepolo, L., Laudani, U.: DNA synthesis in polytenic chromosomes of Anopheles atroparvus. Chromosoma (Berl.) **36**, 305–312 (1972)

Zakian, V.A.: Electron microscopic analysis of DNA replication in main band and satellite DNAs of Drosophila virilis. J. molec. Biol. **108**, 305–331 (1976)

Zhimulev, I.F., Kulichkov, V.A.: Break sites in polytene chromosomes of Drosophila melanogaster: Location and replication features. Genetika (USSR) **13**, 85–94 (1977)

Received April 23, 1981 / Accepted by W. Beermann
Ready for press May 2, 1981