DELAYED ONSET OF REPLICATION OF HUMAN X CHROMOSOMES

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INTRODUCTION

A controversy exists as to whether the human X chromosomes, both normal and abnormal, replicating late in the period of DNA synthesis (S) are also late to start replication at the beginning of S. Petersen (7), Cave (3), Ockey et al. (6), and Priest et al. (8) found evidence for late onset of X chromosome replication. However, Mukherjee and Sinha (5) and Bianchi and de Bianchi (1) did not confirm this late onset. The present studies were designed (*a*) to reconfirm the late onset of replication of one C group chromosome in FUdR-treated XX human cells in fibroblast culture, (*b*) to document when during S replication begins, (*c*) to compare the results with similar studies on XY and XXX human cells, since if no C-group chromosome in XY cells and two C-group chromosomes in XXX cells were late to start replication, the X nature of the chromosome late to start would be substantiated, and (*d*) to study X-chromosome morphology in the absence of tritium incorporation.

MATERIALS AND METHODS

The method used to synchronize human cell lines with 5-fluoro-2′deoxyuridine (FUdR) was reported in detail elsewhere (8). In brief, cultured cells can be partially synchronized at the beginning of S by exposure to FUdR followed by reversal of the block to DNA synthesis by addition of exogenous thymidine (TdR) (4). A maximum of 75% of cells are in S following this synchronization procedure, as compared to 30% for asynchronous cells. All cell lines were established from primary explants in this laboratory. The culture medium was Dulbecco and Vogt's modification of Eagle's medium supplemented with 15% fetal bovine serum (Flow Laboratories, Inc., Rockville, Md.) and 6×10^{-6} M uridine. FUdR in a concentration of 0.1 μg per ml (4×10^{-7} M) was added for 16–20 hr to human XX, XY, or XXX cells in logarithmic growth. The block to DNA synthesis was relieved by pouring off the medium containing FUdR and adding a medium containing 6×10^{-6} M TdR or H_3TdR (0.36 c per μmole) depending

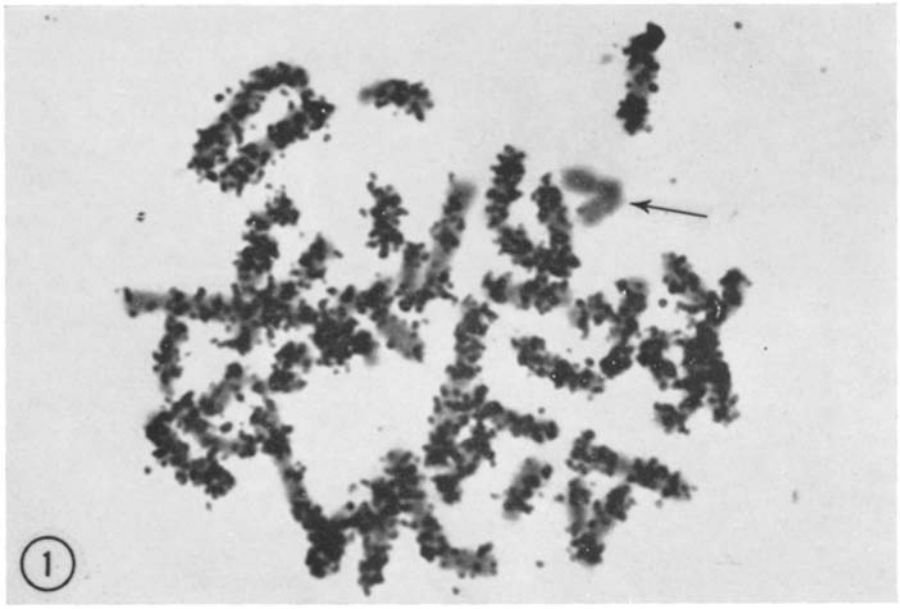

FIGURE 1 XX metaphase, labeled for the first 2 hr of S, containing an unlabeled X chromosome (arrow). Ilford L-4 emulsion; Giemsa stain. × 2,000.

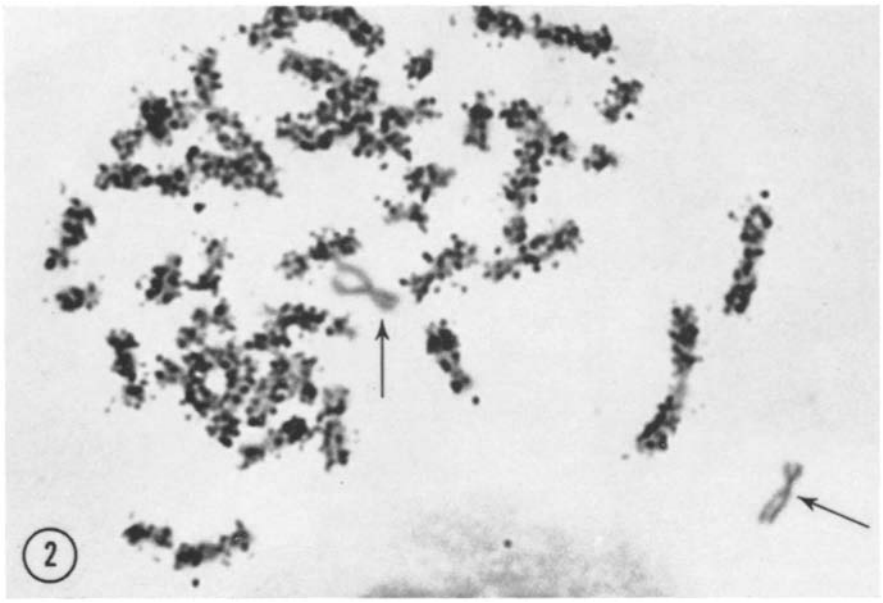

FIGURE 2 XXX metaphase, labeled for the first 2 hr of S, containing two unlabeled X chromosomes (arrows). Ilford L-4 emulsion; Giemsa stain. × 2,000.

on the design of the experiment. The following amounts of H_3TdR were used: for 10- and 20-min pulses 4μc per ml; for 1–2-hr pulses, 0.5 μc; for 3-hr pulses, 0.2 μc; for 4–6-hr pulses, 0.1 μc. A pulse was terminated by pouring off the radioactive medium, rinsing twice with balanced salt solution, and adding medium containing 6×10^{-6} M TdR. 5 hr after introduction of exogenous TdR, vinblastine sulfate (Velban) was added to a final concentration of 0.1 μg per ml. 5 hr later the cells were processed for chromosomes. Air-dried slides were dipped in a 1:1 dilution of Ilford L-4 emulsion:water, exposed for 3 wk, developed, and stained for 20–30 min in Giemsa at pH 6.4. Labeled metaphases were studied for the presence of unlabeled C-group chromosomes (3 gr or less). Grain counts over labeled C-group chromosomes ranged from 8 to 25 gr. Karyotyping was possible in the presence of label because of the fine emulsion grain (0.12 μ). However, double photography was also employed before and after either degraining or application of emulsion.

RESULTS AND DISCUSSION

The presence of one C-group chromosome failing to incorporate H_3TdR at the beginning of S in XX cells (Fig. 1) and two in XXX cells (Fig. 2) was confirmed (Table I). Similar chromosomes were not present in XY cells. By approximately the third hr of S the unlabeled chromosomes were no longer present. Previous studies of the S period in these FUdR-treated cells have indicated its length to be about 6 hr (8). Thus, the failure to label lasts for about one-half of the S period. The comparison of XY, XX, and XXX cells substantiates the X nature of the unlabeled chromosomes. A gradual decline was found in per cent of XX cells containing one unlabeled X chromosome and XXX cells containing two unlabeled X chromosomes through the first 3 hr of S (Table I). One explanation for the decline is "decay" of synchronization as S progresses (8). Another possible reason is the gradual onset of labeling in the unlabeled X chromosomes. Preliminary grain count data suggest the latter to be true during the second and third hours of S. The presence of a few XXX cells with only one unlabeled X chromosome during the second hour of S (Table I) suggests some asynchrony of onset of replication between the two X chromosomes late to start. The unlabeled X chromosomes are a cytologic marker for the first half of S. Furthermore, the per cent of metaphases containing the unlabeled chromosomes probably gives a measure of the degree of synchronization during the first 2 hr of S.

The metaphase position of the unlabeled X chromosome was studied from labeled and unlabeled photographs of 26 XX metaphases. The average distance of the centromere of the unla-

TABLE I

Analysis of Number and Per Cent of Cells Containing Unlabeled C-Group Metaphase Chromosomes After Label of XX, XY, and XXX Cells with H_3TdR During Various Times of S

Analysis does not include unlabeled metaphases, which are presumed not to be synchronized by the FUdR procedure employed

Interval of S during which the label was applied	No unlabeled C-group chromosomes						One unlabed C-group chromosome (rest of group labeled)						Two unlabeled C group chromosomes (rest of group labeled)					
	XX		XY		XXX		XX		XY		XXX		XX		XY		XXX	
	No.	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%
First 10 min	27/57	47	73/75	97	12/52*	23	30/57	53	2/75	3	2/52*	4	0/57	0	0/75	0	38/52*	73
First 1 hr	‡	‡	11/11	100	6/22	27	‡	‡	0/11	0	1/22	5	‡	‡	0/11	0	15/22	68
First 2 hr	32/51	63	11/11	100	19/33	58	19/51	37	0/11	0	4/33	12	0/51	0	0/11	0	10/33	30
First 3 hr	52/52	100	‡	‡	32/36	89	0/52	0	‡	‡	0/36	0	0/52	0	‡	‡	4/36	11
First 4 hr	46/46	100	‡	‡	28/28	100	0/46	0	‡	‡	0/28	0	0/46	0	‡	‡	0/28	0
First 5 hr	51/51	100	‡	‡	‡	‡	0/51	0	‡	‡	‡	‡	0/51	0	‡	‡	‡	‡

* Combined figures for 1st 10 min and 1st 20 min of S, studied separately.

‡ Not studied.

TABLE II

Summary of Distance from the Center of Metaphase to Centromere of Unlabled X Compared, by Cell, to The Average of Distances of the Other C Group Chromosomes in the Same Cell

26 Cells Studied

Average, unlabeled X	Average of average, remainder C group	Difference	Standard deviation difference
mm	*mm*	*mm*	
38	36	2	3

The t value (23 df) was 0.66; thus the *P* value indicated that the difference between two and zero was not significant.

TABLE III

Summary of Length of Unlabeled X Compared, by Cell, to the Average Lengths of Remaining C Group Chromosomes in the Same Cell

23 Cells Studied

Average, unlabeled X	Average of average, remainder C group	Difference	Standard deviation difference
mm	*mm*	*mm*	
18.04	18.31	0.27	0.32

The t value (22 df) was 0.84; thus the *P* value indicated that the difference between 0.27 and 0 was not significant.

beled X from the center of the metaphase was compared to the average for the remainder of the C-group chromosomes in the same cell. No significant difference was found (Table II). However, when late prophases or early metaphases were examined before individual chromosomes could be definitively identified, an unlabeled peripheral DNA mass was consistently noted following label during the first part of S (Fig. 3). These findings suggest that the peripheral position of the X chromosomes in late prophase or early metaphase was not maintained throughout metaphase.

The length of the unlabeled X chromosome was studied from labeled and unlabeled karyotypes of 23 XX metaphases. The average length of the X did not differ significantly from the average lengths of the other C-group chromosomes (Table III). The range of length of the unlabeled X within the C group was between positions 2 and 15 (in order of decreasing length). The position of the late replicating X chromosome at the end of S

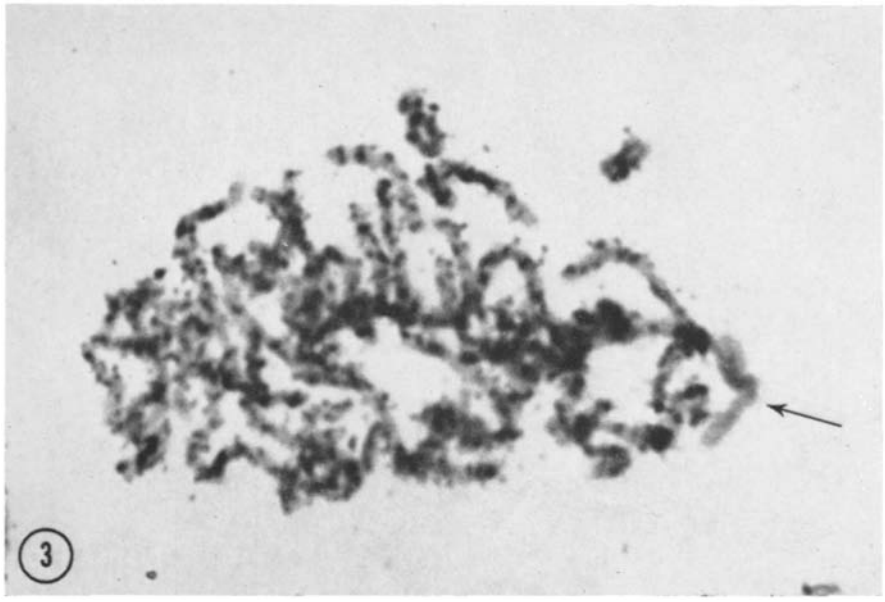

FIGURE 3 XX early metaphase, labeled for the first 2 hr of S, showing an unlabeled peripheral chromosome mass (arrow). Ilford L-4 emulsion; Giemsa stain. × 2,000.

in asynchronous human XX cells, as defined by length and arm ratio, has been found to range over 90% of the X-6–12 group (2). The variation in length reported here cannot be related to the presence of label, since the chromosome remained unlabeled in these studies. Thus, late replicating X chromosomes cannot be identified simply by their position within the C group.

SUMMARY

In XY, XX, and XXX human cell lines, no X, one X, and two X chromosomes, respectively, did not incorporate H_3TdR during the first half of S when FUdR was used to synchronize the cells. The unlabeled chromosome was not found to be peripheral in metaphase position in XX cells, and the average length did not differ significantly from the average lengths of the other members of the C group. The unlabeled X chromosomes are cytologic markers for the first half of S and for the degree of synchronization during the first 2 hr of S in FUdR-treated cells.

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