Timing of replication of beta satellite repeats of human chromosomes

Kelly G.Ten Hagen¹ and Stanley N.Cohen^{1,2*}

Departments of ¹Genetics and ²Medicine, Stanford University School of Medicine, Stanford, CA 94305-5120, USA

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ABSTRACT

The beta satellite sequences of the human genome are a family of genetic elements consisting of 68–69 bp monomeric units repeated contiguously in long arrays up to 1 Mb in length. We have determined the timing of replication of beta satellite subgroups located in the heterochromatic portion of chromosome 9 and on the acrocentric chromosomes in regions both distal and proximal to the rDNA genes. We report that these dispersed subgroups of beta satellite sequences all replicate late during S phase of the cell cycle.

INTRODUCTION

Eukaryotic DNA replication is known to proceed according to a specific temporal order (reviewed in 1–3), which is maintained from one cell cycle to the next (4–6). Cytological present examination (7–10) has suggested that a number of sequences in broad heterochromatic regions of mammalian chromosomes replicate late during S phase of the cell cycle, and density gradient analysis has established the timing of replication for at least one repetitive component of heterochromatic DNA, the human alpha satellite sequences (12). The large distances spanned by these arrays preclude replication of the entire array from external sites and imply that the repetitive units contain replication origins programmed specifically to function late during S phase (12).

The beta satellite repeats of the human genome are a newly identified family of repetitive elements based on a 68–69 bp monomeric repeat unit (13) and containing several distinct subgroups. One subgroup consists of a 2.5 kb higher order repeat of approximately 50–100 copies, located in a heterochromatic portion of the long arm of chromosome 9 (13). Two other subgroups composed of 250–500 copies of a 2.0 kb higher order repetitive unit have been localized cytologically primarily on the short arms of the acrocentric chromosomes, both proximal and distal to repeats encoding ribosomal RNA (13, 14). A fourth subgroup exists which is located primarily, although not exclusively, on the distal side of the rRNA-encoding clusters on each acrocentric chromosome (14). To provide basic information about replication of the beta satellite sequences and also to examine possible effects on replication of the cytological proximity of beta satellite DNA to highly transcribed sequences

that encode ribosomal rRNA, we studied the timing of replication of these beta satellite subgroups. Furthermore, we compared the time of replication of beta satellite sequences to the replication time of alpha satellite repeats, which previously have been shown to replicate late during S phase. Our results indicate that all of the beta satellite subgroups examined replicate predominantly late during S phase.

MATERIALS AND METHODS

Cell preparation and analysis

Cell culture and labeling of TK6 cells by bromodeoxyuridine (BU) was done as described previously (12). Cells prepared as previously described were analyzed and sorted according to DNA content on a fluorescence activated cell sorter (11, 12). The accuracy of the initial sorting was evaluated by mixing 100 μ l volumes of sorted cells with 100 μ l of 2 $\times$ chromomycin A₃ solution and subsequent reanalysis by the cell sorter. DNA from experiments I and II was resuspended in 200 μ l of sterile H₂O and sheared by passage 20 times through a 25 gauge needle before being added to the Cs₂SO₄ gradients.

Hybridization and quantitation

All slot blotting procedures and alpha satellite hybridization procedures have been described previously (12). DNA fragments containing subgroup-specific beta satellite sequences were isolated from plasmids carrying them (p21 β 2 [14], pB3 [13], pB4 [13], and p21 β 7 [14]) and labeled by the method of random priming directly in the agarose as described (15) using 200 ng of DNA in each reaction. The entire BLUR8 plasmid, which carries the human Alu repeat, was labeled by standard nick translation procedures (16). Hybridization to detect subgroup-specific beta satellite arrays was performed as described previously (13, 14). BLUR8 hybridizations were performed at 68°C in 2 $\times$ SSC. Quantitation of the amount of heavy-light (HL) and light-light (LL) DNA present in each gradient was performed by using a Helena Laboratories Quick Scan R&D densitometer.

Southern blot analysis of genomic DNA

TK6 DNA digested with restriction endonuclease *Bgl*II, *Hinf*I or *Hpa*I was electrophoresed in a 0.7% agarose gel at approximately

* To whom correspondence should be addressed

1.5 V/cm; transfer to nitrocellulose filters was performed as described previously (16). Each digest was performed in quadruplicate and Southern blots were hybridized with the plasmid insert of either p21 β 2, pB3, pB4 or p21 β 7 under previously described conditions (13, 14).

RESULTS

To determine the time of replication of B-sat subgroups, approximately 2×10^5 cells were retroactively synchronized in each fraction of the cell cycle (G1, S1 to S4, and G2/M) according to DNA content of nuclei using the fluorescence-activated cell sorter and analyzed as indicated in Materials and Methods. The reanalysis of a typical cell sorting experiment is shown in Fig. 1. DNA extracted from fractionated cells was sheared by passage through a needle before being loaded onto density gradients. A slot blot of Cs₂SO₄ gradient fractionated DNA was hybridized with the ³²P-labeled insert from the plasmid pB3 under stringent conditions (13) to detect the beta satellite subgroup specific to the heterochromatic region of chromosome 9. As shown in Fig. 2A and Table 1, the greatest percentages of HL DNA were seen during the latter portion of the cell cycle, in S3, S4 and G2/M. HL DNA was also seen in S2, with an even smaller amount in S1 (experiment I). These results indicate that this sequence is replicated predominantly, although not exclusively, late during S phase of the cell cycle. Replication detected in the G2/M fraction presumably represents cells that had incorporated BU late during S phase and had moved into the G2/M phase by the end of the labeling period.

Other slot blots were similarly hybridized with the beta satellite inserts from plasmids p21 β 2, pB4 and p21 β 7 under stringent conditions described previously (13, 14). Under these conditions, the p21 β 2 and pB4 inserts should detect arrays located both proximal and distal to the rDNA gene clusters on the acrocentric chromosomes. The p21 β 7 insert will detect an array located predominantly on the distal side of each rDNA cluster. As is seen in Fig. 2B, C and D and Table 1, the timing of replication of arrays detected by these three probes was very similar. p21 β 2 and p21 β 7 showed HL DNA patterns nearly identical to those detected by pB3. The majority of replication occurred in S3, S4 and G2/M with significant amounts in S2 and small amounts present in S1. The replication detected by pB4 was also confined primarily to S3, S4 and G2/M with smaller amounts seen in S2 and S1. The subgroup specificity of each beta satellite probe was verified by hybridization to Southern blots of TK6 genomic DNA (Fig. 3) as described (13, 14).

Slot blots from each sort were then hybridized with the ³²P-labeled alpha satellite insert from pBamX7 as described previously (12). As seen in Fig. 2E and Table 1 the alpha satellite sequences specific to the X chromosome detected HL DNA predominantly during the S3, S4 and G2/M fractions of the cell cycle as determined previously (12). A small amount of HL DNA was also seen in S2. No HL DNA was detected in S1. Thus, the beta satellite sequences examined displayed replication patterns similar to those of the X-chromosome-specific alpha satellite sequences.

To determine that the results obtained were not a consequence of unequal representation of HL DNA in the S phase fractions, slot blots from each TK6 cell-sorting experiment were hybridized with the ³²P-labeled BLUR8 plasmid, containing the Alu repetitive element, as described previously (12). HL DNA

Figure 1. Fluorescence histograms of fractionated TK6 cells. Logarithmically growing TK6 cells (LOG) were labeled for 1 h with BU, stained with chromomycin A₃, and sorted into various fractions of the cell cycle (G1, S1 to S4, and G2/M). The relative number of cells is plotted on the ordinate, and the DNA content is plotted on the abscissa. The reanalysis of each sorted population from cell-sorting experiment I is shown.

detected by the Alu probe was found in all S phase fractions, with a slight skewing toward early S (Fig. 2F and Table 1), as seen before (12). Smaller amounts of HL DNA were also seen in the G1 and G2/M fractions. The HL DNA detected in the G1 fraction may be the result of inefficient sorting of that fraction and the HL DNA detected in the G2/M fraction, as mentioned before, probably represents cells that had moved into the G2/M portion of the cell cycle during the 1 h labeling period.

DISCUSSION

The results reported here indicate that beta satellite subgroups located in the heterochromatic portion of chromosome 9, and regions both distal and proximal to the rDNA repeats on the acrocentric chromosomes all replicate predominantly late during S phase of the cell cycle. A similar timing of replication has been observed for human alpha satellite repeats (12). Earlier work has shown that certain actively transcribed regions of mammalian DNA commonly are replicated early during S phase (17–27), and recent work (R. Little and C.L. Schildkraut, personal communication) has suggested that some of the actively

Figure 2. Time of replication of human beta satellite sequences. HL and LL DNA was separated by density gradient centrifugation in Cs_2SO_4 . Fractions were collected from the bottom of each gradient and applied to nitrocellulose or zeta probe filters, using a slot blot apparatus. Slot blots from cell-sorting experiment I were hybridized with the chromosome 9-specific beta satellite insert from pB3 (A), the beta satellite insert from p21 β 2 (B), the beta satellite insert from pB4 (C), the beta satellite insert from p21 β 7 (D), the X-chromosome-specific alpha satellite insert from pBamX7 (E), and BLUR8, which contains the cloned Alu repetitive element (F). The positions of HL and LL DNA are indicated to the left, and cell cycle stage is indicated above each lane.

transcribed rDNA repeats found by cytological analysis to be in the vicinity of the beta satellite sequences replicate early. The data presented here indicate that if the actively transcribed rDNA clusters flanked by the beta satellite repeats we have studied are early replicating, then this early replication (and transcriptional activity) has little or no influence on the replication time of the beta satellite array. However, notwithstanding the cytological evidence of proximity between the rDNA and beta satellite sequences, the actual distance between these temporally distinct replication units is unknown.

Both the alpha and beta satellite sequences are arranged in long head to tail uninterrupted arrays that span large distances. The specific timing of replication of the beta satellite subgroups examined, some of which may extend up to 1 Mb or more, argues for the existence of origins that initiate replication within the arrays themselves. If this view is correct, the origins within beta satellite repeats must necessarily be programmed to function predominantly late during S phase of the cell cycle. This is the second such example where heterochromatic, simple repetitive units may actually encode the information necessary to allow the initiation of replication (12).

Table 1. Replication time of beta satellite repeats

Expt	Repetitive probe	Relative amt of replication ^a					
		G1	S1	S2	S3	S4	G2/M
I	pB3	0	3	14	20	23	14
	p21 β 2	0	6	24	29	30	16
	pB4	0	4	8	10	13	12
	p21 β 7	0	6	21	26	36	24
	pBamX7	0	0	8	16	23	15
	BLUR8	14	25	25	21	18	13
II	pB3	0	0	6	8	13	7
	p21 β 2	0	9	26	34	29	15
	pB4	0	4	14	24	21	15
	p21 β 7	0	4	11	13	12	15
	pBamX7	0	0	8	15	19	21
	BLUR8	6	15	15	13	10	8

^aData for beta satellite repeats in which four sections of S phase were sorted. Experiments I and II represent separate cell sortings of TK6 cells. In both experiments, DNA was sheared before density gradient centrifugation. Numbers represent densitometric quantitation of the relative amount of replication (HL/HL+LL) during each cell cycle interval.

Figure 3. Southern blots of beta satellite sequences. All subgroup-specific beta satellite probes were checked for specificity by probing Southern blots of total DNA from TK6 cells digested with either *Bgl*I, *Hinf*I or *Hpa*I. Restriction enzymes used are noted at the top of each lane. Lanes denoted pB3 were probed with the insert from pB3, lanes denoted p21β2 were probed with the insert from p21β2, lanes denoted pB4 were probed with the insert from pB4 and lanes denoted p21β7 were probed with the insert from p21β7. Each lane contains 3μg of DNA. Size markers are indicated on the left.

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