

# The Barr body is a looped X chromosome formed by telomere association

(chromosome structure/X-inactivation/centromere/interphase cytogenetics/*in situ* hybridization)

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**ABSTRACT** We examined Barr bodies formed by isodicentric human X chromosomes in cultured human cells and in mouse-human hybrids using confocal microscopy and DNA probes for centromere and subtelomere regions. At interphase, the two ends of these chromosomes are only a micron apart, indicating that these inactive X chromosomes are in a nonlinear configuration. Additional studies of normal X chromosomes reveal the same telomere association for the inactive X but not for the active X chromosome. This nonlinear configuration is maintained during mitosis and in a murine environment.

Barr bodies are unique chromatin structures formed in nuclei of the mammalian female as a means of sex chromosome dosage compensation. First identified as a nucleolar satellite present only in female cells (1), the Barr body represents a single inactive X chromosome. In cultured human cells, it is most easily identified at the periphery of the interphase nucleus, when other chromosomes are not condensed. Because the Barr body is difficult to see among clumped heterochromatin in interphase mouse fibroblasts, and because the silent human X reactivates more frequently in rodent than human cells, Dyer *et al.* (2, 3) suggested that mouse cells may not form proper Barr bodies. Analysis of cultured human cells by electron microscopy (4) or *in situ* hybridization (2) places the Barr body adjacent to the nuclear envelope in 75–80% of interphase cells. Comings (5) suggested that inactive X chromosomes attach randomly to the nuclear membrane, and the multiple Barr bodies in aneuploid cells are widely distributed (6, 7). Nuclear matrix attachment sites are similar for the active and inactive X chromosomes (8). Yet, the configuration of the Barr body has been relatively unexplored. DNA hybridization, *in situ* (9), has provided a powerful method to examine chromosomes during interphase, revealing an orderly arrangement of chromosomes in the interphase nucleus (10–13) and tissue-specific variation (14, 15). Using such methods to explore the human inactive X chromosome, we find that the Barr body consists of a condensed X chromosome in a nonlinear configuration, with telomeres in close proximity.

We examined the Barr body in interphase and mitotic cells using fluorescent probes for centromere and telomere regions of human X chromosomes. In addition to normal X chromosomes, we studied isodicentric X chromosomes (16), which form bipartite Barr bodies (16). Always inactive, they are mirror image duplications with two centromeres (one non-functional) and with two identical telomeres (see Fig. 1). The duplicate centromeres as well as common telomeres and their longer length facilitate structural analysis. To compare distance between hybridization signals with relative physical length we examined three isodicentrics, two joined by their

long arms (3935 and 7213) and the third attached at the short arms (411). We isolated these dicentric chromosomes from their normal homologue in hybrid cells so that all signals would come from the dicentric X chromosome and to examine the human Barr body in a mouse cell environ. Finally, we simultaneously hybridized centromere and subtelomere probes using differential labels and confocal microscopy.

## MATERIALS AND METHODS

**Cell Lines.** These are characterized in Table 1. The hybrids derived from A9 mouse fibroblasts were selected in hypoxanthine/aminopterin/thymidine medium, back selected in 6-thioguanine to eliminate the active X; to retain the inactive X, the silent *HPRT* locus was reactivated by 5-azacytidine. Inactive X hybrids derived from tsA1S9T mouse cells were selected directly at 39°C for activity of the *A1S9T* locus at Xp11 (17).

**Preparation of Slides.** *Interphase cells.* Confluent cells in LabTek slide chambers were fixed in methanol/acetic acid (3:1) and air dried.

*Mitotic cells.* Logarithmic-phase cells were treated with colcemid (1 hr), and mitotic cells were detached by shake-off, fixed in methanol/acetic acid, dropped onto slides, and air dried.

**Probes.** For simplicity, the human X-specific probes used to mark the centromeres and ends of the short and long arms of the chromosome are called X<sup>cen</sup>, Xp<sup>tel</sup>, and Xq<sup>tel</sup>, respectively. Fig. 1 shows the location of sequences homologous to these probes (labeled XC, 29C1, and F8).

X<sup>cen</sup>. The XC probe for the centromere region is a 2-kilobase (kb) *Bam*HI fragment (19) homologous to alphoid DNA; under stringent conditions XC hybridizes specifically with the X chromosome (19).

Xp<sup>tel</sup>. The 29C1 probe used to mark the short arm (Xp) telomere is a 1.8-kb *Pst* I fragment hybridizing to a subtelomeric sequence located about 20 kb from the end of the short arms of X and Y chromosomes (20); each X chromosome has 3–10 tandem copies.

Xq<sup>tel</sup>. The F8c probe used to mark the long arm (Xq) telomere is a 1.4-kb *Eco*RI fragment containing exon 26 of the blood-clotting factor VIII locus (21), which hybridizes exclusively to the locus in Xq28, near the telomere.

**Nick-Translation.** XC and 29C1 inserts and the entire F8c plasmid were labeled with Biotin-11-dUTP using the BRL nick-translation kit. The probes were purified in a spin column containing 50 mM Tris, 10 mM EDTA, and 0.1% SDS (pH 7.4) and stored at 4°C for up to 1 month.

Abbreviations: DNP, dinitrophenyl; DAPI, 4',6-diamidino-2-phenylindole.

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Table 1. Characteristics of cell lines

Cell line*	Human chromosome content	Inactive X chromosome
F411	46X,dic(X)(qter>p21.2::p21.2>qter) <sup>†</sup>	Dicentric X
GM3935	46X,dic(X)(pter>q27.1::q27.1>pter)	Dicentric X
7213	46X,dic(X)(pter>q26.3::q26.3>pter)	Dicentric X
F411-A9	Dicentric X; five autosomes	Dicentric X
GM3935-A9	Dicentric X; five autosomes	Dicentric X
GM7213-tsA1	Dicentric X; four autosomes	Dicentric X
DB1214-A9	Normal active X; one autosome	None
GL-A9	Normal inactive X; one autosome	Normal X

\*The first three entries are fibroblast cell lines; the remaining entries are hybrids.

<sup>†</sup>Breakpoints of the three dicentric chromosomes were defined using molecular probes.

**In Situ Hybridization. Biotinylated probes.** Slides were immersed for 2 min in 70% formamide/2× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate), dehydrated in a series of 70–100% ethanol, and air dried. Hybridization was as described by Devilee *et al.* (22) (37°C overnight) using 80–90 ng of probe per slide in formamide (60% for XC, 50% for 29C1, and F8c). For hybridizations with XC, the slides were washed (30 min, 20°C) in 2× SSC/0.1% SDS and then washed in 0.1× SSC/0.1% SDS (30 min, 42°C). The third wash was like the first. Hybridizations with 29C1 and F8c were as for XC, except all washes were in 2× SSC/0.1% SDS. Slides were washed in a fluorescein buffer of 3 M NaCl/20 mM Tris, pH 8. The biotin label was detected as described by Pinkel *et al.* (23), except that fluorescein buffer was substituted for BN buffer in all solutions and washes.

**Dinitrophenyl (DNP)-labeled probes.** XC was labeled with DNP by reaction with 2,4-dinitrophenylbenzaldehyde (DN-BAL, Aldrich) as described by Shroyer *et al.* (24) and purified in a spin column with TE buffer. The hybridization mixture (50% formamide, 0.4 mg of salmon sperm DNA per ml, 2× SSC, and 60 ng of biotinylated 29C1 and 60 ng of DNP-labeled XC per slide) was heated to 70°C, iced, and placed under coverslips. For double labels, slides were incubated, rinsed, and labeled with fluorescein as for single probes. After the last wash in fluorescein buffer, 100 μl of 1:50 rabbit anti-DNP/5% bovine serum albumin/and 0.04% goat serum was applied (overnight, 4°C). Slides were rinsed three times in

phosphate-buffered saline, treated with 100 μl of anti-rabbit IgG conjugated to a Texas red label in PBS, and counterstained with propidium iodide in phenylenediamine antifade solution.

**4',6-Diamidino-2-phenylindole (DAPI) Staining.** Cells fixed on slides, pretreated with RNase, were stained (10 min) with DAPI in 150 mM NaCl/10 mM Tris/1% Triton X-100/0.1% bovine serum albumin, and Mowial solution was placed under the coverslip before sealing.

**Analysis. Single label.** Slides were examined microscopically for number and position of signals. The two signals from the two ends (or two centromeres) of isodicentric chromosomes were usually in the same focal plane (those that were not could not be measured). In contrast, if also present, the signal from the normal X chromosome was often in another focal plane. The distance between signals was determined from photomicrographs (1200× magnification); distance was measured from the center of one signal to the center of the other. In some cases, measurements were made directly from superimposed confocal images, using the "length" program, and were similar to those obtained from photomicrographs.

**Double label.** Signals obtained by labeling Xp<sup>tel</sup> with biotin and X<sup>cen</sup> with DNP were analyzed using a Nikon Optiphot microscope mounted to a laser-scanning confocal imaging system (Bio-Rad MRC 500). We obtained z and xz series from computer-assisted images taken simultaneously from two channels. The images were subtracted, one from the other to eliminate cross signals, and then merged.

## RESULTS

**Signals in Control Cells.** The mean percent of cells with at least one signal was 37 (range, 17–76) with X<sup>cen</sup>, 25 (range, 16–34) with Xp<sup>tel</sup>, and 13 with Xq<sup>tel</sup> probes. For all probes, and in all cell lines, the signals were discrete. Cells from normal males and females labeled with X<sup>cen</sup> showed the expected X dosage. The number of Xp<sup>tel</sup> signals was similar in both sexes reflecting the locus on two X chromosomes in females and on X and Y chromosomes in males; most often the two signals were in separate parts of the cell, consistent with separate domains for the two X chromosomes in females and the X and Y chromosomes in males (25, 26). Hybrids with only a normal human active X chromosome (DB1214-tsA1S9) had only a single signal with either X<sup>cen</sup> (Fig. 2A) or Xp<sup>tel</sup> in >90% of labeled cells.

**Signals in Interphase Cells with Isodicentric Chromosomes.** Cells with three discrete X<sup>cen</sup> signals were seen in all three dicentric cell lines. The signal for the normal X is not always seen in photographs as it may not be in the same focal plane as the dicentric signals. Fig. 2D shows a cell with three signals, including two that are close. This characteristic close double signal (in a single focal plane) was seen in 41% of cells with an X<sup>cen</sup> signal (shown in Figs. 2C and E and 3: X<sup>cen</sup>). Often peripheral, the double signal resembles the bipartite Barr body formed by these chromosomes in interphase nuclei (16) (visualized with DAPI in Fig. 2B). Two close Xp<sup>tel</sup> signals were also seen in 37% of labeled cells (Fig. 3: Xp<sup>tel</sup>) and in cells labeled with Xq<sup>tel</sup> (Fig. 2G).

**Double Signals Originate from Isodicentric Chromosomes.** As it is absent in control fibroblasts, this characteristic double signal is not due to replicated or diffuse signals. Double signals were seen in hybrid cells with a dicentric chromosome but no normal X chromosome (66% of cells labeled with X<sup>cen</sup> and 37% of cells labeled with Xp<sup>tel</sup>); in cells with two signals, the two were invariably close. As expected, two sets of paired (four) signals were common in mitotic cell hybrids (Fig. 4). That the close double signals were rare in cells lacking dicentric chromosomes and frequent in cells with only dicentric chromosomes indicates they come from

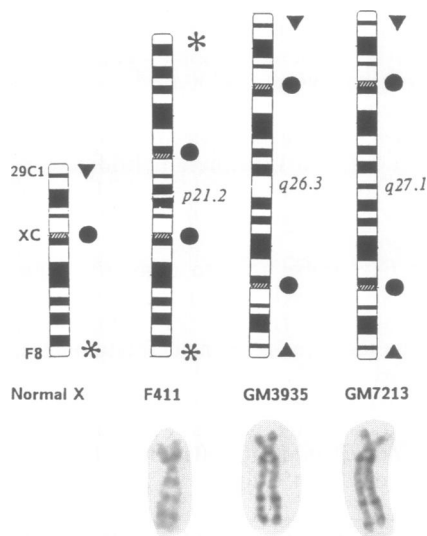


FIG. 1. Diagram (Upper) and photographs (Lower) of G-banded isodicentric X chromosomes showing breakpoints (in italics) and location of sequences homologous to probes [Xp<sup>tel</sup> (▼), 29C1; X<sup>cen</sup> (●), XC; Xq<sup>tel</sup> (\*), F8].

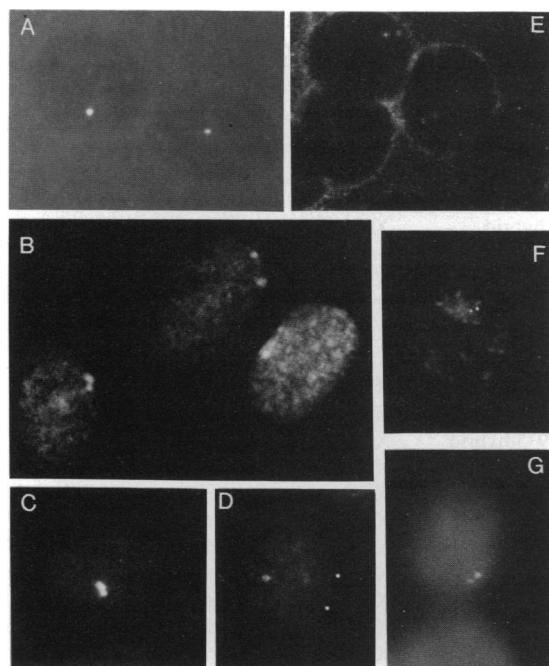


FIG. 2. (A) Control hybrids with normal active X chromosome (interphase) showing single  $X^{cen}$  signal. (B–D) The 411 fibroblasts (interphase) stained with DAPI (B) to show bipartite Barr bodies and labeled with  $X^{cen}$  to mark centromeres (C and D). (E–G) The 411 hybrid cells labeled with  $X^{cen}$  in interphase (E) and mitosis (F). (G) Same hybrids labeled with  $Xq^{tel}$ . Note: The variable distance between  $X^{cen}$  signals (C vs. D) resembles that seen with DAPI in B. The signal for the normal X not seen in C is seen in D. ( $\times 750$ .)

the two centromeres (or the two telomeres) of the dicentric chromosomes.

**Interphase Position of Isodicentric X Centromeres.** The distance between the two centromeres varied among cell lines, ranging in fibroblasts from 0.9 to 2.2  $\mu\text{m}$  (Table 2). Unexpectedly, it was greater in the 411 chromosome, joined by the short arms, than in 3935 and 7213 chromosomes with centromeres separated by the long arms (Fig. 1).

**Interphase Position of Isodicentric X Telomeres.** The two signals from chromosomes labeled with  $Xp^{tel}$  were surprisingly close; the distance was like that between centromeres, both about 1  $\mu\text{m}$  (Table 2). When both telomeres of 411 were labeled with  $Xq^{tel}$ , the distance between them ( $1.0 \pm 0.3 \mu\text{m}$ ) was less than expected for a chromosome of its size and was considerably less than between centromeres ( $2.3 \pm 0.7 \mu\text{m}$ ) (Table 2).

**Isodicentric Human X Chromosomes in Hybrid Cells.** Hybrid cells also let us examine the human inactive X chromosome in a foreign environment. In hybrids derived from mouse A9 cells by 5-azacytidine treatment, the two  $X^{cen}$  signals were as close as in human parent cells (2.2 vs. 2.3  $\mu\text{m}$  and 1.3 vs. 1.3  $\mu\text{m}$  for 411 and 3935, respectively) (Table 2 and Fig. 3:  $X^{cen}$ ). However, the centromeres were significantly further apart in the 7213 hybrid (derived from mouse tsA1 cells) than in parent human cells (2.6  $\mu\text{m}$  vs. 0.9  $\mu\text{m}$ ,  $P < 0.0005$ ). The  $Xp^{tel}$  signals were only slightly farther apart in hybrids than in parent cells (1.5 vs. 1.1,  $P < 0.005$ , and 1.6 vs. 1.1,  $P < 0.0005$ , for 3935 and 7213, respectively) (Table 2 and Fig. 3:  $Xp^{tel}$ ).

**Mitotic Cells.** To examine the inactive X chromosome in mitosis, cells from mitotic shake-offs were fixed without hypotonic treatment. One caveat is that if sister chromatids are still joined, signals due to replication of the sequence at one telomere (signal on each sister chromatid) are not easily distinguished from those at two telomeres. However, this ambiguity can be resolved when the two chromatids disjoin

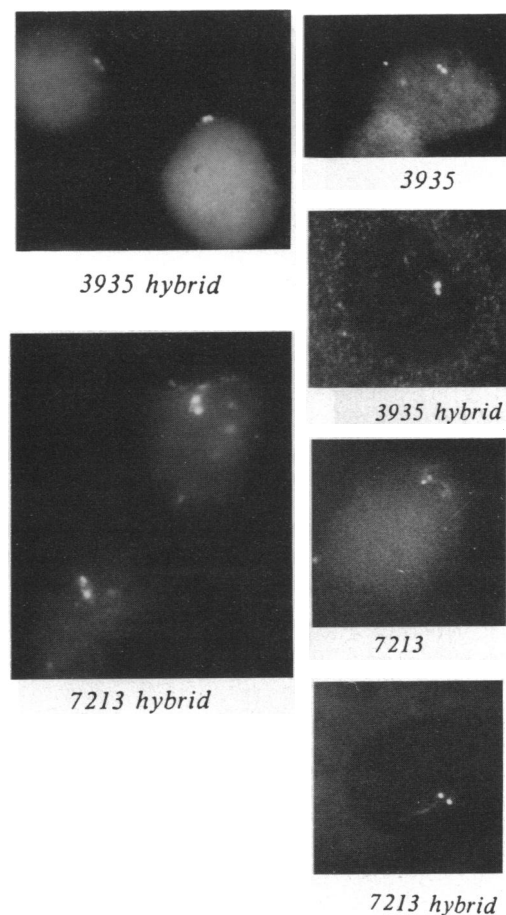


FIG. 3. Biotinylated signals for  $X^{cen}$  (Left) and  $Xp^{tel}$  (Right) in 7213 and 3935 hybrids and parental human cells, as indicated. ( $\times 750$ .)

in anaphase, seen in cells with four signals (i.e., Fig. 4). For all three isodicentrics, the mean distance between double signals, whether telomeric or centromeric, was consistently about 1  $\mu\text{m}$ . Therefore, at mitosis, when chromosomes are most condensed, the two ends (which may be as close as the replicated chromatids—i.e., Fig. 4C) are only slightly closer than in interphase. The distance between centromeres changed little from interphase to mitosis for the 3935 chromosome (1.3 vs. 1.1  $\mu\text{m}$ ,  $P \leq 0.05$ ) but decreased significantly for the 411 chromosome with centromeres separated by the short arms (2.3 vs. 1.1,  $P \geq 0.0005$ ).

**Relationship of Centromere and Telomere Analyzed by Simultaneous Hybridization with  $X^{cen}$  and  $Xp^{tel}$  Probes.** When the 3935 chromosome was doubly labeled [DNP-labeled  $X^{cen}$  conjugated with Texas red and biotin-labeled  $Xp^{tel}$  conjugated with fluorescein isothiocyanate (FITC)-avidin] the four signals obtained were close but difficult to resolve with the compound microscope. Using confocal microscopy, we could superimpose Texas red and FITC-avidin signals and optically section the cell from top to bottom along the z axis by 0.5- $\mu\text{m}$  intervals to examine the three-dimensional relationships. Signals were often at the periphery of the nucleus and most often near the top, perhaps an ascertainment bias favoring brighter signals. Fig. 5A shows that all four signals were adjacent. Although cells were acid fixed, we could detect differences in the depth of signals. Telomere signals (green) came into view before those from the centromeres (red), suggesting that telomeres were closer to the nuclear membrane; this was supported by an xz image (optical section in the vertical plane), which also revealed a greater distance from centromere to telomere than between the two centromeres or the two telomeres (Fig. 5B). However, the span

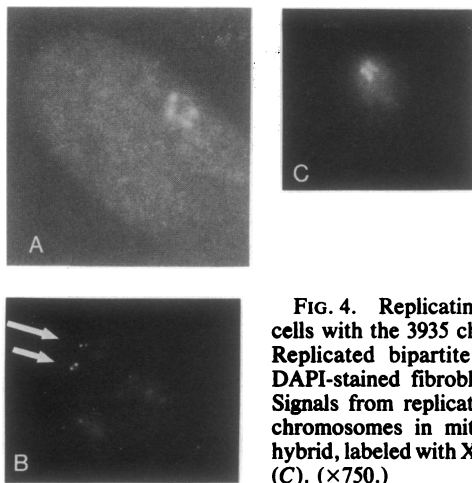


FIG. 4. Replicating Barr bodies in cells with the 3935 chromosome. (A) Replicated bipartite Barr body in DAPI-stained fibroblast. (B and C) Signals from replicating isodicentric chromosomes in mitotic cells from hybrid, labeled with  $Xp^{tel}$  (B) and  $X^{cen}$  (C). ( $\times 750$ .)

between  $X^{cen}$  and  $Xp^{tel}$  signals was not large compared to the widely separated signals seen in hybrids with the normal human active X chromosome (data not shown).

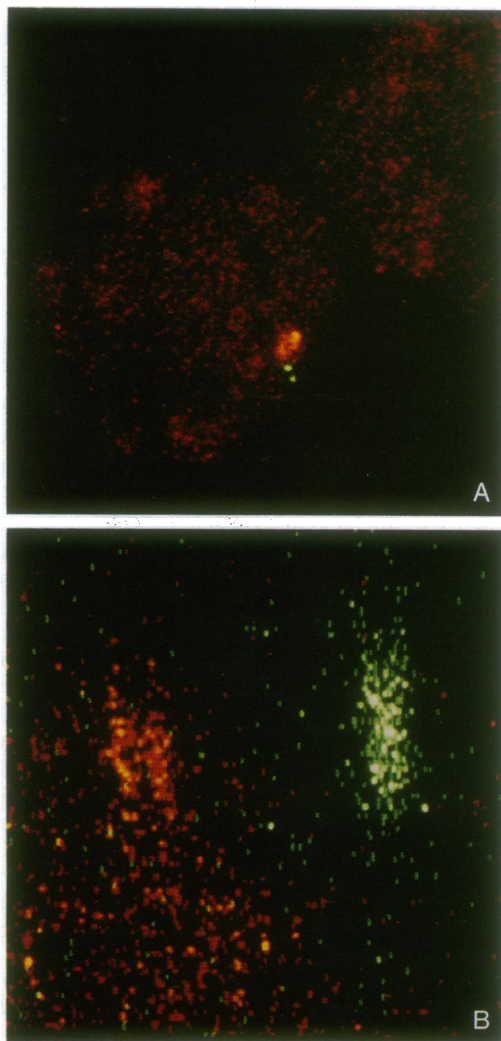


FIG. 5. Confocal images of 3935 hybrid simultaneously labeled with  $Xp^{tel}$  and  $X^{cen}$ . (A) z series image showing superimposed telomere (green) and centromere (red) double signals. Nucleus visualized with propidium iodide. ( $\times 1700$ .) (B) Superimposed xz vertical images of the same cell, taken through telomere and through centromere signals, showing close proximity of the two centromere signals (seen as two red parallel lines) and the greater distance from centromere to telomere (green signal). ( $\times 6000$ .)

Table 2. Distance between double signals resulting from *in situ* hybridization of biotinylated probes

Cell line	Probe	Phase of cell cycle	Distance,* $\mu\text{m}$	n
<i>Active normal X</i>				
DB hybrid	$Xp^{tel} + Xq^{tel}$	Interphase	$10.4 \pm 5.3$	10
<i>Inactive normal X</i>				
G1 hybrid	$Xp^{tel} + Xq^{tel}$	Interphase	$1.5 \pm 0.3$	22
<i>Inactive dicentric X</i>				
F411	$X^{cen}$	Interphase	$2.2 \pm 1.7$	24
411-hybrid	$X^{cen}$	Interphase	$2.3 \pm 0.7$	28
411-hybrid	$X^{cen}$	Mitosis	$1.1 \pm 0.3$	35
411-hybrid	$Xq^{tel}$	Interphase	$1.0 \pm 0.3$	20
GM3935	$X^{cen}$	Interphase	$1.3 \pm 0.5$	23
3935-hybrid	$X^{cen}$	Interphase	$1.3 \pm 0.5$	16
3935-hybrid	$X^{cen}$	Mitosis	$1.1 \pm 0.3$	22
GM3935	$Xp^{tel}$	Interphase	$1.1 \pm 0.3$	28
3935-hybrid	$Xp^{tel}$	Interphase	$1.5 \pm 0.7$	20
3935-hybrid	$Xp^{tel}$	Mitosis	$1.1 \pm 0.3$	36
GM7213	$X^{cen}$	Interphase	$0.9 \pm 0.3$	21
7213-hybrid	$X^{cen}$	Interphase	$2.6 \pm 0.4$	41
7213-hybrid	$X^{cen}$	Mitosis	$1.1 \pm 0.2$	10
GM7213	$Xp^{tel}$	Interphase	$1.1 \pm 0.4$	24
7213-hybrid	$Xp^{tel}$	Interphase	$1.6 \pm 0.4$	27
7213-hybrid	$Xp^{tel}$	Mitosis	$1.0 \pm 0.2$	13

n, Number of cells analyzed.

\*Mean  $\pm$  SD.

**Interphase Position of Telomeres in Normal X Chromosomes.** Because either Xp or Xq telomere of isodicentrics was capable of telomere association, we analyzed hybrids having normal X chromosomes, using a mixture of  $Xp^{tel}$  and  $Xq^{tel}$  probes. The mean distance between these signals in hybrids with the wild-type active X chromosomes was  $10.4 \pm 5.3 \mu\text{m}$ , with a wide range from 3 to  $22 \mu\text{m}$  (DB hybrid in Table 2). In striking contrast, the two signals were close ( $1.5 \pm 0.3 \mu\text{m}$ ) in hybrids with only the normal inactive X chromosome, showing proximity of long- and short-arm telomeres (Fig. 6A and Table 2, G1 hybrid).

## DISCUSSION

**Evidence That the Inactive X Chromosome Forms a Loop Structure with Telomeres Associated.** These studies show that the inactive X chromosome, whether isodicentric or structurally normal, is not a linear structure. The studies with single probes show that the two ends of these chromosomes are too close together for linear structures. Because we only measured distance between two discernible signals and our probes were subtelomeric, the distance between telomeres may be even less than measured. In any event, based on interphase studies of Lawrence *et al.* (27, 28), Trask *et al.*

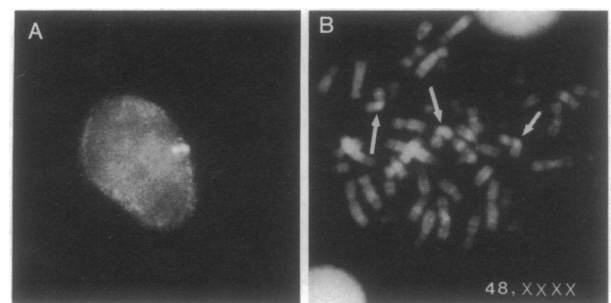


FIG. 6. Telomere association of the normal inactive X chromosome. (A) G1-A9 hybrid labeled with  $Xp^{tel}$  and  $Xq^{tel}$  showing proximity of signals from the two ends of the chromosome. (B) Characteristic bends in all three inactive X chromosomes (arrows) in a hypotonic-treated metaphase from a 48, XXXX human cell. ( $\times 750$ .)



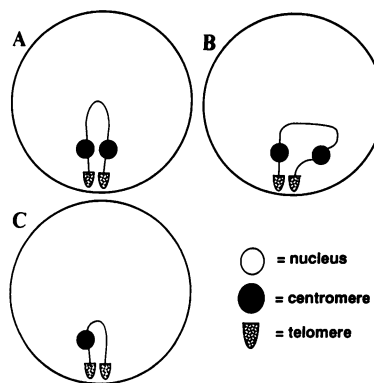


FIG. 7. Diagram showing one simple model that fits our observations of dicentric (A and B) and normal (C) inactive X chromosomes. The model in B accounts for the greater variability observed for centromere signals.

(28), and others [reviewed by Trask *et al.* (29) and Manuelidis and Cher (30)], the 1- $\mu\text{m}$  distance between inactive X telomeres is roughly equivalent to one or two megabases of interphase DNA. This is only slightly greater than the 700-nm fiber that characterizes metaphase chromosomes and is considerably less than the  $>10\text{-}\mu\text{m}$  length of chromosome 1 (smaller than the smallest isodicentric chromosome analyzed) measured by electron microscopy in an acid-fixed prometaphase preparation. In fact, at interphase, the mean distance between telomeres of the normal active X is 10-fold greater ( $10.4 \pm 5.3 \mu\text{m}$ ; Table 2, DB hybrid, Xp<sup>tel</sup> plus Xq<sup>tel</sup>).

The small distance between centromeres of dicentrics means that these chromosomes must be enfolded so that centromeres are also adjacent. The simplest nonlinear model to fit our observations is a loop (modeled in Fig. 7). As the telomere signals are always close, whereas the distance between centromeres is variable among the three dicentrics, the base of the loop is at the telomeres; the centromeres may be closer (Fig. 7A) or farther apart (Fig. 7B). Supporting the looped structure are the characteristic bends in the proximal long arm of normal inactive X chromosomes observed in metaphase preparations (31). Fig. 6B shows a human metaphase with four normal X chromosomes in which all three inactive X chromosomes are bent. That the telomeres are not closer together is probably due to disruption of telomere association by hypotonic treatment used to prepare metaphases.

**Relationship Between Chromosome Configuration and Transcription.** Similar location of signals in hybrid and human cells for 411 and 3935 chromosomes indicates that cell environment alone does not determine chromosome configuration. However, the position of centromeres may be affected by transcriptional activity. Selection of the 7213 hybrid required activity of the *A1S9T* locus, not far from the centromere, and greater transcriptional activity of this gene might be responsible for the greater distance between X<sup>cen</sup> signals in hybrid cells. Similarly, the relatively greater distance between centromeres of the 411 chromosome in human and hybrid cells ( $>2 \mu\text{m}$ ) could be due to expression of some loci on the intervening short arm (17). In both cases, this distance decreases considerably in mitotic cells, when euchromatic (transcribed) regions condense, as expected, if in fact, transcriptional activity influences position of centromeres.

**The Barr Body in Mitosis.** Although centromeres of the isodicentric chromosomes are closest in mitosis, the distance between the telomeres changes relatively little from interphase to mitosis. That the distance between telomeres in mitotic cells is also 1  $\mu\text{m}$  (Table 2 and Fig. 4) suggests that the loop structure is maintained during mitosis.

**Significance of Telomere Association.** Although both telomeres of the inactive X chromosome are close to the nuclear membrane, we have no evidence of membrane attachment. There is some electron microscopic evidence for a network of filaments emanating from the membrane toward the Barr body (3), and in meiosis telomeres are frequently reversibly associated with the nuclear envelope (18, 32). Yet, the proximity of telomeres in mitotic cells (in absence of nuclear membrane) suggests that the nuclear envelope is not required to maintain telomere association. Hinton (33) showed that terminal adhesions between nonhomologous ends of polytene chromosomes were inherent in the nature of the telomere. The common DNA sequence (telomere) at the ends of all human chromosomes could predispose to this kind of association; however our observations that the telomeres of the active X chromosome are not close suggest that association of the two ends of the inactive X chromosome is not a general characteristic of interphase chromosomes and may be a unique attribute of inactive chromosomes. How telomere association occurs and what role the looped configuration of the chromosome plays in silencing transcription of the inactive X chromosome are subjects for further study.

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