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Compound Lateral Asymmetry in Human Chromosome 6: BrdU-Dye Studies of 6q12→6q14

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INTRODUCTION

Incorporation of the thymidine analog 5-bromodeoxyuridine (BrdU) into chromosomal DNA causes alterations both in chromosomal morphology and stainability [1, 2]. When stained with the bisbenzimidazole dye, 33258 Hoechst, BrdU substituted chromosomes and chromosomal regions fluoresce less intensely than their thymidine containing counterparts [3]. Staining with the Hoechst dye, exposure to light, and subsequent staining with Giemsa reveals a similar pattern of decreased stainability of the BrdU substituted chromosomal regions [4].

Considerable information about the underlying DNA base content in specific chromosomal regions has been obtained using these new BrdU-dye techniques. Following a single cell cycle in the presence of BrdU, asymmetric 33258 Hoechst fluorescence of chromatids in the centromeric region of mouse chromosomes has been observed [5]. This asymmetry of fluorescence has been interpreted as visual confirmation of previously determined unequal distribution of thymidine residues in polynucleotide chains of that region. These centromeric regions contain thymidine-rich mouse satellite DNA [6].

Similar findings have been seen in human chromosomes especially in highly polymorphic regions. Lateral asymmetry has been reported for the long arm of the Y chromosome [7], the secondary constrictions of 1 and 16, and the centromeric regions of chromosomes 15 and 19 [8, 9]. These are all sites of constitutive heterochromatin, composed of highly repetitive DNA sequences [10]. Human satellite DNA species rich in A-T base pairs have been localized by in situ hybridization techniques to secondary constrictions of chromosomes 1, 9, and 16, the long arm of the Y, and the short arm of the acrocentric chromosomes [11]. As a result of these findings, it has been suggested that lateral asymmetry on staining of these human chromosomal regions reflects interstrand differences in thymidine distribution within these regions.

Thus far, the asymmetric distribution of thymidine as reflected by asymmetric stainability has been observed in regions of constitutive heterochromatin. The compound lateral asymmetry observed in the polymorphic heterochromatic secondary

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constriction of chromosome 1 has been interpreted as reflective of alternating regions of interstrand differences in thymidine distribution [8]. In the present paper, we report our observations of compound lateral asymmetry in a chromosomal region which is neither a site of constitutive heterochromatin nor a recognized polymorphic region of the human chromosomal complement. We describe compound lateral asymmetry of bands 6q12→6q14 which contain a late replicating autosomal region, 6q12. Our observations on normal and structurally altered no. 6 chromosomes indicate that there are alternating interstrand differences in thymidine concentration in this region as reflected by staining pattern, and that the timing of replication and the compound lateral asymmetry of 6q12→6q14 are characteristics which are not affected by intrachromosomal position.

MATERIALS AND METHODS

Peripheral blood leukocytes were grown in McCoy's modified 5a medium supplemented with 15% fetal calf serum and PHA for 72 hr. BrdU (Gibco, Grand Island, N.Y.) was added to the culture medium during the last 7 hr of culture to achieve a final concentration of 200 μg/ml. Colchicine was added 1 hr prior to termination of the cultures, and the cells were harvested in the standard manner [12] in subdued light. Air dried preparations were made. The slides were stained in 33258 Hoechst dye (American Hoechst Company, Somerville, N.J.) at a concentration of 2.0 μg/ml in 0.5 × SSC for 4 hr while being exposed to a UV light source (G.E. GRO and SHO, F15T8·PL) at a distance of 1–3 inches. The slides were rinsed, placed in 0.5 × SSC at 55°C for 1 hr, and stained in 4% Giemsa (Gurr's) in Gurr's pH 6.8 phosphate buffer for 6 min.

Blood was cultured from 12 individuals: 10 control subjects and two individuals with previously detected abnormalities of the no. 6 chromosome. The 10 controls included two karyotypically normal 46,XX individuals (C1 and C2) and eight individuals with another chromosomal abnormality unrelated to the no. 6 chromosome (C3–C10). The karyotypes are: C3: 46,XY, del(2)(p23); C4: 46,XY, 8p$^+$; C5: 45,XX, t(15q21q); C6:46,XY, inv dup (13q)(q2105→q34); C7: 46,XX, t(10:21)(q24:q22); C8: 46,XY, t(10:17) (q24;p13); C9: 45,XO; and C10: 46,X, dir dup (Xp)(p11→pter). The karyotypes of the two individuals with abnormalities of the no. 6 chromosome are: P1: 46,XX, inv(6)(p21;q25); and P2: 46,XY, var(6)(p11,q11). (P2 has a very large centromeric region which includes an increase in the length of bands p11 and q11 identified by trypsin Giemsa banding and C-banding as seen in figure 3.)

RESULTS

Chromosomes of cells grown in the presence of thymidine except for a terminal BrdU pulse demonstrate extensive decondensation of the BrdU containing late replicating chromosomal regions. These regions correspond to the regions reported by investigators using BrdU incorporation during most of the S period and a terminal pulse of thymidine. Using the terminal BrdU pulse and the Hoechst plus Giemsa staining procedure described in this report, the late replicating regions are visualized as palely stained regions against a darker staining, thymidine containing, background. The secondary constrictions of chromosomes 1, 9, and 16 and the long arm of the Y chromosome stain palely in most cells. Bands 3p12, 4p15, 4q13, 4q28, 5p14, 13q21, and 13q31 which are the negatively stained bands marked by the large arrows in figure 1, have been previously designated as late replicating using fluorescence techniques [13]. The present technique results in separation of bands 3p12 and 13q21 into two late replicating regions by an earlier, darker staining sub-band noted by the small arrows in figure 1.

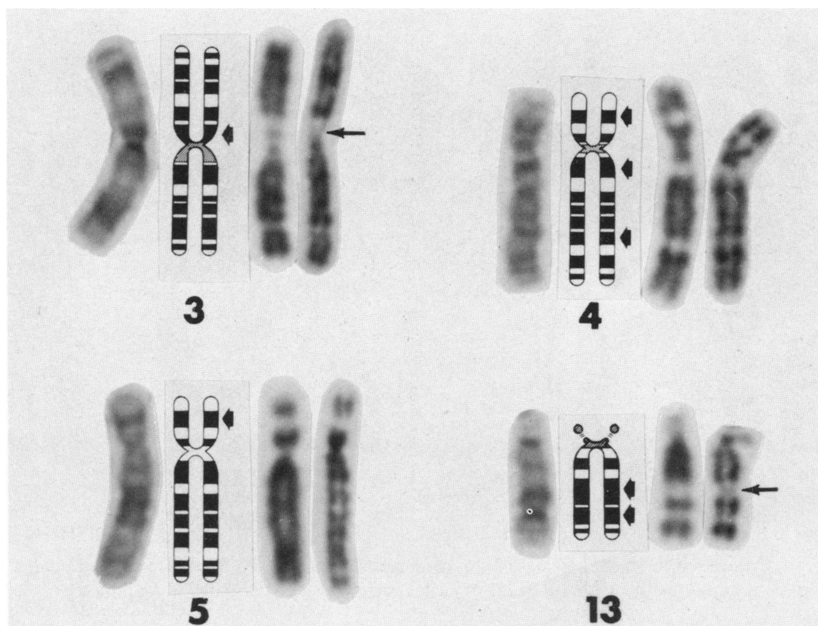

FIG. 1.—G-banded (*left*), idiogrammatic (*middle*), and Hoechst plus Giemsa stained (*right pair*) representations of four autosomes and their late replicating bands. The G-banded example demonstrates the bands shown in the idiogram. The heavy arrows on the idiogram indicate bands that appear destained in the BrdU treated chromosomes as a result of late replication. The thin arrows indicate darker, earlier replicating regions within such destained regions (see text for details).

Band 6q12 is a region also previously described as late replicating. Although it does not replicate as late as the regions just described, its stainability is suppressed in more than 50% of the cells after a terminal BrdU pulse. In addition, the region 6q12→6q14 shows compound lateral asymmetry with a contralateral pattern (fig. 2). The region is composed of two equally sized, contiguous, laterally asymmetric regions. Hence, region 6q12→6q14 is divided into two laterally asymmetric sub-regions, such that the darkly stained portion is on the opposite chromatid in each sub-region.

Figure 2 shows the no. 6 chromosomes from 10 control subjects. The pattern of compound lateral asymmetry is always observed in metaphase spreads where 6q12 is late replicating; this phenomenon occurs in 50% of the no. 6 chromosomes of a given preparation. There is often homologue asynchrony for 6q12 with the result that in a given cell 6q12 is late replicating in only one of the homologues (fig. 2, C10). The pattern of asymmetry does not vary from one individual to another and has been seen with similar frequency in all individuals tested.

Chromosome 6 pairs for patient P1 are seen in figure 3. Figure 3*A* shows G-banded no. 6 chromosomes which identified the patient's karyotype as 46,XX, inv(6) (p21;q15). Figure 3*B* shows BrdU-dye treated chromosomes. The inv(6) shows the asymmetrical pattern for 6q12→6q14 repositioned in the short arm of the inverted chromosome. The intrachromosomal shift of the late replicating band 6q12 does not affect the timing of its replication.

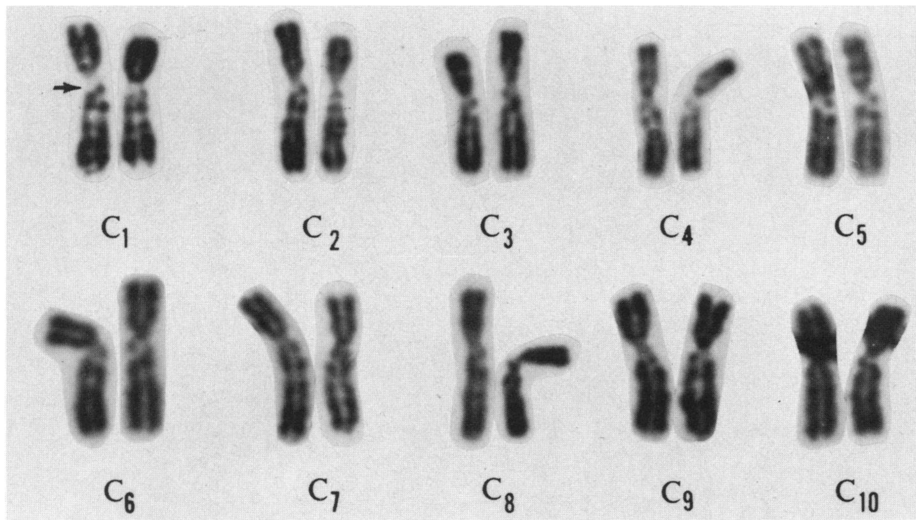

FIG. 2.—Partial karyotypes showing no. 6 chromosomes of the 10 control individuals after BrdU Hoechst plus Giemsa treatment. Arrow in C1 indicates region of compound lateral asymmetry.

No. 6 chromosomes of patient P2 are seen in figure 4. Figure 4*A* shows their G-banded pattern, and the arrow indicates an apparently enlarged centromeric, negatively G-banded region of one member of the pair. Figure 4*B* indicates that this region is C-band positive. This region of chromosome 6 which corresponds to 6p11 and perhaps 6q11 represents a region of constitutive heterochromatin. Figure 4*C* shows by BrdU-dye studies that the replication pattern is not altered by the extra heterochromatin, nor does the extra heterochromatin appear to be late replicating. Both homologues exhibit compound lateral asymmetry.

DISCUSSION

Several investigators have described lateral asymmetry of stainability in a number of regions in the human complement using BrdU-dye techniques, but not to our

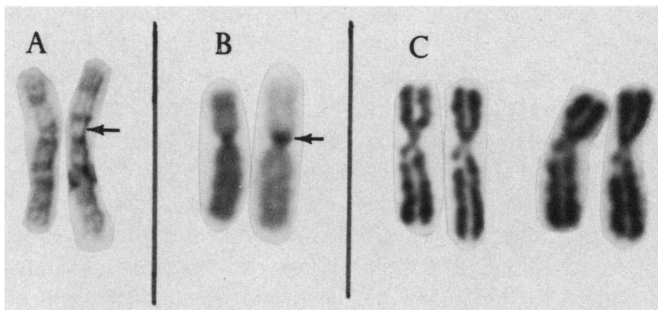

FIG. 3.—Partial karyotypes for patient P1: 46,XX, inv(6)(p21;q25). *A*, Trypsin G-banded examples with inv(6) on the right; and *B*, BrdU Hoechst plus Giemsa treated examples with inv(6) on the right.

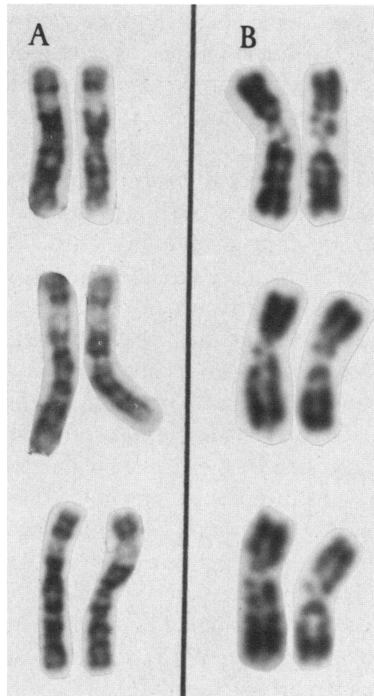

FIG. 4.—Partial karyotypes for patient P2: 46,XY, var(6)(p11,q11). *A*, Trypsin G-banded example with arrow indicating the enlarged variable region; *B*, C-banded example with arrow indicating extra constitutive heterochromatin; and *C*, BrdU Hoechst plus Giemsa treated examples with var(6) chromosome on the right of each pair.

knowledge in chromosome 6. We suspect that the particular BrdU-dye protocol described in this report has been responsible for this new observation. The use of a terminal, concentrated BrdU pulse causes the despiralization or decondensation of late replicating regions. Subsequent Hoechst plus Giemsa staining allows more detailed visualization of these late replicating regions. Regions previously perceived as single late replicating bands are subdivided into several bands separated by earlier replicating bands; examples are 3p12 and 13q21 (see fig. 1). It is quite likely that this degree of resolution is not possible with other described BrdU-dye protocols. Where fluorescence microscopy is used, the limitations of photographic resolution probably preclude resolution of the small regions we have described [13]. The use of lower concentrations of BrdU do not result in extensive despiralization of late replicating regions. Procedures in which a terminal thymidine pulse is applied on a BrdU suppressed background incorporates thymidine into late replicating regions. These chromosomes would not be despiralized in late replicating regions [13–15].

The extreme decondensation of late replicating regions by our protocol allows visualization of the compound lateral asymmetry of 6q12→6q14, consistent with an alternating asymmetric distribution of thymidine residues in the chromosomal DNA of that region. It is unlikely that the staining pattern is the result of a sister chromatid

exchange (SCE) in a region of simple lateral asymmetry. There is no evidence for a site specific exchange in 6q12→6q14, and exchanges are believed to be less frequent near the centromere [16]. If the observed pattern was due to a site specific exchange, one would expect to find individual no. 6 chromosomes with a simple asymmetry when no exchange had occurred or when a second exchange had occurred at the same site. Such patterns are never seen. One does occasionally see no. 6 chromosomes without any asymmetry, but in these chromosomes, 6q12 is darkly stained presumably because 6q12 has completed replication prior to addition of BrdU.

Extending the period of BrdU incorporation beyond what we have used in this study should reveal other areas of asymmetry which replicate earlier in the DNA synthesis period. Examination of such areas and their placement relative to one another should produce additional information about chromosomal substructure.

The studies of the patient with the inv(6) reveal the applicability of this approach as another banding technique for diagnostic purposes. The BrdU-dye studies confirmed the pericentric inversion suggested by the G-band pattern and ruled out the possibility of an interstitial deletion with insertion of nonhomologous material. These results further imply that the control of replication of 6q12 is localized to that band since an intrachromosomal shift of the band did not alter its timing of replication. If this autonomous control of replication kinetics is generalizable to other structural rearrangements, it provides a method of identifying chromosomal segments in de novo rearrangements with partial aneuploidy. We have, in fact, already successfully applied this technique to several such rearrangements [17, 18].

The studies of patients P1 and P2 were initiated because of significant phenotypic manifestations. They were both found to have an anomalous no. 6 chromosome. P1 was evaluated for severe hypotonia and developmental delay. It is difficult to attribute her clinical problems to the inversion, especially when these studies reveal no position effect on replicative timing. If her phenotypic manifestations are the result of position effect they are altered at the level of control or accuracy of transcription or translation. Patient P2 was evaluated for multiple congenital anomalies. The heterochromatic region variant observed in one of his no. 6 chromosomes did not replicate late, nor appear to influence the replicative pattern of 6q12. Despite these negative results, we believe that further studies using various times of analog application may reveal regional alterations of DNA replication patterns in such patients, providing a cytogenetic basis for their phenotypic manifestations.

SUMMARY

The Giemsa stainability of late replicating chromosomal regions can be suppressed by the incorporation of BrdU as a terminal pulse at the end of S period. The 200 μg/ml terminal pulse of BrdU and subsequent Hoechst plus Giemsa staining reveals a uniquely informative pattern of chromosomal bands coupled with specific decondensation of late replicating regions.

Band 6q12 is late replicating in at least 50% of the cells examined from 12 individuals. The region 6q12→6q14 shows compound lateral asymmetry of stainability which presumably reflects an alternating pattern of thymidine asymmetry in the chromosomal DNA of that region. An intrachromosomal shift of 6q12→6q14 due to a

pericentric inversion does not affect its temporal replicative pattern or its compound lateral asymmetry.

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