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INCREASED AMOUNTS OF HYBRID (HEAVY/HEAVY) DNA IN BLOOM'S SYNDROME FIBROBLASTS[1]

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SUMMARY: The nuclear DNA of fibroblasts from patients suffering with Bloom's syndrome, density labeled for less than one round of DNA replication to give heavy/light molecules, was examined for spontaneous amounts of heavy/heavy DNA (hybrid DNA). When compared to normal fibroblasts the Bloom's syndrome cells exhibited a sixfold increase in such DNA.

Exchange of pieces of DNA between sister chromatids was shown to occur in mammalian cells by Taylor (1) shortly after his original demonstration of this phenomenon in plant cells (2). In 1964, German (3) reported that exchange can occur between chromatids of homologous human chromosomes, presenting this as cytological evidence for somatic crossing-over, although genetic evidence for crossing-over in mammalian somatic cells has not yet been reported. At the molecular level, some evidence has been presented for the exchange of small amounts of DNA between parental and daughter strands in dividing cells (4, 5).

Certain human genes now have been recognized which can influence the frequency

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Abbreviations used: BS, Bloom's syndrome; hybrid DNA, heavy/heavy DNA.

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with which chromatid exchange occurs (6, 7). Cultured cells from patients suffering with BS, which is characterized clinically by small size, a sun-sensitive skin disorder, disturbed immunity, and cancer proneness, display a striking increase in the number of spontaneously occurring chromatid exchanges between both sister and homologous-but-nonsister chromatids (6, 7). In the experiment reported here, we have employed cells homozygous for this disorder in an attempt to clarify the relationship between chromatid exchanges observed in the microscope and molecular mechanisms of recombination. The results suggest that, when compared to normal fibroblasts, BS cells exhibit an enhanced amount of hybrid DNA under conditions where semiconservative replication should result in only heavy/light molecules.

Hybrid DNA was measured in normal and BS cells by density labeling the replicating DNA for 8 h, chasing with unlabeled medium for a further 14 h, and subjecting extracted DNA to isopycnic cesium chloride centrifugation. The density label was thus present for less than one round of DNA replication so that all replicated, double-stranded DNA should have one DNA strand of normal density and the other of a heavier density as a result of BrdUrd incorporation (heavy/light DNA). Any spontaneous hybrid DNA containing density label in both of its strands could occur by an exchange mechanism, one model of which has been described by Holliday (8). After the centrifugation in cesium chloride gradients of density-labeled, extracted DNA, a small amount of hybrid DNA occurs at densities heavier than the heavy/light main peak. This hybrid DNA was present regardless of whether whole cells or isolated nuclei were used for the extraction, and, therefore, it is not cytoplasmic in origin. Accurate estimates of the amount of hybrid DNA are difficult because of cross contamination from the trailing of the heavy/light peak. In an effort to improve resolution, fractions at densities heavier than the heavy/light peak were pooled, dialysed twice against 0.01 M Tris HCl, 0.001 M EDTA, 0.04 M NaCl, pH 8.0, and recentrifuged in a second cesium chloride gradient. Profiles after the second centrifugation are shown in Fig. 1. Any remaining heavy/light DNA is seen to have been separated from the hybrid

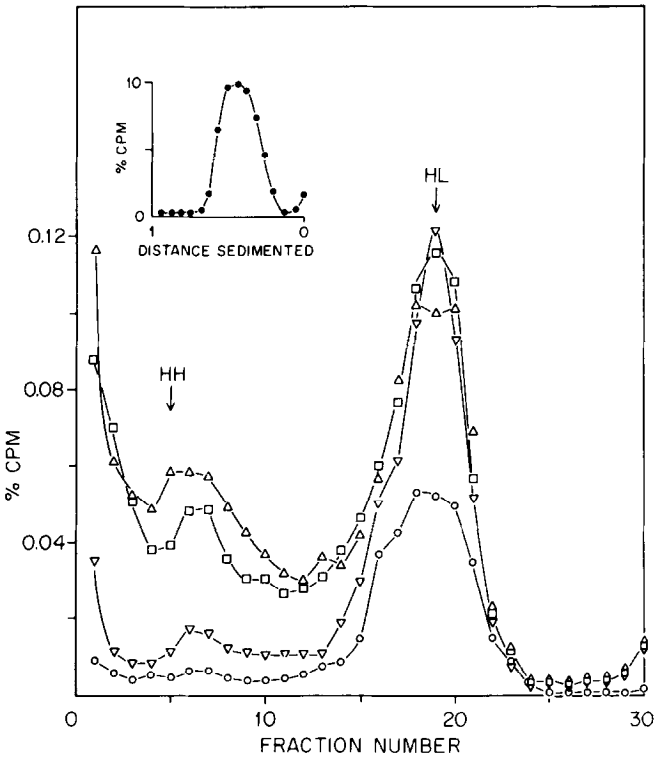

Fig. 1. Normal fibroblasts HSBP (O, ▽) or BS fibroblasts H369 (□) and HG1014 (△) were plated at 3.10^5 per 100-mm dish in Eagle's MEM supplemented with 10% calf serum and dishes incubated at 37° in an atmosphere containing 2% CO_2. After 40 h 3 μg/ml bromodeoxyuridine and 0.25 μg/ml fluorodeoxyuridine were added and 1 h later 20 μCi/ml of [^{3}H]thymidine (Schwartz/Mann 60 Ci/mmol) was added for 8 h. This results in the density-labeled (i.e., BrdUrd-containing) DNA strand also becoming radioactively labeled with [^{3}H]thymidine. After incubation for 9 h with BrdUrd and FUrd and 8 h with [^{3}H]-thymidine, the medium was removed; the cells were rinsed with and transferred to medium, containing 4 μg/ml thymidine, where they remained for 15 h. Cells were then lysed in 0.5% Sarkosyl, 0.4 M EDTA, and 200 μg/ml of Proteinase K (E. M. Biochemicals) and incubated on a rotator at 60 rpm at 37° for 2 h. Phenol equilibrated with 0.01 M Tris-HCl, 001 EDTA, 0.04 M NaCl at pH 8.0 was added and the incubation continued another h. The phenol layer was separated by centrifugation, and the aqueous phase was removed and dialyzed against buffer containing 0.01 M Tris-HCl, 0.001 M EDTA, 0.04 M NaCl at pH 8.0 to remove any remaining phenol. The aqueous DNA-containing portion was sheared by repeated passage through a 27-gauge needle. Cesium chloride was then added to 1.39 gm/ml (refractive index 1.4046) and centrifugation was performed using a Beckman type 40 rotor at 35,000 rpm for 66 h. The gradients were fractionated to give 30 portions of 0.225 ml each. A small amount of each fraction was utilized to determine the position of the DNA in the gradient, after which those containing DNA heavier than heavy/light were pooled, the density adjusted to a refractive index of 1.4046, and the volume made up to 7.0 ml with a solution of cesium chloride of the same density. These pooled fractions were recentrifuged and fractions collected as above. The DNA in each fraction was then precipitated with TCA and its radioactivity determined. The profiles of such a second centrifugation are presented and the data expressed as percentage of cpm found after the first centrifugation.

DNA, and amounts of the latter may be accurately estimated. BS cells clearly have elevated amounts of hybrid DNA banding at densities greater than semiconservatively replicated heavy/light DNA. In order to determine whether the hybrid DNA was double stranded, the single strand-specific nuclease S1 was incubated with native and heat-denatured hybrid DNA obtained from the second centrifugation. As shown in Table 1, radioactivity which was found as hybrid DNA represented double-stranded DNA because the S1 nuclease (Cal Biochem.) digests only DNA rendered single stranded by heat denaturation. Radioactivity recovered at densities greater than heavy/heavy was believed to represent single-stranded DNA because the number of acid-precipitable cpm in these densities was reduced by incubation with S1 nuclease. A similar single-stranded fraction detected by Moore and Holliday (5) was thought by them to have arisen during the shearing of the DNA.

The inset to Fig. 1 shows the profile of this hybrid DNA after alkaline sucrose sedimentation. This DNA has a single strand weight-average molecular weight of 5.10^6. The result indicates that exchanged regions are of about 5.10^6 daltons or smaller, because most of the hybrid DNA bands at densities less than that of totally heavy/heavy DNA.

In conclusion, the density-label experiments reported here suggest that human fibroblasts labeled for less than one round of DNA replication contain a small amount of double-stranded, hybrid DNA which band at densities greater than heavy/light. Only heavy/light DNA would be expected. This result suggests the operation of an exchange mechanism. The spontaneous amount of hybrid DNA (0.04%) occurring in our normal human fibroblasts compares well with earlier values obtained by other investigators for Chinese hamster cells in culture (4, 5). Although this amount is somewhat greater than expected during the early steps of any sister-chromatid exchange observed cytologically, a relationship between the amount of hybrid DNA and sister-chromatid exchange was suggested by the data of both the reports just cited. This conclusion is supported by our finding that fibroblasts from individuals with BS, which have a greater than tenfold increase in the spontaneous frequency of exchange between both sister and homologous-but-nonsister chromatids (6, 7)

Table 1

Effect of S1 nuclease on acid precipitable radioactivity banding at densities greater than heavy/light after isopycnic centrifugation

Banding	Acid precipitable cpm
Between heavy/heavy and heavy/light	
Untreated	1843
Untreated + S1 nuclease	1744
Heat denatured	1790
Heat denatured + S1 nuclease	564
Banding heavier than heavy/heavy	
Untreated	592
Untreated + S1	113

Prior to treatment with the S1 nuclease, the DNA was dialyzed against 0.4 mM $ZnSO_4$, 0.25 M NaCl, 0.03 M $NaCH_3COOH$ at pH 4.6. S1 nuclease at 0.5 I.U./ml was then added to native or heat-denatured DNA in this buffer and incubated for 30 min at 37°. Acid precipitable cpm were then estimated.

also have six to tentimes more spontaneously occurring hybrid DNA after density labeling than do normal cells.

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