SHORT PAPERS

BANDING PATTERNS OF CHINESE HAMSTER CHROMOSOMES

SURABHI KAKATI AND ANIL K. SINHA

Department of Pathology, Pediatrics and the Division of Experimental Biology, Baylor College of Medicine
and
Department of Pathology, Texas Children's Hospital, Houston, Texas 77025

Manuscript received May 1, 1972

SPECIFIC Giemsa-stained cross bands (G) have been induced in human and mouse chromosome complements by denaturating and reassociating techniques (RIDLER 1971; SCHNEDL 1971a; 1971b; DRETS and SHAW 1971) or by simply incubating the aceto-alcohol fixed chromosomes in a saline solution (SUMNER, EVANS and BUCKLAND 1971; BUCKLAND, EVANS and SUMNER 1971). Various concentrations of trypsin have also been utilized to produce similar bands within the human chromosomes (SEABRIGHT 1971; WANG and FEDOROFF 1972). Thus, the G-band technique, like the quinacrine mustard technique (Q), can equally be utilized for the identification of individual homologs.

In this communication we present G as well as C (Centromeric heterochromatin blocks) bands of the Chinese hamster chromosomes. A comparative study of the two types of bands has revealed some interesting differences.

MATERIALS AND METHODS

Chromosome Preparation: Young Chinese hamsters *Cricetulus griseus*, of both sexes were killed for initiating cell cultures of kidney, peritoneum and testis. The cultures were treated with colchicine, trypsinized and passed through the sodium citrate solution. The cells were then fixed with 1:3 acetic-alcohol and flame-dried on slides.

The diploid male (Don) and female (Daisy) cell lines were obtained through the courtesy of Dr. T. C. HSU. The procedure for chromosome preparation was the one described for primary cell cultures.

Direct chromosome preparations were made from the bone marrows and testes of the animals that had received colchicine for three and one half hours prior to their sacrifice. The marrow cells and the minced testes were suspended in the sodium citrate solution. The rest of the procedure for chromosome preparations was identical to the one used for cell culture samples.

Induction of C and G Bands: The flame-dried slides were dipped in NaOH solution (0.07 N) for 1.15 min. They were then kept in a mixture of sodium chloride and sodium citrate solutions (12 × SSC) at 65°C for 48 or 72 hr. At the end of the incubation periods the slides were rinsed in several changes of 70% ethanol and then in 90% ethanol. The slides were then stained with Giemsa solution (pH = 7.0) for 30 min, washed briefly with distilled water, air dried and mounted in Permount.

Supported in part by the Robert A. Welch Foundation, The National Foundation and USPHS Grant No. FR-05425.

RESULTS

The slides incubated in SSC solutions for 48 hr or 72 hr exhibited metaphases with C as well as G bands. Hence, the incubation periods of C band (48 hr) and G band (72 hr) in case of human cells do not seem applicable to the Chinese hamster cells.

The chromosome preparations made from cells of different tissues did not respond alike to Giemsa stain treatment. The spermatogonial cells in culture and the Don cell line gave the best results. It was, however, noted that the bands were nonspecific to a particular type of tissue, i.e. all the metaphases irrespective of their origin exhibited a similar banding pattern. Some minor variations, of course, were noticeable even in individual metaphases of the same cell type.

C Band: In Figure 1 it can be observed that all the autosomes except pairs 1 and 2 have darkly stained centromeric regions. Each arm of the pair 1 possess an

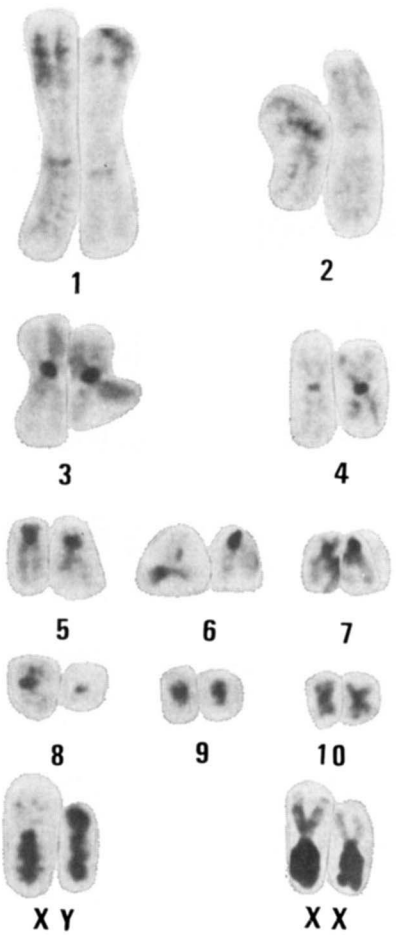

FIGURE 1.—Chromosome complement of a male Chinese hamster bone marrow cell exhibiting the C band; two X chromosomes are inserted from a female peritonium cell.

interstitial lightly stained band. This finding is in agreement with the earlier report of HSU and ARRIGHI (1971). The pair number 2, on the other hand, does not show any localized heterochromatin. The long arm and the proximal end of the short arm of the male X and the entire Y chromosome are darkly stained. The two X chromosomes of the female are indistinguishable from the male X chromosome. Most probably the facultative heterochromatin of the female X chromoosme does not respond to C-band treatment.

G Band: The specificity of bands for homologous chromosomes become apparent merely by surveying the banding pattern of a total complement (Figure 2). The major bands are the land marks for each pair. The minor bands, quite often, fuse to form a single broad band and show some variation in their expression from one homolog to the other. It can also be noted that the C-band positive or negative homologs (Figure 1) are lacking the bands around their centromeres during G-band formations. The individual chromosome pairs are identifiable by virtue of their following characteristic G patterns.

Chromosome 1: Each arm of this chromosome has two distinct dark bands. The distal band of the short arm is followed by a conspicuous negative band beyond which many minor bands fuse to form a broad band that extends approximately distal two third of the short arm. The telomeric end of the long arm is pale. This is followed by two positive bands being separated by a negative band. Besides these two major bands, the proximal two third of the long arm has many minor bands.

Chromosome 2: Both the arms of the chromosome exhibit a fairly uniform banding pattern. The distal half of each arm contains a conspicuous negative band in between two positive bands. The negative band of the long arm is, however, wider than that of the short arm. The telomeric ends are pale.

Chromosome 3: This has a wide dark band occupying about the distal one third of the short arm. Beyond this band there is a distinct negative band. There is another dark band present proximal to the centromere. The long arm also has a dark band at the distal end. This is followed by a conspicuous negative band. Two more wide bands are present at approximately two thirds of the proximal end of the long arm.

Chromosome 4: The distal end of the short arm stains dark then gradually gets paler down to the centromeric region. The long arm, on the other had, usually exhibits two distinct bands; one at the distal end and another at the proximal end.

Chromosome 5: This is the largest of the acrocentric chromosomes. Its long arm has a series of positive bands of which the one proximal to the centromere is the most prominent. The distal end of the short arm is paler than the proximal end.

Chromosome 6: The area extending from the centromere down to the proximal one third of the long arm stains dark. This is immediately followed by a negative band. A narrow but distinct positive band is present in the center of the long arm; the rest of this arm is pale. The short arm is darkly stained.

Chromosome 7: One of the distinctive features of this pair is a broad negative band that occupies about the proximal one fourth of the long arm. The rest of the

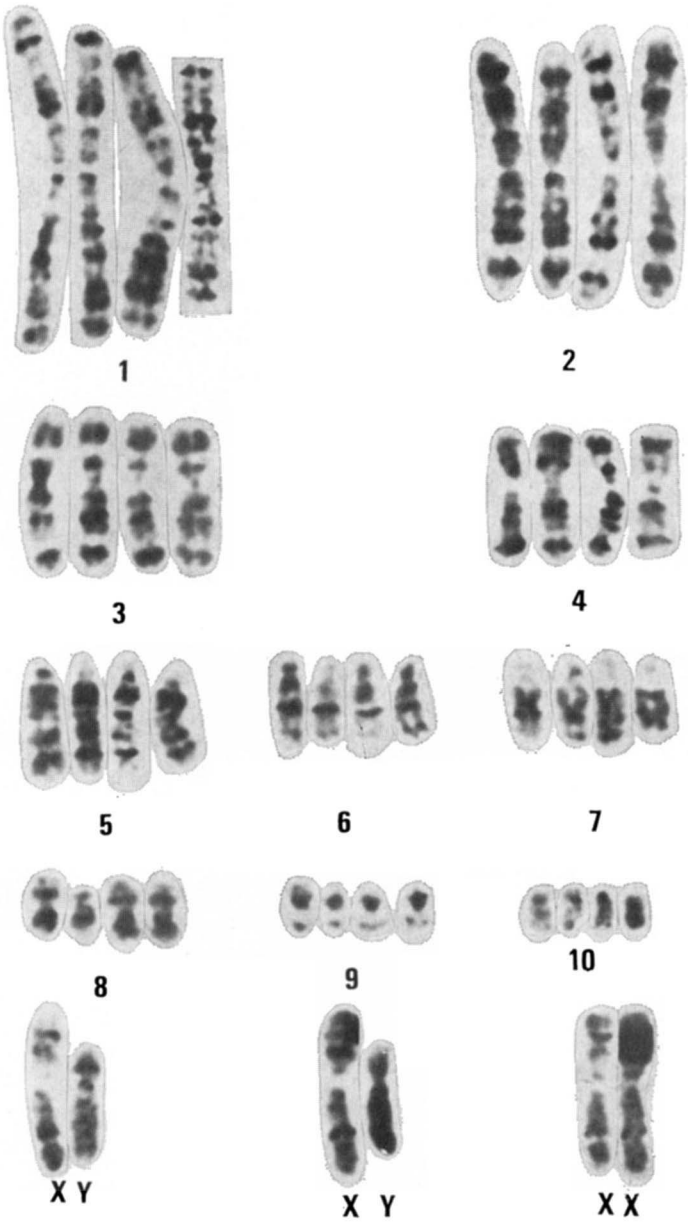

FIGURE 2.—Chromosomes of male Chinese hamster showing G bands. The first pair of each set belongs to a single cell (Don) where as others are derived from testicular cells grown *in vitro*. Two female X chromosomes (Daisy) are inserted. The wide dark regions in the short arm of one of the Xs are due to overlapping.

long arm is dark but occasionally the distal one fourth gets paler. The short arm is pale.

Chromosome 8: One arm is completely dark whereas the other arm has a posi-

tive band. The telomeric ends of both the arms are otherwise lightly stained.

Chromosome 9: A negative band is present at the proximal end of an arm which is followed by a narrow positive band. This positive band terminates at the telomeric end. The other arm is completely dark.

Chromosome 10: This pair of the autosomes does not exhibit any distinct band.

X chromosome: By conventional staining the X chromosome is not always distinguishable from pair 4; the X chromosome sometime exhibits a secondary constriction in its long arm. However, the X chromosome bears a distinguishable banding pattern. It is characterized by a dark band located at the distal one third of the long arm and above the secondary constriction. This band often forms an arch. Two positive bands are present on the short arm. The telomeric ends are slightly stained. The male X and the female Xs stain alike.

Y chromosome: The Y chromosome does not seem to reveal any distinct band. It is darkly stained throughout its entire length and thus behaves alike during G- and C-band formation.

DISCUSSION

Upon induction of G bands in human as well as in mouse chromosomes one notes that the G bands are invariably associated with the C bands. The present observation on the banding patterns of the Chinese hamster suggests that the C-band positive chromosomes of this mammal show a tendency to lose their C bands during G-band formation despite the fact that both types of bands were induced by the same technique. The query as to whether or not such a differential mode of band formation is attributable to Chinese hamster alone can not be resolved until more species have been studied for these two types of bands.

It is interesting to note that the Y chromosome of the Chinese hamster behaves similar to that of the man (SCHNEDL 1971a) and mouse (SCHNEDL 1971b; BUCKLAND *et al.* 1971) in the sense that it maintains a uniform pattern during C- and G-band formation. This does not seem to be true with regard to the X chromosome.

The C band of human and mouse X chromosome is restricted to the centromeric areas. The X chromosome of the Chinese hamster, on the other hand, occupies a band that extends from the proximal end of the short arm to the entire long arm. Such a band of the X chromosome is, however, not maintained during the G-band formation. Instead, the X chromosome exhibits the cross-bands like the mouse and human X chromosome.

LITERATURE CITED

BUCKLAND, R. A., H. J. EVANS and A. T. SUMNER, 1971 Identifying mouse chromosomes with the ASG technique. Exptl. Cell Res. **69**: 231–236.

DRETS, M. E. and M. M. SHAW, 1971 Specific banding patterns of human chromosomes. Proc. Natl. Acad. Sci. U.S. **68**: 2073–2077.

HSU, T. C. and F. E. ARRIGHI, 1971 Distribution of constitutive heterochromatin in mammalian chromosomes. Chromosoma **34**: 243–253.

RIDLER, M. A. C., 1971 Banding patterns of metaphase chromosomes in Down's syndrome. Lancet **11**: 354–356.

SCHNEDL, W., 1971a Banding pattern of human chromosomes. Nature **233**: 93–94. ——, 1971b The karyotype of the mouse. Chromosoma **35**: 111–116.

SEABRIGHT, M., 1971 A rapid banding technique for human chromosomes. Lancet **11**: 971–972.

SUMNER, A. T., H. J. EVANS and R. A. BUCKLAND, 1971 New technique for distinguishing between human chromosomes. Nature **232**: 31–32.

WANG, H. C. and S. FEDOROFF, 1972 Band in human chromosomes treated with trypsin. Nature New Biol. **235**: 52–53.