COMPARATIVE BANDING PATTERN ANALYSES IN HUMAN CHROMOSOMES INDUCED BY UREA AND TRYPSIN TREATMENTS¹⁾

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Studies of banding patterns in human chromosomes have advanced rapidly since the introduction of the quinacrine fluorescence technique (Caspersson *et al.* 1970, 1971) and several different procedures based upon incubation in warm saline or buffer solutions followed by Giemsa staining (Sumner *et al.* 1971; Schnedl 1971; Drets and Shaw 1971). These techniques have become available to identify each chromosome pair in the human complement. However, these methods either require special equipment (an ultraviolet microscope) or are time-consuming, and sometimes obscure the banded appearance. A rapid and simple method should therefore be of advantage for the production of banding patterns.

Recently two rather simple kinds of techniques have been developed, which reveal the characteristic banding patterns within chromosomes. The first technique is that developed by Seabright (1971), and Wang and Fedoroff (1972) with trypsin treatment. The second, developed in this laboratory (Shiraishi and Yosida 1971, 1972; Kato and Yosida 1972), has been devised as a result of experiments with urea solution supplemented with saline or phosphate buffer solution. Both techniques were essentially the same as those used for the extraction of protein from the cell. Banding patterns produced by these two techniques appeared to be very similar to each other. The present study was undertaken to compare the banding patterns produced by these two techniques and was also done to confirm the specificity as well as constancy of these patterns.

MATERIALS AND METHODS

Human peripheral leucocytes from 10 healthy subjects (5 females and 5 males) were cultured according to the routine phytohemagglutinin method. Chromosome preparations and the urea banding technique were performed by the same methods as those

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previously used (Shiraishi and Yosida 1972).

The technical procedure for trypsin banding was performed according to Seabright (1971) but modified slightly. Within 24 hours after air drying, chromosome preparations were treated for 5-10 seconds with cold trypsin-versene (0.05% trypsin solution in 0.02% EDTA solution) kept in refrigerator (0-4°C). Then, the slides were rinsed thoroughly with tap water and stained with Giemsa diluted about 50 times with Sørensen's buffer (pH 6.8) for 5-10 min. The trypsin treatment needed some cautions in handling. When freshly prepared slides were immediately immersed in the treatment solution, most of bands appeared to be obscure or took a fuzzy appearance, which was no longer informative. This could be avoided to a certain degree by drying the slides for many hours. Best results were obtained when slides were kept drying overnight (for about 12 hours) at room temperature before use. Natural drying is desirable for these methods.

RESULTS

Banding patterns of normal female and male karyotypes produced by these treatments are presented in Figures 1 to 3. Those produced by the urea and trypsin treatments are shown in "a" and "b" in these figures, respectively. As seen in the figures, each chromosome pair showed a specific pattern of bands within chromosomes and close similarity in homologues, and the patterns produced by the two methods seemed to be essentially the same, though the pattern produced by the urea technique is slightly clearer than that produced by trypsin. A diagram depicted in the last column of Figs. 1-3 was determined based on more than 40 suitable metaphase cells. Banding patterns produced by the present two methods were in general good agreement with those obtained in our previous work (Shiraishi and Yosida 1972). Chromosome pairs 4, 5, 9, 11, 12, 19, 20, 21 and 22 in the previous description were designated in the present study as pairs No. 5, 4, 11, 12, 9, 20, 19, 22 and 21, respectively, referring to the banding patterns published by Caspersson *et al.* (1970, 1971), Seabright (1971) and Paris Conference (1971). In the present study, diagrams of banding patterns in pairs 5 and 13 and the short arm of the X chromosome were slightly revised as follows. Chromosome 5 shows in its long arm five dark bands and two dark bands in the short arm. The three characteristic dark bands in the middle of the long arm are helpful for identification. Close to the distal part of both arms and the centromere of the short arm unstained regions are visible. Chromosomes 4 and 5 display a close morphological similarity, but they are distinguishable by the banding patterns (Fig. 1). Chromosome 13 has three or four dark bands in the long arm. The distal part of the long arm is sometimes unstained. The X-chromosome exhibits three dark bands in the long arm and one dark band in the middle of the short arm. The distal part of the short arm is unstained. No difference has been observed between the two X-chromosomes in the female.

Figs. 1-3. Four normal karyotypes of females and males after treatment with two techniques and diagram of most reliable patterns for each chromosome pair.

a. Urea-treatment

b. Trypsin-treatment

DISCUSSION

There was no difference in banding pattern between the urea and trypsin treatment techniques. The patterns produced by these two methods are also similar to those shown by quinacrine fluorescence (Caspersson *et al.* 1970, 1971) and other techniques by Giemsa staining (Sumner *et al.* 1971; Schnedl 1971; Drets and Shaw 1971). In contrast to the fluorescence technique, dark stained bands can also be detected by the present techniques at the centromere regions of most chromosomes. A striking peculiarity is found in the secondary constriction adjacent to the centromere of the long arm of chromosomes 1 and 16. The regions, very poorly stained with quinacrine, are darkly stained by the present techniques.

The most important observation in the present study was that the procedures described induced the characteristic banding pattern in all individual human chromosomes, and these patterns enabled the identification of all chromosome pairs. Although the chromosomes in the groups A and E are ascertained from arm ratio and chromosome length, the chromosome pairs within groups B, C, D, F and G display rather close morphological similarities. It is therefore necessary and important to standardize the system of chromosome numbering in groups B, C, D, F and G based upon the characteristic banding patterns in each chromosome pair. Consequently the banding patterns are very helpful for accurate identification and designation of individual chromosomes and detection of specific chromosome segments involved in rearrangements such as translocations and inversions (Paris Conf.).

The procedures used in the present study are essentially the same as those employed for protein extraction from cells. Although we do not know the exact molecular mechanisms, the banding patterns revealed by Giemsa staining technique might be related to the physical state of constituent DNA and its association with proteins, rather than to the denaturation and renaturation of DNA. Recently Wang and Fedoroff (1972) suggested that removal of protein component of the nucleoprotein complexes making up the chromosomes might allow Giemsa stain to react directly with the nucleic acid component. According to Kato and Moriwaki (1972) the solubilization or extraction of some chromosomal proteins, probably of acid nature, would be the primary cause of the appearance of banded structure of metaphase chromosomes. However, the relation of the banding patterns to functional aspects of specific chromosomes requires further study.

SUMMARY

The present study was undertaken to compare the banding patterns of human chromosomes produced by trypsin and urea treatment techniques. Banding patterns produced by these two techniques appeared to be essentially the same and specific in each pair of homologues. These patterns also enabled the recognition of specific segments of all individual human chromosomes. From the banding pattern produced by the two techniques a diagram of the pattern was proposed by a slight modification from our previous presentation (Shiraishi and Yosida 1972).

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