DA/DAPI-Fluorescent heteromorphism of human Y chromosome

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Summary. Variation of DA/DAPI intensity in the Yq12 band was observed in five amniotic cell specimens and one blood specimen from the father of one fetus. Three distinct classes of Yq heterochromatin were identified by distamycin A (DA) treatment of the cell cultures and various staining techniques. The heterochromatin in the Yq11.23 sub-band does not undercondense when exposed to DA, and shows pale fluorescence with quinacrine staining, positive C-banding, and bright fluorescence with DA/DAPI technique. This class of heterochromatin was consistently observed in all specimens studied. The other two classes of heterochromatin are in the Yq12 band. Both show undercondensation when exposed to DA, quinacrine-bright fluorescence, and positive C-banding; however, one class of heterochromatin shows DA/DAPI-bright fluorescence and the other shows pale fluorescence. The size and banding intensity of the two classes of heterochromatin in Yq12 are variable. These results provide cytological evidence of heterogeneity within the Y heterochromatin region containing AT-rich DNA.

Materials and methods

Five amniotic cell specimens and one blood specimen were studied. The cells were grown in Eagle's MEM medium supplemented with 20% fetal bovine serum, streptomycin and penicillin. For blood, phytohemagglutinin was added to the culture medium. Duplicate sets of cultures were set up from each amniotic cell specimen. DA (Sigma) was dissolved in Hank's solution (1 mg/ml), filtrated, and added to one set of cultures at a final concentration of 100 µg/ml for 24 h before fixation. Colcemid at a final concentration of 0.05 µg/ml was added to the cultures 3–4 h before harvest. Chromosome spreads were prepared by the air-dry technique.

The metaphases were stained with quinacrine di-HCl (Q-banding), conventional Giemsa stain, a modified C-banding method, and DA/DAPI technique (Schweizer et al. 1978; Schweizer 1980). From each staining technique 15–20 metaphases were examined. At least 5 metaphases showing the best differentiation were photographed.

Results and discussion

Brilliant quinacrine fluorescence in Yq12 was found in all specimens studied. When DA/DAPI technique was employed, two different fluorescence intensities in Yq12 were found in one of the amniotic cell specimens and the blood from the father of the fetus. The fluorescence was bright in the proximal part and pale in the distal part of Yq12 (Fig. 1). The heteromorphic variant was inherited from father to fetus. Recently, DA/DAPI-fluorescent heterochromatism of human chromosomes 13p and 15p has also been reported (Babu et al. 1986; Pérez-Castillo et al. 1987).

To rule out a possible Y-autosome translocation in the present case and to study the organization of Y heterochromatin, cells were pretreated with DA (100 µg/ml) at the final concentration for 24 h before fixation. DA in tissue culture apparently causes undercondensation of the AT-rich region of Y chromosomes (Prantera et al. 1979; Schmid 1979). Undercondensed Y heterochromatin was found in all mitoses analyzed, including the specimen with heteromorphic DA/DAPI fluorescence. As shown in Fig. 2, the bright fluorescence of the Q-banded Y heterochromatin was not changed by DA. The Y heterochromatin showed lighter staining with the conventional Giemsa stains, as compared with the euchromatic regions (bands Yp11 and Yq11). A Y-autosome translocation was ruled out in the present case since brightly Q-fluorescing satellites in D and G group chromosomes do not show undercondensation after DA treatment (Schmid 1979).
Fig. 1A–C. G group and Y chromosomes from an amniotic cell specimen (A, B) and the blood from the father of the fetus (C). A Q-banding shows Yq12 with brilliant fluorescence. B DA/DAPI staining shows two fluorescing intensities in Yq12. The proximal part of Yq12 is bright and the distal part is pale. C The father’s Y chromosome after DA/DAPI staining shows the same pattern as B.

Fig. 2A, B. Y chromosomes from prometaphases and metaphases from an amniotic cell culture treated with 100 µg/ml distamycin A for 24 h before fixing. The distal part of heterochromatin in the long arm is undercondensed. A Quinacrine staining; B conventional Giemsa staining.

Fig. 3A, B. Sequentially stained Y chromosomes by Q- and C-banding (left) and C-banding (right) from metaphases of distamycin A-treated amniotic cell cultures. A and B were from two different amniotic cell specimens. The C-banded positive material in the Y chromosome extends interstitially in the long arm beyond the quinacrine-bright segment. This extended segment with normal condensation is insensitive to DA.

Sequential staining of Y chromosomes by Q- and C-banding after DA-induced undercondensation is presented in Fig. 3. Q-banding revealed a bright fluorescence with undercondensed heterochromatin in Yq12. When C-banding was applied, a small spot of centromeric heterochromatin and a large block of heterochromatin in the long arm of the Y terminal were observed. C-banded Yqter heterochromatin extended further to the proximal portion of the Y chromosome, as compared with the Q-banded brightly fluorescing region. The extended segment of C-banded heterochromatin in the proximal portion that showed Q-pale fluorescence did not undercondense when exposed to DA, as demonstrated also in Fig. 4 (DA/DAPI stain). The undercondensed heterochromatin appeared as a thin chromatin thread adjoined to the normally condensed heterochromatin, and showed bright Q-fluorescence.

According to the current international nomenclature (Paris Conference 1971; ISCN 1985), the long arm of the human Y chromosome can be distinguished into two bands (bands q11 and q12). Yq11 shows dull Q-fluorescence and Yq12 shows bright fluorescence. Therefore, undercondensed heterochromatin is restricted to Yq12 and the normally condensed heterochromatin is located at Yq11.23. These features were consistently observed in all specimens studied. Our results confirm the previous reports (Soudek and Laraya 1976; Limon et al. 1979; Bühl 1980). Those investigators demonstrated that the Yq constitutive heterochromatin segment after C-banding is larger than the Q-banded fluorescing segment.

The patterns of DA/DAPI fluorescence of the Y chromosomes after DA-induced undercondensation are shown in Fig. 4. The centromere of the Y chromosome appeared as a small DA/DAPI band separating the dull short arm and proximal long arm segments. The long arm heterochromatin revealed several bright and pale bands. The pattern of the bright DA/DAPI fluorescence in Yq12 is variable among the Y chromosomes from different amniotic specimens. However, the particular pattern for a given Y chromosome is consistent within the individual amniotic cell specimen. The Y chromosomes from amniotic cells with DA/DAPI-heteromorphic fluorescent...