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Human Chromosome Banding by Feulgen Stain Aids in Localizing Classes of Chromatin

Abstract. *The band patterns of human chromosomes displayed by the Feulgen stain are similar to but not identical with those of the conventional quinacrine and Giemsa preparations. The parallelism among the three is principally that of prominent negative bands that appear consistently at characteristic loci throughout a wide range of chromosome compaction. The correlation of those bands with regions of low optical density in nonbanded Fuelgen-stained chromosomes suggests that they are loci of DNA that is inherently diffuse or readily labile. The instances of disparity among the patterns of the three modes of banding occur at the telomeres and at known regions of heterochromatin, and are interpreted here as reflecting the heterogeneity of the protein moieties in their reactivity to the cytochemical treatments.*

As has been demonstrated for the chromosomes of the mouse (*1*), the correspondence among the Feulgen banding patterns of human chromosomes (F bands) and those produced by other methods in general use—quinacrine fluorescence (Q bands) and Giemsa chromaticity (G bands)—is sufficiently close for pairing and identification of F banded chromosomes to be made in terms of the standard karyotypes and idiogram established for the Q and G bands (*2*). On analysis, the correspondence proves to be based principally on certain negative regions that are constant and so characteristic that they have been designated as "landmarks" (*2*). However, there are some chromosome segments in the complements of both mouse and man where the tripartite correspondence does not persist. Those regions in the chromosomes of man are well defined in the Feulgen banded karotype and have been identified as heterochromatin (*3*) or highly repetitious DNA, a fraction that has been found by biochemical analysis to display considerable heterogeneity with respect to base ratios (*3, 4*). With the parameter of DNA density established by the specificity and stoichiometry of the Feulgen reaction, a study of the instances of correspondence and disparity in the banding patterns produced by the three cytological stains allows for localization of different classes of chromatin and provides a basis for their cytochemical characterization. My observations provide support for the hypothesis (*1*) that the mechanism of banding, analyzed in terms of the Feulgen method, is bimodal. The negative bands are those inherently sparse in DNA or those from which the DNA has been removed by one or several steps of the cytochemical procedure, while the dark bands result from precipitation in situ of a selective group of chromatinic proteins with the consequent condensation of their associated DNA.

Lymphocytes were harvested after 3 to 4 days of culture with and without a 2-hour incubation with Colcemid. Chromosome spreads were prepared as described (*1*). Some of each set of slides were treated prior to Feulgen staining to induce banding (*1*). Karyotypes were assembled from micrographs of well-spread metaphase sets of varying degrees of compaction. Designation of chromosome number was made by reference to the standard karyotype (*2*) and to those appearing in original publications (*5–9*). Of the preparations obtained from seven series, representing four subjects, 40 complete metaphase sets that had been treated for banding were analyzed for the presence of the characteristic negative bands. Those included preparations that had been treated with Colcemid and preparations not treated with Colcemid, with values for total length of the chromosome complement ranging from 163 to 335 units (*10*).

The parallelism between the prominent negative regions of the Feulgen banded preparations and those of the standard Q and G banded chromosomes is reported in Table 1. With one exception, 2q distal, each of the negative regions designated as "landmarks" (*2*) of the Q and G bands (Table 1, column A) was detectable in one or both homologs of each chromosome pair of all Feulgen banded sets examined

Table 1. Characteristic negative bands of human chromosomes. (Column A) Characteristic and consistent negative bands of both G and Q patterns of human chromosomes. No marking (no asterisk) indicates designated negative landmarks (*2*). The (*) indicates other regions consistently seen as negative (*21*). (Column B) Negative regions detected in one or both homologs in 40 Feulgen banded human karotypes (Fig. 1). (Column C) Negative or pale regions seen in a nonbanded Feulgen stained set (Fig. 2, series b).

Arm	A	B	C
1p	Distal (tip)*	Distal	Distal
q	Proximal	Proximal	Proximal
2p	Median	Median	Median-distal
q	Proximal	Proximal	
	Distal		
3p	Median	Median	Median
q	Median	Median-distal	Median-distal
4q	Proximal	Proximal	Proximal
	Distal	Distal	
5q	Distal	Distal	
6p	Median	Median	Median
q	Median	Median	
7q	Median*	Median	Median
8p	Median	Median	Median
9q	Median*	Median	
10q	Proximal*	Proximal	Proximal
11q	Median	Median	Median
12q	Proximal*	Proximal	Proximal
14q	Median-distal*	Median-distal	Median
15q	Median*	Median	Median
17q	Proximal*	Proximal	Proximal
18q	Median	Median	Median

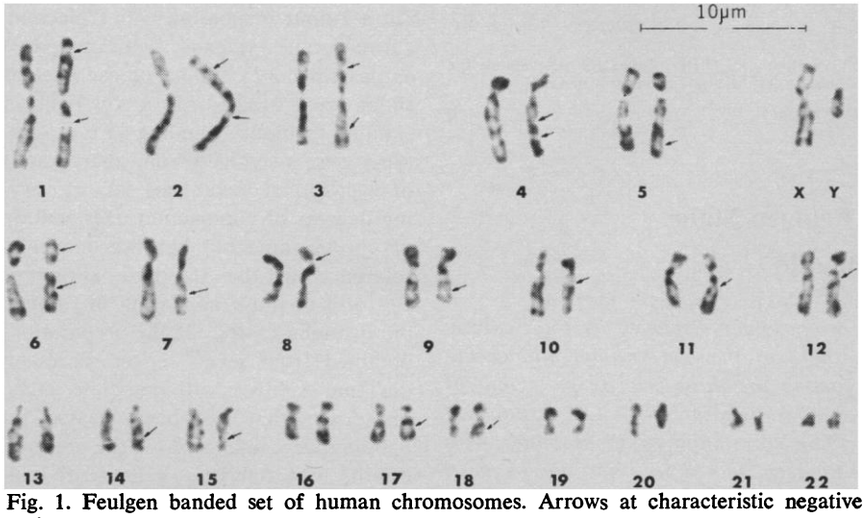

Fig. 1. Feulgen banded set of human chromosomes. Arrows at characteristic negative regions.

(Table 1, column B; Fig. 1). Although the number of bands increases with degree of decompaction (*1, 9*), giving rise to additional negative bands, those are distinguishable by the very fact that they do not appear with the fidelity of the prominent or "landmark" (*2*) negative bands.

I have reported (*11*) some differential staining, albeit not sharply delineated as bands, in mouse metaphase chromosomes treated by the conventional Feulgen reaction, with no prior band-inducing treatment. Since nonbanded mouse chromosomes are not readily identified, the specificity or randomness of location of the Feulgen pale regions could not be determined. In the human complement, however, when similar Feulgen diffuse regions were observed in nonbanded chromosome preparations, it was possible, particularly in those chromosomes identifiable by relative size and arm ratio, to relate the Feulgen pale regions of the nonbanded sets to the characteristic negative bands of the banded sets (Fig. 2). Of 21 prominent negative regions established as characteristic and consistent by analysis of 40 Feulgen banded karyotypes, 16 were identified as diffuse or negative regions in the single nonbanded set represented in Fig. 2, series b, and Table 1 (*12*).

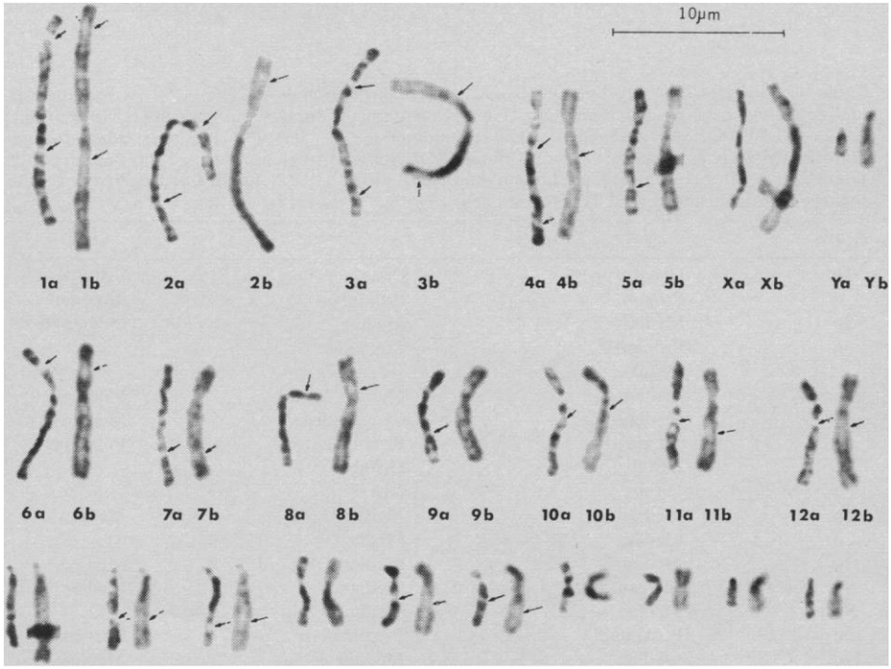

Fig. 2. The left-hand member of each chromosome pair (series a) is from a Feulgen banded set (alkaline-saline procedure prior to Feulgen staining). Arrows at characteristic negative bands. The right-hand member (series b) is nonbanded (conventional Feulgen stain). Arrows at diffuse regions corresponding to characteristic negative bands of series a.

The reproducible localization of the prominent or landmark negative bands (Figs. 1 and 2, series a) and their correlation with pale or diffuse regions in nonbanded chromosomes (Fig. 2, series b) indicate specificity in the nature or organization of the DNA-protein complex at those loci. In terms of the Feulgen banding method, that specificity may represent one or more of the following: (i) chromosome loci of low DNA density in the intact chromosomes due, possibly, to a diffuse, highly decompacted state; (ii) regions at which the protein moiety of the chromatin has been removed in the fixation, allowing the DNA fibers to relax or stretch, or alternatively, regions at which the DNA-protein is displaced in fixation; (iii) a class of uniquely labile DNA that is readily extracted under such varying conditions as (a) the hydrolysis of the Feulgen reaction, (b) incubation in buffer at *p*H 10 or in saline solution of high ionic strength, (c) methanol-acetic fixation, or (d) the removal of its associated "protective" (*13*) protein by any of the foregoing. Each of those interpretations, implying that the negative bands represent regions of low DNA density, is valid in view of the specificity and stoichiometry of the Feulgen stain for DNA. The correspondence between the Feulgen negative regions and the landmark negative G and Q bands (*2*) indicates that extrapolation might be made to interpret the latter as also representative of regions of low DNA density, inherent or induced. In view, however, of the reports that reverse patterns, or R bands, are produced by acridine orange fluorescence (*14*) and by Giemsa stain after incubation in *p*H 6.5 buffer at 87°C (*15*), that extrapolation must be held in abeyance until the substrates of Giemsa chromaticity and quinacrine fluorescence are more specifically defined than is at present possible.

The assumption that the display of discrete dark bands is dependent upon some factor in addition to that of DNA density is supported by the appearance

of the nonbanded chromosomes as shown in Fig. 2, series b. Although differential staining is apparent, it is not sharply delineated into bands and interbands as in the banded chromosomes (Figs. 1 and 2, series a). It was previously suggested that, although the Feulgen dense bands display concentrated DNA, their formation is due to the interaction of specific proteins with the cytochemical treatment (*1*). Consonant with that suggestion is the fact that, whereas the "landmark" negative regions are demonstrated by all of the banding methods considered here, the production of the dark bands is variable, showing dependency upon the preparative cytochemical treatment as well as upon the stain itself. All of the heterochromatic regions displayed by the C band Giemsa technique (*16*) are seen in the Feulgen banded (and nonbanded) sets as densely stained. Of those, the large dense bands associated with the secondary constrictions of 1q, 9q, and 16q are not stained by the Q banding method (*2, 8, 9*) and are variably stained in the ASG (acetic acid–saline Giemsa), alkaline-saline, and protease Giemsa methods (*5–8*). The distal Yq, the most brilliant Q band of the human complement (*2*) is also variably stained by those Giemsa techniques (*5–8*). With the Feulgen, the distal Yq is somewhat less dense than the proximal Yq or Yp (Figs. 1 and 2) and is frequently seen as subdivided into two bands. Analysis of the various cytochemical treatments indicates that the variable Giemsa display of any single heterochromatic region, for example, the large block on chromosome 9, is primarily dependent on the *p*H and temperature of the preparative treatment (*17*).

Another significant observation of my study is that of Feulgen dense staining of telomeres, particularly those of 1p,q, 2p,q, 3p,q, 4p,q, 5p,q, 6p,q, 7p, 8p, 9p, 10p, 11p, 13p, 14p, 17p, 19p, 20p, 22p (Figs. 1 and 2). In both Q and G banding patterns, even in those Giemsa methods utilizing a procedure similar to that of the band inducing steps of this study (alkaline treatment followed by incubation in high ionic salt solution) (*6, 8*), the tips of most of the arms "fade out." On the other hand, a high proportion of the telomeres are densely stained by the Giemsa R band technique (*15*) that includes controlled heating in buffer at *p*H 6.5, but no alkaline or saline incubation. In view of the specificity of the Feulgen reaction, there is little doubt that the densely stained telomeres, following alkaline-saline treatment, are DNA rich. The negative Giemsa staining of most of those telomeres following alkaline-saline treatment (*6, 8*), and their positive staining following treatment in acid buffer at 87°C (*15*), therefore confirms that positive Giemsa staining is dependent upon some factor or factors other than that of DNA density, with both *p*H and temperature critical. The negative or pale Giemsa staining of most of the telomeres after a band preparative treatment consisting only of exposure to a proteolytic enzyme (*5*) suggests that the factor involves the protein moieties of chromatin. The observations of this study, therefore, support the suggestions of other investigators (*18*) that Giemsa staining reflects the DNA-protein associations. In more specific terms, the substrate for the Giemsa stain is the DNA-protein complex, the stainability of which is governed by the protein moieties, which, in turn, show specificity in response to the *p*H and temperature of the cytochemical treatments.

The chromosome set of Fig. 2, series b, provides a reference for analysis of the effect of the methanol-acetic fixation on the Q band patterns. It is clear from the positive Feulgen stain that the DNA of neither the centric nor the telomeric heterochromatin is removed by that fixative. Therefore, whereas the negative fluorescence of the landmarks discussed above might be due to low density of DNA, absent or dull fluorescence at those regions of heterochromatin may not be so attributed. In fact, many of the telomeres are more densely stained by Feulgen than are those regions corresponding to the bright Q bands of the intermediate portions of the arms. The absence of fluorescence at the telomeric heterochromatin, therefore, appears to be due to some factor other than low DNA concentration. It has been suggested that quinacrine fluorescence reveals regions that are relatively rich in the adenine-thymine base pair (A·T) (*19*). However, the secondary constrictions of chromosomes 1, 9, and 16 shown by biochemical study (*4*) to be A·T rich, and shown by this study (Figs. 1 and 2) to be DNA dense, do not fluoresce with quinacrine (*2*). It appears, therefore, that even though DNA moieties may be the substrate for quinacrine, the production of the fluorochrome in situ is dependent on the availability of the potentially stainable groups. That availability, in turn, may be determined by the molecular nature or steric relation of the chromosomal proteins (*20*).

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20. M. A. Ferguson-Smith and B. M. Page [*J. Med. Genet.* **10**, 282 (1973)] have presented evidence of correlation between G bands and chromosomeres of pachytene chromosomes, lending support to the concept expressed here that chromosome banding is based, at least in part, upon differential DNA densities inherent in the chromosome.
21. These regions appeared in all of the Feulgen banded sets and, although not included in the list of "landmarks" (*2*), are present in all Q and G banded sets reviewed in this study (*5–8*).
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