LIGHT AND SCANNING ELECTRON MICROSCOPY 
OF THE SAME HUMAN METAPHASE 
CHROMOSOMES

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SUMMARY

A technique has been developed to examine the same G-banded human metaphase chromosomes, 
first in the light microscope and then in the scanning electron microscope (SEM). A structural 
involvement in chromosome banding was confirmed by a positional correlation between the G-
positive bands observed in the light microscope and the circumferential grooves between the quater-
nary coils of the metaphase chromosomes, observed in the SEM. In further support of this the 
regions between the grooves showed a positional relationship with the G-negative or reverse (R) 
bands.

The examination of slightly extended metaphase chromosomes in the light microscope demon-
strated that the G-banding pattern corresponded to that described by the Paris nomenclature for 
metaphase chromosomes. The arrangement of the circumferential grooves of the same 
chromosomes, observed in the SEM, was shown to relate to that described by the Paris nomenclature for 
prometaphase chromosomes. Therefore, using the SEM it is possible to demonstrate the 
details of prometaphase banding in metaphase chromosomes.

INTRODUCTION

High-resolution banding techniques have been developed to study human 
chromosomes in the light microscope (LM) (Yunis & Sanchez, 1973; Yunis, Sawyer 
& Ball, 1978). These techniques have made it possible to visualize previously un-
detectable chromosome defects in relation to clinical syndromes (Buhler, Buhler & 
Christen, 1983; Fryns, Heremans, Marien & Van den Berghe, 1983) and cancer 
(Yunis, Bloomfield & Ensrud, 1981; Yunis et al. 1982).

The limited resolving power of the LM restricts the precise location of minute 
chromosome abnormalities and for this reason several authors have recently attempted 
to observe banded human chromosomes by transmission electron microscopy 
(TEM) (Burkholder, 1981; Goyannes & Mendez, 1982; Xu & Wu, 1983) and scan-
ing electron microscopy (SEM) (Harrison, Britch, Allen & Harris, 1981; Harrison, 
Allen, Britch & Harris, 1982; Harrison, Allen & Harris, 1983; Sweney, Lam &

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Shapiro, 1979; Utsumi, 1982). In a previous paper we presented the first three-dimensional human karyotype, from metaphase chromosomes observed in the SEM (Harrison et al. 1981). A relationship was demonstrated between metaphase chromosome quaternary coiling, as described by DuPraw (1965, 1966), and the LM G-banding pattern. Parallel circumferential grooves along the chromatids demarcated the individual gyres of the coils, which were shown to correspond to the LM G-positive bands. This technique involved the preparation of parallel samples for examination by LM and SEM. An improved technique has been developed that allows observation of the same G-banded metaphase in the LM and the SEM. The details of this technique are presented here. Using this method we were able to confirm our initial findings and also demonstrate a positional correlation between the intergroove regions and the reverse banding (R-banding) pattern. As a result of the high resolution available with the SEM we are able to show that the G bands observed in prometaphase chromosomes in the LM are present in mid-metaphase chromosomes, when viewed in the SEM.

MATERIALS AND METHODS

Heparinized peripheral blood was cultured in RPMI 1640 medium (Gibco, Scotland) supplemented with 20 % foetal calf serum (FCS) (Gibco, Scotland) containing phytohaemagglutinin (PHA) (Wellcome, Beckenham, Kent) for 72 h at 37 °C. Colcemid (0.01 μg/ml) (Gibco, Scotland) was added for 1-5 h. Cells were incubated in 75 mM-KCl for 8 min at room temperature, followed by three washes in methanol/acetic acid fixative (3:1, v/v). Between the second and third wash cells were incubated for 1 h at 4 °C. Circular coverslips (15 mm diam.) were divided into numbered sections using an engraving pen. Chromosome spreads were made by dropping cells onto these coverslips, followed by air-drying. It is uncommon to air-dry specimens before preparation for SEM but this step was necessary for spreading and adherence of the chromosomes from complete metaphases, onto the glass surface. Air-drying of coverslips was prevented at all subsequent stages.

Two to three days later the metaphases were G-banded using 0.025 % trypsin (Wellcome) in 0.85 % saline for 5–10 s at room temperature, modified from the technique of Seabright (1971). This was the optimum time in trypsin to reveal the major LM G-positive bands, previously described as 'just banded' metaphases (Harrison et al. 1982) and to provide the best chromosome morphology in the SEM. The coverslips were then fixed in 3 % glutaraldehyde (Polaron Equipment Ltd, Watford) in 0.1-mol-Sørensen's phosphate buffer (pH 7.4) for 20 min, to arrest the action of the trypsin immediately, then rinsed in Gurr's buffer (pH 6.8) (Searle, Bucks). The methods of rinsing in Gurr's buffer or saline alone, as applied in routine LM G-banding, or the addition of soybean trypsin inhibitor (Sigma Chemical Co.), were found to be insufficient to inactivate the trypsin completely. They were then stained in 2 % Giemsa (R66, Hopkins and Williams, Essex), in Gurr's buffer (pH 6.8) for 8 min. Fixation in glutaraldehyde slightly reduced the uptake of Giemsa stain by the chromosomes. A further rinse in Gurr's buffer was given, then the coverslips were mounted, in the buffer, onto a slide and sealed around the edge with nail varnish. Thus, throughout the G-banding procedure, the chromosomes were prevented from air-drying. A number of suitable G-banded metaphases were selected and photographed in the LM. The nail varnish was removed and the coverslips were dropped into fresh 3 % glutaraldehyde in Sørensen's buffer, again without air-drying, for a minimum of 30 min; they were processed for SEM by osmium tetroxide-thiocarbohydrazide (Osmium-TCH) fixation, as described in detail elsewhere (Harrison et al. 1981), which allows direct SEM visualization of uncoated chromosomes.

Briefly: (1) specimens removed from glutaraldehyde were rinsed in Sørensen's buffer, followed by (2) fixation in 1 % osmium tetroxide in the same buffer, (3) three rinses in distilled water, followed by (4) 5 min incubation in a freshly prepared, saturated solution of TCH in distilled water. (5) Three further rinses in distilled water, and (6) additional fixation in 1 % osmium tetroxide in
LM and SEM of human metaphase chromosomes

distilled water were carried out. Steps (3) to (6) were repeated. The specimens were then dehydrated through a graded acetone series (20% to 100%), then critical-point dried from liquid carbon dioxide with absolute acetone as the transitional fluid. Coverslips were examined in an ISI SS-40 SEM. The same metaphases observed in the LM were relocated in the SEM using recognizable markings engraved on the coverslips. The individual chromosomes were rotated within the SEM and photographed to facilitate direct correlation with the chromosomes photographed in the LM.

RESULTS

Metaphase spreads from human peripheral blood were G-banded, photographed in the LM and, after further processing, the same metaphases were examined in the SEM, as shown in Fig. 1. To obtain chromosomes of optimum morphology in both the LM and the SEM several modifications of the routine G-banding technique for LM were necessary.

(1) It has been shown that to achieve the best chromosome morphology to demonstrate the 'G-banding pattern', or arrangement of circumferential grooves in the SEM, metaphases were required to be 'just-banded' in the LM (Harrison et al. 1983). This effect was produced here by reducing the exposure to trypsin.

(2) After pretreatment with trypsin the specimens were fixed in glutaraldehyde, which immediately arrested the action of the enzyme. Rinsing in buffer or saline alone, as in routine G-banding, enabled gradual enzymic breakdown of chromosome structure to continue, resulting in a variable morphology in the SEM.

(3) Repeated air-drying was found to produce collapse of chromosome structure (Harrison et al. 1982). Therefore, after the initial air-drying step, which is essential to obtain complete metaphases on the coverslips, the chromosomes were prevented from further air-drying. This required the metaphases to be immersed in buffer during photography.

As a result of these modifications the degree of contrast between G-positive and G-negative bands in the LM photographs was reduced (Fig. 1A) when compared with conventionally G-banded metaphases. However, chromosome structure was consistently preserved to a sufficiently high standard to allow detailed cytogenetic analysis to be carried out in the SEM (Figs 1B, 2, 3).

After relocation of the same metaphase spread in the SEM, selected chromosomes were examined in detail at a higher magnification (Fig. 2). A correlation was demonstrated between the G-positive LM bands (black arrows, Fig. 2, insets) and the circumferential grooves along the chromatids, when observed in the SEM (white arrows, Fig. 2). Chromosome 1, with a large number of G bands, clearly demonstrated this relationship (Fig. 2A). Certain G-positive bands were shown to correspond in position to two circumferential grooves, indicated by connected white arrows (Fig. 2A). This feature was also observed in the smaller chromosomes, as demonstrated in a chromosome 15 from this metaphase (Fig. 2B), with one prominent G-positive band (black arrows, Fig. 2B, inset).

Generally, the circumferential grooves were situated at parallel positions on sister chromatids. Distortion of the chromosomes was found to shift the grooves from this
precise alignment, as observed in chromosome 15, leaning to one side (Fig. 2a) and chromosome 5 (Fig. 2c). The LM G-positive bands were shifted an equal distance in the same direction (Fig. 2b, c, insets).

Chromosome 5 was characterized by one large G-positive band in the centre of the long (q) arm in contracted metaphase chromosomes (connected black arrows, Fig. 2c, inset). In the SEM this region was shown to correspond to three circumferential grooves in both chromatids (connected white arrows, Fig. 2c). Three G-positive bands, 5q14, 5q21 and 5q23, are characteristic of this region when elongated metaphase chromosomes are observed in the LM (black arrowheads, Fig 3A, B, insets), as described by the International System for Human Cytogenetic Nomenclature (ISCN, 1978, 1981), shown diagramatically in Fig. 3c(1). The white arrowheads (Fig. 2c) demonstrate the positional relationship between the circumferential grooves and the ISCN (1981) mid-metaphase banding pattern (black arrowheads, Fig. 3c, chromosome (1)). Therefore, the details of mid-metaphase chromosome banding were observed in contracted chromosomes in the SEM. Certain grooves appeared to be more pronounced when observed from alternative directions. Fig. 2d shows the same number 5 chromosome as Fig. 2c, viewed upside down. From this angle the circumferential groove at the distal end of the q arm was more prominent (Fig. 2d) than when observed in the upright position (Fig. 2c). Fig. 3 shows a homologous pair of elongated number 5 chromosomes from a different metaphase. These chromosomes, when compared with the more contracted number 5 chromosomes in Fig. 2c, showed an increase number of G-positive bands in the LM (black arrowheads, Fig. 3A, B, insets), as described above. A corresponding increase in the number of circumferential grooves was also observed in these extended chromosomes, when viewed in the SEM (white arrows, Fig 3A, B). An identical pattern of coiling was present in both members of the homologous pair (compare grooves in Fig. 3A, B). This close correlation was shown in all homologues, in this and numerous other metaphases.

The diagrammatic representation of chromosome 5 (Fig. 3c) clearly demonstrates the relationship between the mid-metaphase (chromosome (1)) and prometaphase (chromosome (2)) banding patterns, which have been described in detail (ISCN, 1981). That is, the G bands observed at mid-metaphase are made up of sub-bands in prometaphase. Each LM G-positive mid-metaphase band, observed in the extended chromosomes (black arrowheads, Fig. 3A, B, insets; and chromosome (1), Fig. 3c) was found to correspond in position to more than one circumferential groove when viewed in the SEM (white arrows, Fig. 3A, B). These in turn were correlated with the sub-bands of the prometaphase chromosomes (similarly connected black arrows, chromosome (2), Fig. 3c). For example, G bands 5p14, 5q12, 5q14, 5q21 and 5q23, visible in the LM, were represented by two circumferential grooves.

Fig. 1. The same G-banded human metaphase spread observed by LM(a) and then by SEM (b). One chromosome, 4, is off the photograph. The chromosomes show reduced contrast between G-positive and G-negative bands (a) as a result of the modifications used to preserve the best chromosome morphology for SEM (b). ×2000.
Fig. 2. Chromosomes from the metaphase in Fig. 1. G-positive LM bands (black arrows, insets) correspond to circumferential grooves in the chromatids (white arrows).

A. Chromosome 1 shows a large number of grooves. Certain G-positive bands correspond in position to two circumferential grooves, indicated by connected white arrows.

B. Chromosome 15 shows one prominent G band (black arrows, inset). This corresponds to two circumferential grooves in each chromatid (connected white arrows). The chromosome is leaning to one side and the circumferential grooves are shifted from parallel alignment. The G-band is shifted an equal distance in the same direction.

C. Chromosome 5 is characterized by one large G-positive band in the centre of the q arm (connected black arrows, inset). This corresponds to three circumferential grooves, in both chromatids, in the SEM (connected white arrows). The white arrowheads demonstrate the positional relationship between the grooves and the ISCN mid-metaphase banding pattern (black arrowheads, Fig. 3c(1)).

D. The same number 5 chromosome viewed upside down. From this angle the groove at the distal end of the q arm is more pronounced (white arrow) than when observed in the upright position (see c). ×7000.
in the SEM (white arrows connected in pairs, Fig. 3A,B), and corresponded to two sub-bands in prometaphase (black arrows connected in pairs, Fig. 3c). G bands 5q32 and 5q34 were visible in the LM photographs (Fig. 3A,B, insets). However, in the SEM, circumferential grooves were demonstrated, within the 5q3 region (single white arrows at distal portion of the q arm, Fig 3A,B) corresponding to 5q32 and 5q34 and also the sub-bands 5q31-2, 5q33-2 and 5q35-2 not visible in the LM (compare single white arrows, Fig 3A,B, and single black arrows, Fig. 3c(2) at distal end of q arm). Circumferential grooves corresponding in position to sub-bands 5p15-32, 5p13-2 and 5q13-2 were also observed in the SEM (compare single arrows, as above) that were not resolved in the LM photographs. Therefore, the details of prometaphase banding were observed in mid-metaphase chromosomes in the SEM.

Elongated chromosomes are more prone to banding of the chromatids than the more contracted chromosomes and, as a result, at several points along the sister chromatids the grooves were slightly shifted from positions directly parallel to each other (Fig. 3A,B). This was reflected by an equivalent distortion in the G bands in the LM (Fig. 3A,B, insets).

Those grooves, indicated by a small white arrowhead (Fig. 3A,B), were resolved more clearly when the chromosomes were viewed from one side (small white arrowheads, Fig. 3D,E). Numerous discrepancies in number and precise position of grooves between sister chromatids and homologues were excluded by rotational viewing in the SEM.

In support of this conclusion, that the circumferential grooves correspond to the LM G-positive bands, it was also demonstrated that the regions between the grooves were related to the G-negative or reverse bands (R bands). Fig. 3r demonstrates the R-banding pattern as described (ISCN, 1978). The R-positive bands, indicated by black arrowheads, correspond in position to the raised regions of the chromatids between the circumferential grooves (large white arrowheads, Fig 3D,E). Identical patterns were demonstrated in both members of this homologous pair (compare large arrowheads in Fig 3D,E). A similar correlation was also observed in other homologous chromosome pairs taken from different metaphases.

DISCUSSION

Examination of human metaphase chromosomes prepared directly in parallel for LM and SEM, revealed a relationship between chromosome quaternary structure and the LM G-banding pattern (Harrison et al. 1981). In this paper the use of an improved technique to examine the same chromosomes first in the LM and then in the SEM, has confirmed our original findings and has proved to be a more convenient and precise method for application to clinical cytogenetics.

The new procedure is ultimately time-saving, as suitable metaphases are initially selected by LM and easily relocated in the SEM. This eliminates the tedious scanning of coverslips for suitable metaphases by SEM alone.

All chromosomes examined in detail confirmed a positional correlation between the
LM G-positive bands and the circumferential grooves between the gyres of the quaternary coils. This relationship persisted even when the chromosomes were distorted, providing conclusive evidence for the involvement of chromosome structure in the production of LM banding patterns. In further support of this the regions between the grooves were shown to correlate in position to the G-negative or R bands.

Frequently, in contracted metaphase chromosomes only the major G-positive bands may be observed in the LM. Examination of the same contracted chromosomes
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in the SEM revealed that in certain regions along the chromatids one LM G-positive band was related to a series of circumferential grooves. These grooves, in turn, showed a positional relationship to the mid-metaphase banding pattern, as described (ISCN, 1978, 1981). Therefore the details of the mid-metaphase chromosome banding pattern were observed in contracted metaphase chromosomes in the SEM. This correlation may be applied to those cases in which contracted chromosomes predominate, for example, leukaemic bone marrows, preparations from solid tumours or chorionic villi samples, to provide increased details of the metaphase G-banding pattern. The examination of homologous pairs of slightly extended metaphase chromosomes in the LM demonstrated that the G-banding pattern corresponded to the Paris nomenclature for mid-metaphase chromosomes (Paris Conference, 1971; ISCN, 1978). The arrangement of the circumferential grooves of the same chromosomes, when observed in the SEM, was related to that described by the Paris Conference.

Fig. 3. A,B. A homologous pair of elongated number 5 chromosomes from a different metaphase. An increased number of G-positive bands in the LM (black arrowheads, insets) and a corresponding increase in number of circumferential grooves in the SEM (white arrows) are observed, when compared with the more contracted number 5 chromosome (Fig. 2c). Identical patterns of coiling are demonstrated in both members of the homologous pair; compare position and number of grooves in A and B. Each LM G-positive mid-metaphase band (black arrowheads, insets) corresponds to more than one circumferential groove in the SEM (connected white arrows). For example, G bands 5p14, 5q12, 5q14, 5q21 and 5q23, indicated in the LM by black arrowheads (see insets) are represented by two circumferential grooves (white arrows connected in pairs), and correspond to two sub-bands in prometaphase (see Fig. 3c, black arrows connected in pairs).

G-bands 5q32 and 5q34 are visible in the LM photographs (black arrowheads, distal portion of q arm, insets). In the SEM, circumferential grooves are present corresponding to these bands and also the sub-bands 5q31-2, 5q33-2 and 5q35-2, which are not visible in LM (compare single white arrows in these figures and single black arrows, in Fig. 3c, distal portion of q arm). X10,000.

Circumferential grooves corresponding in position to sub-bands 5p15-32, 5p15-2, 5p13-2 and 5q13-2 are also present in the SEM (compare black, in c, and white arrows, as above), which are not observed in the LM photographs (insets). These elongated chromosomes are prone to bending and at several points along the sister chromatids the grooves are shifted from positions directly parallel to each other, reflected by an equivalent distortion in the G bands in the LM (insets).

c. Diagramatic representation of chromosome 5. Chromosome (1) shows the banding pattern at mid-metaphase and chromosome (2) represents the banding pattern at prometaphase. The black arrowheads on the left-hand chromosome correspond to the G bands observed in the LM (black arrowheads, Fig. 2a, insets). The connected black arrows on the right-hand chromosome indicate those sub-bands correlated with each mid-metaphase G band (black arrowheads, left-hand chromosome) and correspond in position and number to those circumferential grooves on the chromosomes in A and B, shown by connected white arrows.

d,e. Chromosomes from A and B viewed from one side. Those grooves shown by a small white arrowhead (see B) are resolved more clearly from this angle. The raised regions between the grooves, indicated by large white arrowheads, correspond in position to the G-negative or R bands (see r). X10,000.

f. Diagramatic representation of an R-banded chromosome 5 (from ISCN, 1978). The black arrowheads, indicating the positions of the R bands, correlate with the large white arrowheads on d and e.
nomenclature for prometaphase chromosomes (ISCN, 1981) and showed a precise correlation between homologous chromosome pairs. The rotation of individual chromosomes in the SEM highlighted different regions of the chromatids from different angles. As a result the discrepancy in number and location of individual grooves was accurately resolved in all chromosomes. Therefore, using the SEM it is possible to demonstrate the details of prometaphase G-banding pattern in metaphase chromosomes and so to overcome the technical difficulties associated with the analysis of prometaphase chromosomes in the LM. By permitting detailed comparison of homologous chromosomes in the LM and SEM, this improved technique potentially allows study of minute chromosome aberrations, and the precise location of breakpoints involved in structural chromosome rearrangements. It can readily be applied to those cases in ante-natal diagnosis and oncological cytogenetics that have complex or unusual translocations.

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REFERENCES


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