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THE ULTRASTRUCTURAL ORGANIZATION OF PREMATURELY CONDENSED CHROMOSOMES

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SUMMARY

In an effort to understand the arrangement of the basic 30 nm chromatin fibre within metaphase chromosomes, changes in the organization of prematurely condensed chromosomes (PCC) were examined as a function of progression through the cell cycle. The structural features of PCC observed under the light microscope were compared with those obtained by scanning electron microscopy. PCC with varying levels of condensation were obtained by fusing mitotic HeLa cells with interphase cells synchronized at different times in the cell cycle. PCC from G_1 cells are composed of rather tightly packed bundles of tortuous chromatin fibres. The density of fibre packing along the longitudinal axis of G_1-phase PCC is lower and less uniform than that of metaphase chromosomes. Early G_1 PCC exhibit gyres suggesting a despiralized chromonema. The condensed domains in G_1 PCC appear to be organized as supercoiled loops; whereas fibre-sparse domains consist of longitudinal fibres running along the chromosome axis. As cells progressed towards S phase, a greater proportion of highly extended regions containing prominent longitudinal fibres became evident in the PCC. The pulverized appearance of S-phase PCC under the light microscope corresponded to the highly condensed, looping fibre domains separated by more extended segments containing longitudinal fibres that are visualized using the scanning electron microscope. Active sites of DNA synthesis are implicated to be localized within extended longitudinal fibres. Post-replicative chromosome maturation extends through the G_2 period and appears to involve rearrangement of the extended longitudinal fibres into packed looping-fibre clusters, which then coalesce.

These observations support the model for packing DNA into chromosomes proposed in 1980 by Mullinger & Johnson. Briefly, this model suggests that the chromonema of each metaphase chromatid contains regions composed of folded longitudinal chromatin fibres as well as looping fibres that emerge from the axis at distinct foci. The final level of chromatin packing in metaphase chromosomes is attained by spiralization of the chromonema.

INTRODUCTION

Metaphase chromosomes and interphase chromatin are constructed from 10 nm nucleosome fibres coiled in the form of a 30 nm solenoid, stabilized by histone H1 and divalent cations (Finch & Klug, 1976; Worcel & Benyajati, 1977; Ris & Korenberg, 1979; Hamkalo & Rattner, 1980). Despite numerous investigations, the arrangement

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of the 30 nm chromatin fibre within metaphase chromosomes remains unclear. The nature of this higher order organization has been difficult to ascertain due to the compact packaging of chromatin fibres within metaphase chromosomes. Techniques designed to loosen the structure of metaphase chromosomes and thus reveal the underlying fibre organization are fraught with a spectrum of problems ranging from extensive dispersal of chromosomes beyond recognition to aggregation of non-histone chromosomal proteins.

One approach to understanding the arrangement of the 30 nm chromatin fibre within metaphase chromosomes is to visualize the chromosome condensation cycle, which is tightly coupled to the mammalian cell cycle. According to the model for the chromosome condensation cycle proposed by Mazia (1963), chromosomes begin to decondense during the telophase stage of mitosis, and gradually continue this process throughout G_1 and into S phase until maximum dispersion is reached at the time of DNA replication. Following replication, chromosomes begin a gradual recondensation process, which culminates in the formation of metaphase chromosomes. This model is supported by results from a number of investigations using a variety of experimental methods (Ringertz, Darzynkiewicz & Bolund, 1969; Pederson & Robbins, 1972; Zetterberg & Auer, 1970; Alvarez, 1974; Moser, Muller & Robbins, 1975; Hildebrand & Tobey, 1975; Nicolini, Ajiro, Borun & Baserga, 1975). The phenomenon of premature chromosome condensation provides direct visual evidence for cell-cycle-specific changes in the higher order arrangement of chromatin fibres within interphase chromosomes. Fusion of mitotic cells with interphase cells results in a rapid breakdown of the interphase nuclear framework and organization of interphase chromatin into prematurely condensed chromosomes (PCC) (Johnson & Rao, 1970). The process of premature chromosome condensation closely resembles the entry of the nucleus of a cycling cell into mitotic prophase (Johnson & Rao, 1970; Matsui, Yoshida, Weinfeld & Sandberg, 1972; Obara, Chai, Weinfeld & Sandberg, 1974). The only apparent difference is that the morphology of PCC is determined by the position of the interphase cell in the cell cycle at the time of fusion (Johnson & Rao, 1970; Stenman & Saksela, 1971).

Prematurely condensed chromosomes are an elegant model system for ultrastructural analysis of the organization of interphase chromatin. In the process of premature chromosome condensation, the nuclear membrane breaks down and the chromatin condenses, so that the resulting PCC may reflect the actual intranuclear organization before fusion. This characteristic permits investigation of changes in the arrangement of the chromatin fibre within PCC as a function of progression through the cell cycle. Previous electron-microscope studies of PCC were not specifically designed to address this question. Matsui *et al.* (1972) studied thin sections of CHO cells during PCC formation in order to compare the events associated with the disruption of the nuclear framework during premature chromosome condensation with the events occurring during normal mitotic prophase. Schwarzacher, Ruzicka & Sperling (1974) and Ruzicka (1977) investigated ultrastructural features of metaphase chromosomes and PCC from unsynchronized human fibroblasts following G-banding of methanol/acetic acid-fixed, air-dried chromosome spreads. They demonstrated that PCC are

composed of the same 30–100 nm fibrils as metaphase chromosomes and interphase chromatin, and that G-bands appear thicker and contain relatively more tightly packed fibrils than R-bands. Mullinger & Johnson (1983) studied the organization of chromatin fibres in *S*-phase PCC and discussed the relationship between condensed fibre aggregates and replication clusters.

Changes in the ultrastructural arrangement of the basic 30 nm chromatin fibre that parallel the well-characterized changes in PCC morphology during the cell cycle by light microscopy have been analysed in our laboratories (Hanks, Gollin, Rao, Wray & Hittelman, 1983). We used high-resolution scanning electron microscopy (SEM) to examine the three-dimensional relationship of chromatin fibres within chromosomes prepared from synchronized HeLa cells using standardized, conventional cytogenetic spreading techniques.

METHODS

Preparation of chromosome spreads for light and electron microscopy

These studies were performed on HeLa cells grown in monolayer cultures. Procedures for cell culture, synchronization and fusion to induce PCC have been described (Hanks *et al.* 1983). Analysis of PCC morphology by light microscopy was performed on chromosome spreads prepared using classical cytogenetic procedures. Briefly, cells were fixed in methanol/acetic acid (3:1, v/v), dropped on wet microscope slides, air-dried and stained with Giemsa. Chromosome spreads were prepared for electron microscopy using the same procedures, except that cells were dropped onto glass coverslips and immediately immersed in fresh methanol/acetic acid (3:1) fixative to avoid the drastic alterations in specimen structure produced by air-drying. The chromosomes were then stabilized in 2 % aqueous uranyl acetate, dehydrated in a graded series of acetone solutions, critical-point dried, sputter-coated with 15–20 nm gold/palladium, and observed using a JEOL JEM-100CX scanning-transmission electron microscope operating at 40 kV (for details, see Hanks *et al.* 1983).

Technical problems in the preparation of PCC for scanning electron microscopy

PCC induction and preparation of chromosomes for SEM are characterized by occasional technical problems. The capricious step in PCC induction is that of fusion of mitotic with interphase cells. The amount of fusion, that is, the number of cells that fuse to each other (from two to ten, for example) varies between different lots of Sendai virus or polyethylene glycol and also fluctuates for reasons that remain unclear. The difficulty in the preparation of chromosomes for SEM is based on the necessity to preserve the fragile, three-dimensional structure of chromosomes. Air-drying damages the ultrastructure of specimens due to fierce surface-tension forces inherent in the process. Standard chromosome-spreading techniques utilize air-drying as a means of achieving flat chromosome spreads that adhere to microscope slides. In the interest of preserving chromosome ultrastructure, we chose not to air-dry the chromosomes and consequently, our chromosome spreads were often incomplete.

The ultrastructure of chromatin fibres observed within chromosomes might depend on other factors in the method of preparation. Two potential artifacts inherent in our method of chromosome preparation should be mentioned. First, fixation of chromosomes with methanol/acetic acid (3:1) is known to extract some histone proteins (Dick & Johns, 1968; Brody, 1974; Burkholder & Duczek, 1982). In addition, methanol/acetic acid is a coagulant fixative, and therefore not generally useful for extremely high-resolution transmission electron-microscopic analyses of structures such as macromolecules. However, methanol/acetic acid has been widely used for ultrastructural studies of chromosomes, since it is the only fixative that preserves chromosomes *in situ* and also permits chromosome spreading (Burkholder, 1977; Ruzicka, 1977; Harrison, Allen, Britch & Harris, 1982). Cheung, Gollin & Wray (1981 and unpublished observations) found that fixation with methanol/

acetic acid does not alter the arrangement of the basic 30 nm chromatin fibre within PCC or metaphase chromosomes compared to fixation with the Wray-Stubblefield hexylene glycol buffer, although it decreases chromatin fibre diameter. These results confirm those of Ris (1978), who suggested that methanol/acetic acid is extremely useful for investigating the arrangement of the basic chromatin fibre in chromosomes although it is not suitable for the study of nucleohistone structure. The second potential artifact is that the hypotonic treatment used to swell cells before fixation and chromosome spreading may affect chromosome structure. Barnitzke, Bullerdiek & Schloot (1981) have shown a strong correlation between chromosome length and increasing hypotonicity. This finding suggests that the PCC we observed may have an extended morphology relative to their state *in situ*. This extension of length is most probably achieved at the level of chromomere clustering or uncoiling of the chromonema (Jorgensen & Bak, 1982). To avoid this potential artifact, we have prepared all chromosomes for examination using standard cytogenetic techniques and have attempted to limit discussion of our data to a description of changes in the arrangement of chromatin fibres associated with changes in PCC morphology during the cell cycle.

Other methods have been used to prepare PCC for SEM. Most recently, Mullinger & Johnson (1983) used a technique adapted for chromosome preparation by Harrison *et al.* (1982). The procedure involves preparation of standard, air-dried chromosome spreads after fixation in methanol/acetic acid, followed by trypsin–Giemsa banding, fixation in glutaraldehyde and osmium tetroxide, sequential incubations in sodium thiocarbohydrazide, distilled water, osmium tetroxide and distilled water, dehydration in a graded series of acetone solutions and, finally, critical-point drying. This technique eliminates the need to coat the specimen with a conductive layer of metal, since the sodium thiocarbohydrazide–osmium tetroxide complex imparts conductivity to the specimen. This feature would permit resolution of finer surface details.

Problems may be associated with the following steps in this preparative technique. Treatment with hypotonic solution and fixation in methanol/acetic acid may have inherent problems as previously discussed. Air-drying may distort the fine surface structure of the chromosomes leading to inability to distinguish individual chromatin fibres and the paths they traverse. Trypsin–Giemsa banding is known to alter chromosome morphology, primarily by extraction of chromosomal proteins. However, this step is necessary to visualize surface details of chromosomes prepared by this procedure.

Thus, it is apparent that a completely satisfactory method of preparing chromosomes for analysis by scanning electron microscopy has not been developed. Therefore, the results of SEM analysis of chromosomes must be interpreted cautiously in the light of the artifacts that can be produced by current preparative techniques.

RESULTS AND DISCUSSION

The structure of prematurely condensed chromosomes

G_1-phase PCC. Under the light microscope, PCC of G_1 phase cells appear as single chromatids. A closer analysis of G_1 PCC morphology reveals a relationship between chromatid length and thickness, and the degree of advancement of the cell toward *S* phase at the time of fusion (Schor, Johnson & Waldren, 1975; Hittelman & Rao, 1976, 1978; Rao, Wilson & Puck, 1977; Rao & Hanks, 1980; Sperling, 1982). As a cell progresses through G_1, its PCC become more extended. The early stages of this decondensation process appear to involve an uncoiling of the chromonema, resulting in gyred chromatids characteristic of those produced after specific treatments of chromosomes (Manton, 1950; Ohnuki, 1968; Goradia & Davis, 1977) (Fig. 1A). Immediately before entry into *S* phase, the PCC are highly extended and individual chromosomes are no longer distinguishable. This decondensation process appears to be necessary, although not sufficient for the initiation of DNA synthesis (Hanks & Rao, 1980).

At the ultrastructural level, early G_1-phase PCC are composed of a rather tightly packed bundle of highly tortuous chromatin fibres with a diameter of about 30 nm (Fig. 2). G_1 PCC show a general resemblance to individual metaphase chromatids. However, the density of fibre packing along the longitudinal axis of PCC is lower and less uniform than that of metaphase chromatids. The fibres composing the more condensed domains of the early G_1 PCC often appear to be organized as supercoiled loops that emerge from within the chromosome axis, while in fibre-sparse domains, longitudinal fibres running along the chromonemal axis are often visible (Fig. 2). In more extended regions along the longitudinal axis of early G_1-phase PCC, gyres are apparent (Fig. 2, closed arrows). The coiled appearance seems to arise from a uniform unwinding of the chromosome. The packing of the basic chromatin fibre within this uncoiled chromosome appears much less dense than in the more highly condensed PCC domains.

As cells progress to late G_1, the PCC appear more attenuated, as revealed by light microscopy (Fig. 1B). SEM analysis reveals that late G_1 PCC have a greater proportion of highly extended regions containing prominent longitudinal fibres (Fig. 3, open arrow). Domains composed of tightly packed looping fibres are diminished in size. In some regions, small clusters of loops are observed that appear to arise from a single point of origin (Fig. 3, closed arrow).

S-phase PCC. PCC from *S*-phase cells exhibit a characteristic 'pulverized' morphology. This appearance results from variable levels of condensation in different chromosome segments and is precisely related to their position in *S* phase and to their replicative state at the time of fusion (Johnson & Rao, 1970; Sperling & Rao, 1974; Sperling, 1982).

During early *S* phase, PCC reach their overall minimum level of condensation (Figs 1C, 4). Mid-*S*-phase PCC are characterized at the light-microscopic level by the presence of many condensed segments, often appearing double, which represent regions of the chromosome that have completed replication (Figs 1D, 5A, boxed regions). The highly condensed segments are separated by more extended domains, which represent regions that have not yet initiated replication as well as 'gap' regions. Autoradiographic analyses of PCC spreads from *S* phase cells pulse-labelled with [^{3}H]thymidine immediately before fusion clearly reveal a heavy localization of silver grains within the gap regions of the PCC (Sperling & Rao, 1974; Lau & Arrighi, 1981; Mullinger & Johnson, 1983). Thus, the sites of active DNA replication at the time of fusion appear as gaps in the continuity of the chromosome when viewed under the light microscope.

Ultrastructurally, mid-*S*-phase PCC consist of alternating domains of sparse longitudinal fibres and packed looping fibres. Regions of low-density longitudinal fibres correspond to the gap regions detected using light microscopy. Presumably, the gaps represent sites where the density of nucleoprotein fibres is too low to be resolved, rather than actual breaks in the continuity of the chromosome (Rohme, 1975). In some cases, the continuity of PCC is apparently maintained by a single longitudinal fibre linking two looping-fibre domains (Fig. 6, bracketed region). Looping-fibre

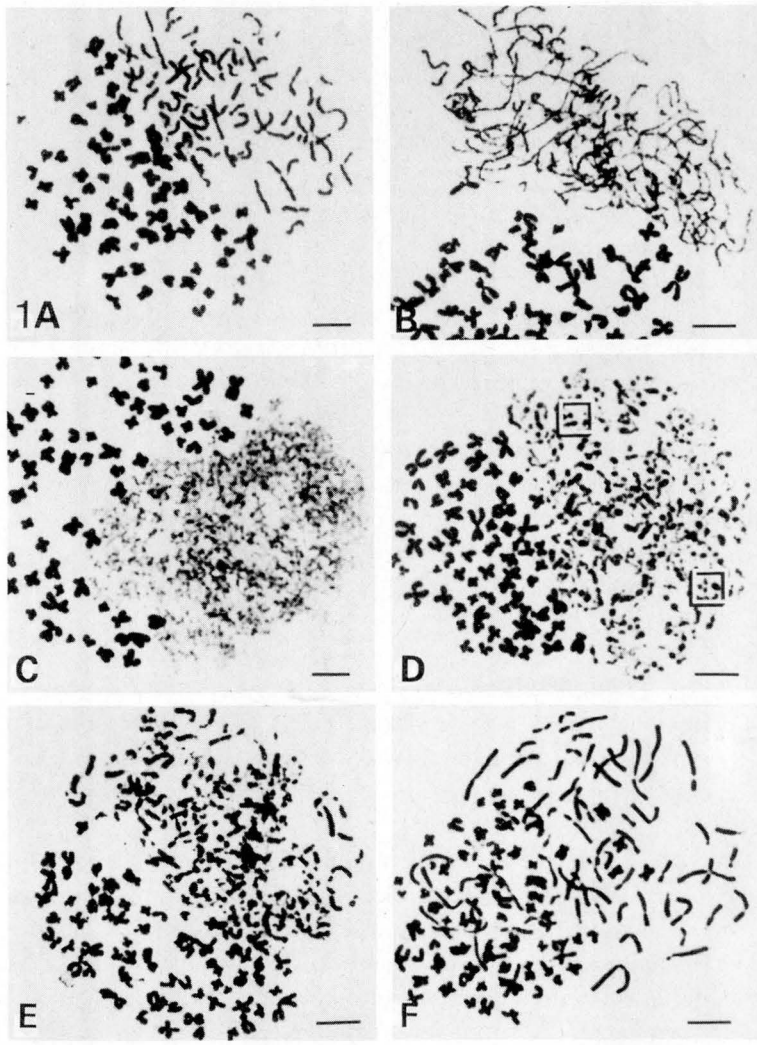

Fig. 1. Light micrographs of PCC spreads at various phases of the cell cycle. A. Early G_1; B, late G_1; C, early S; D, mid-S; E, late S; F, G_2. The darkly stained, highly condensed metaphase chromosomes at the left of each figure are from the mitotic cell used to induce premature chromosome condensation. Bars, 5 μm. (From Hanks *et al.* (1983).)

domains show various degrees of compaction (Fig. 6). In some cases, clusters of looping fibres appear to emerge from a single point of origin (Fig. 6, arrow *b*). Many parallel longitudinal fibres can be seen within larger clusters (Fig. 6, arrow *a*).

The light-microscopic morphology of late-*S*-phase PCC differs from that of mid-*S*-phase PCC in that the condensed, replicated chromosome segments are much longer and thus constitute a greater fraction of the total chromosome length (Figs 1E, 7). Ultrastructural analysis of late *S* PCC indicates that the long stretches of condensed,

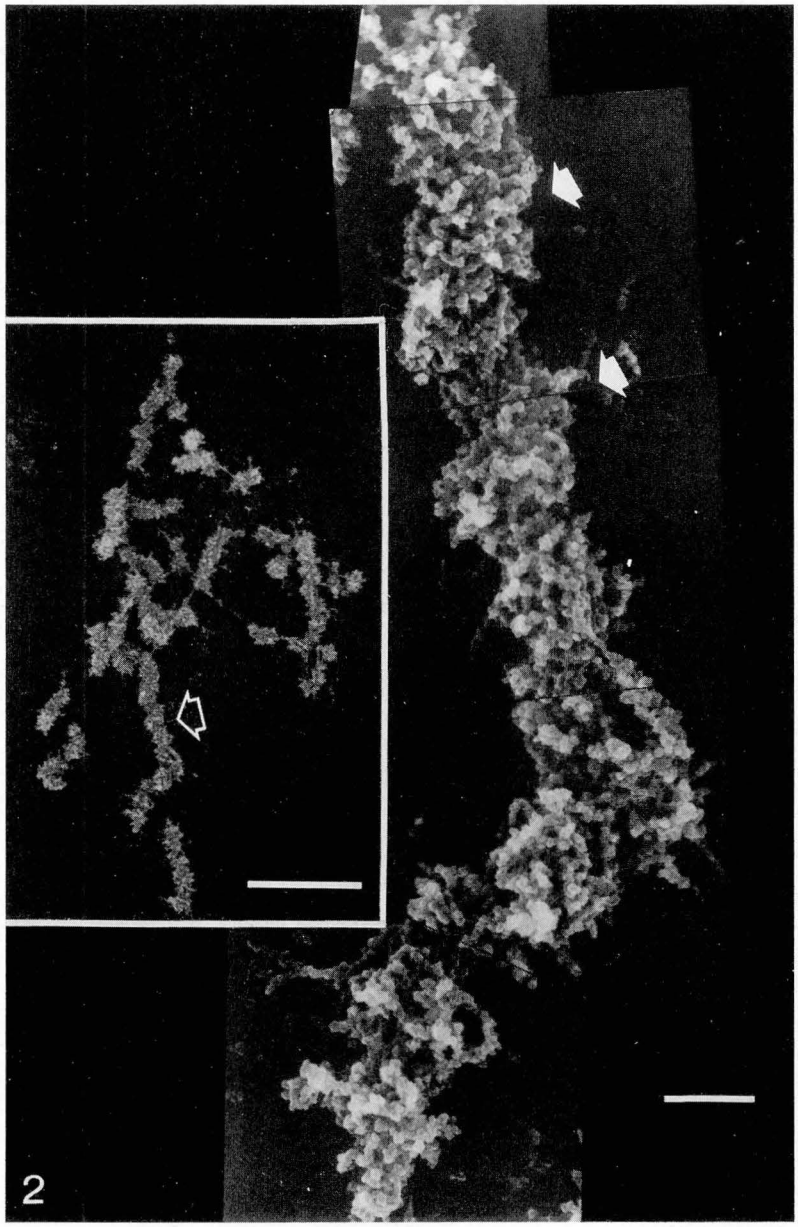

Fig. 2. Ultrastructure of early G_1 PCC showing features suggesting chromonema spiralization (indicated by closed arrows). Bar, 0·5 μm. Inset illustrates position of enlarged chromosome in spread (open arrow). Bar, 5 μm. (From Hanks *et al.* (1983).)

replicated chromosomes are composed of nucleoprotein fibres organized primarily as packed loops (Fig. 8). The fibres are packed together much more loosely than in metaphase chromatids, however, and some degree of longitudinal organization is observed. In regions where single, unreplicated segments merge into condensed,

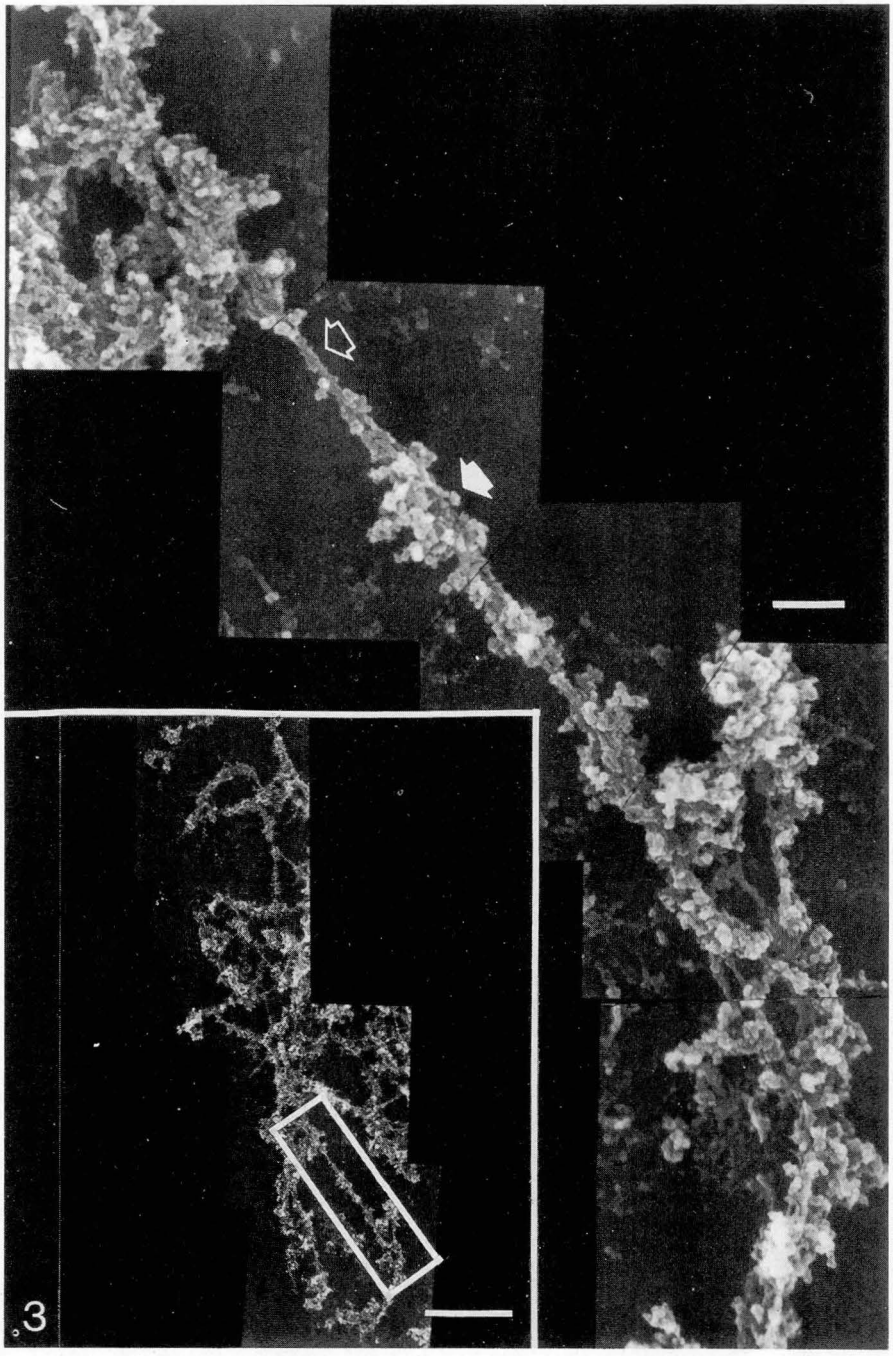

Fig. 3. Ultrastructure of late G_1 PCC. The region shown is an enlargement of the boxed area of the inset. Bars, 0·5 μm; inset, 5 μm. (From Hanks *et al.* (1983).)

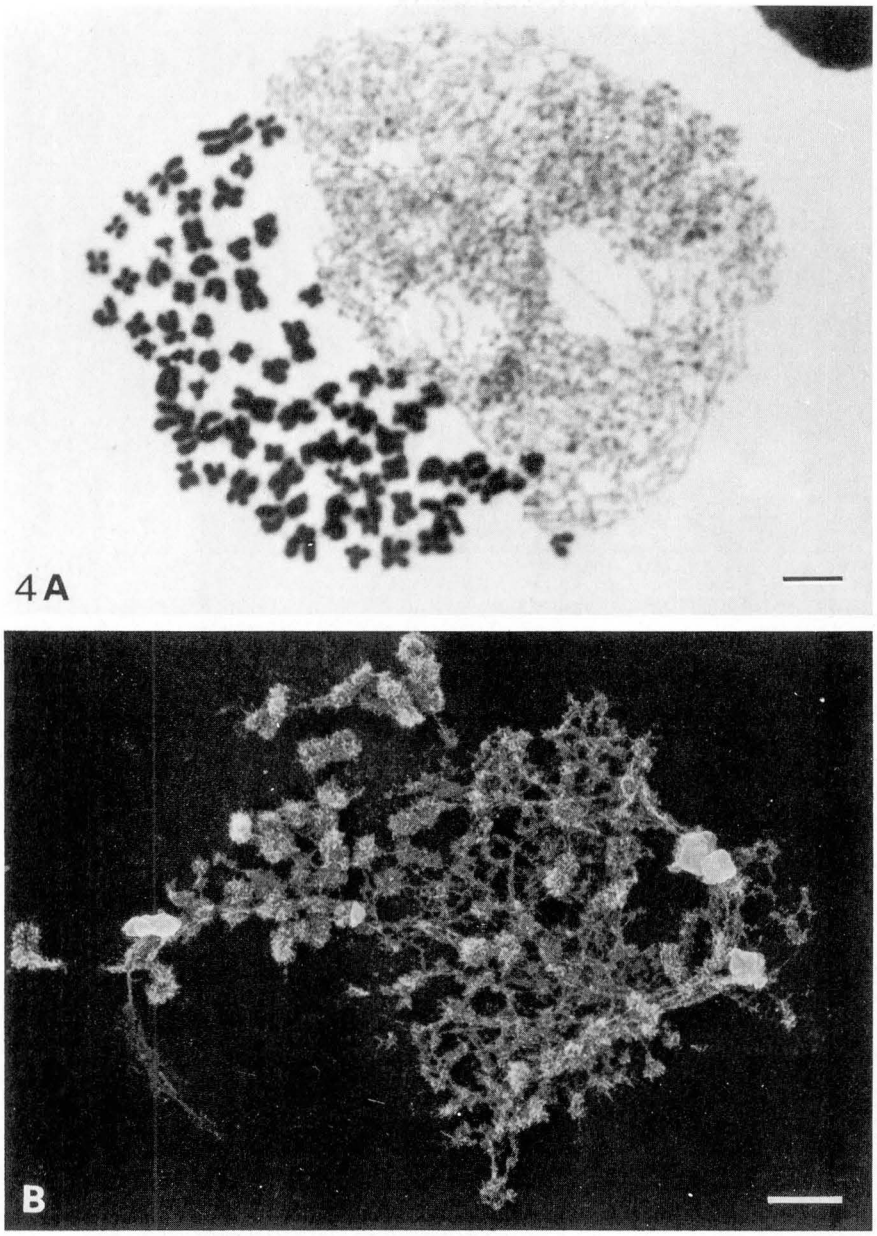

Fig. 4. Light (A) and SEM (B) micrographs of early *S* PCC spreads. Bars, 5 μm.

replicated domains, the unreplicated chromatid appears to be contiguous with one of the doubled segments, while the other replicated chromatid segment appears to be situated laterally (Fig. 8, inset).

G_2-*phase PCC*. When viewed by light microscopy, G_2 PCC resemble early prophase chromosomes and are characterized by extended double chromatids of rather uniform

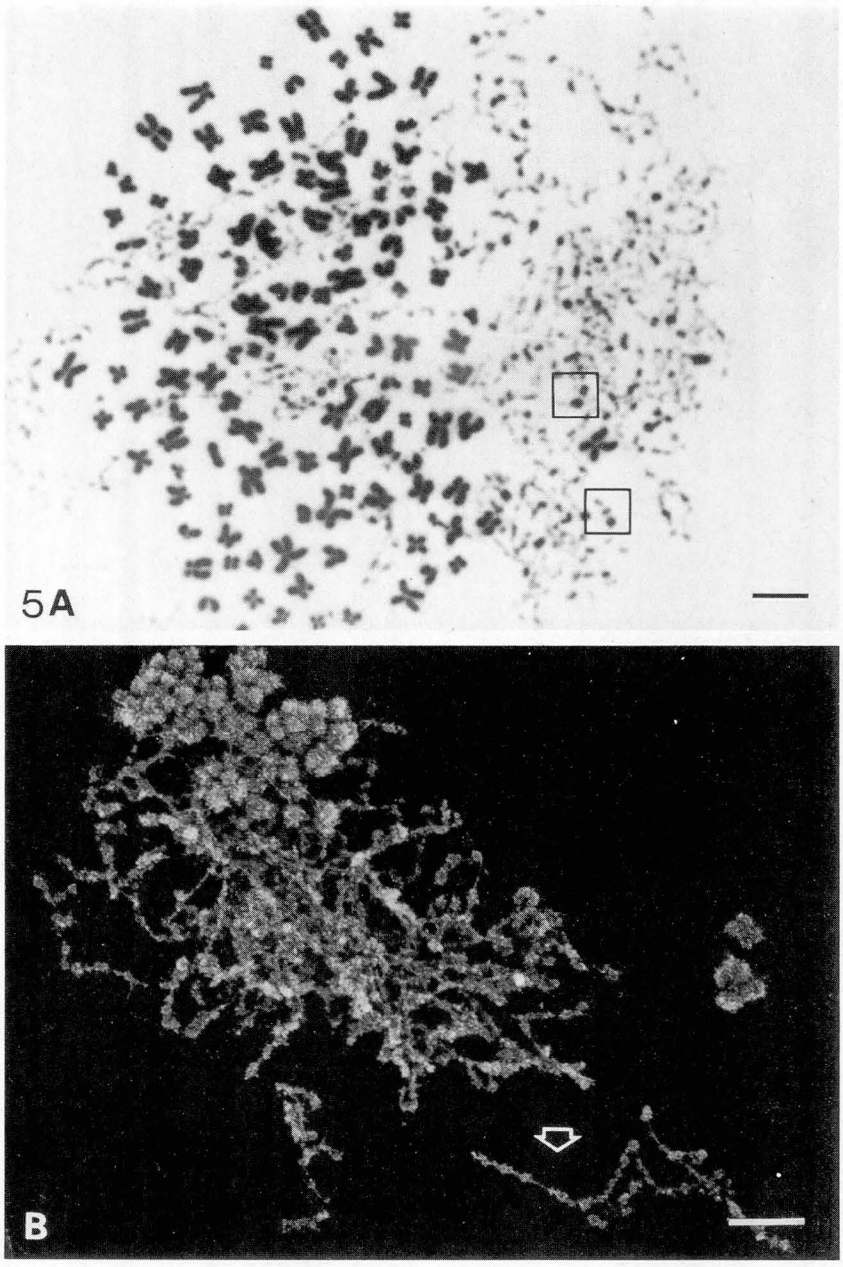

Fig. 5. Light (A) and SEM (B) micrographs of mid-*S* PCC spreads. Bars, 5 μm.

thickness throughout the length of the chromosome (Fig. 1F). At the ultrastructural level, G_2 PCC appear to consist primarily of packed looping fibres very similar to the replicated segments of late *S* PCC (Fig. 9). In contrast to late G_1 and *S*-phase PCC, the fibre density is quite uniform throughout the length of the G_2 chromosome.

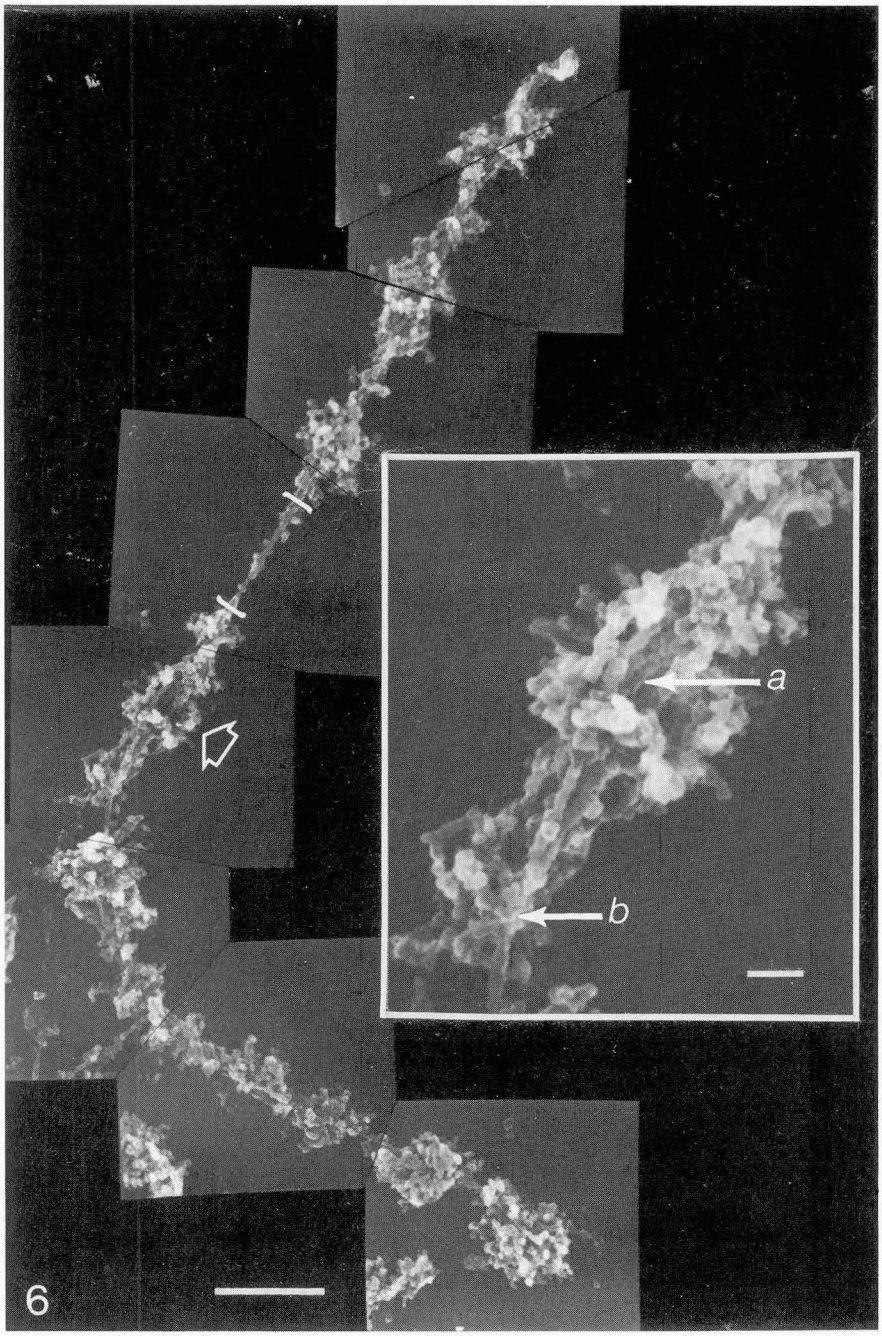

Fig. 6. Ultrastructure of mid-*S* PCC illustrating continuity of a single longitudinal fibre. The chromosome shown is indicated by an arrow in Fig. 5B. Bar, 1 μm. The inset is a further magnification of the region indicated by the arrow in the main part of the figure. Arrow *a* indicates a cluster with many parallel fibres; arrow *b*, a cluster of looping fibres with a common origin. Bar, 0·2 μm. (From Hanks *et al.* (1983).)

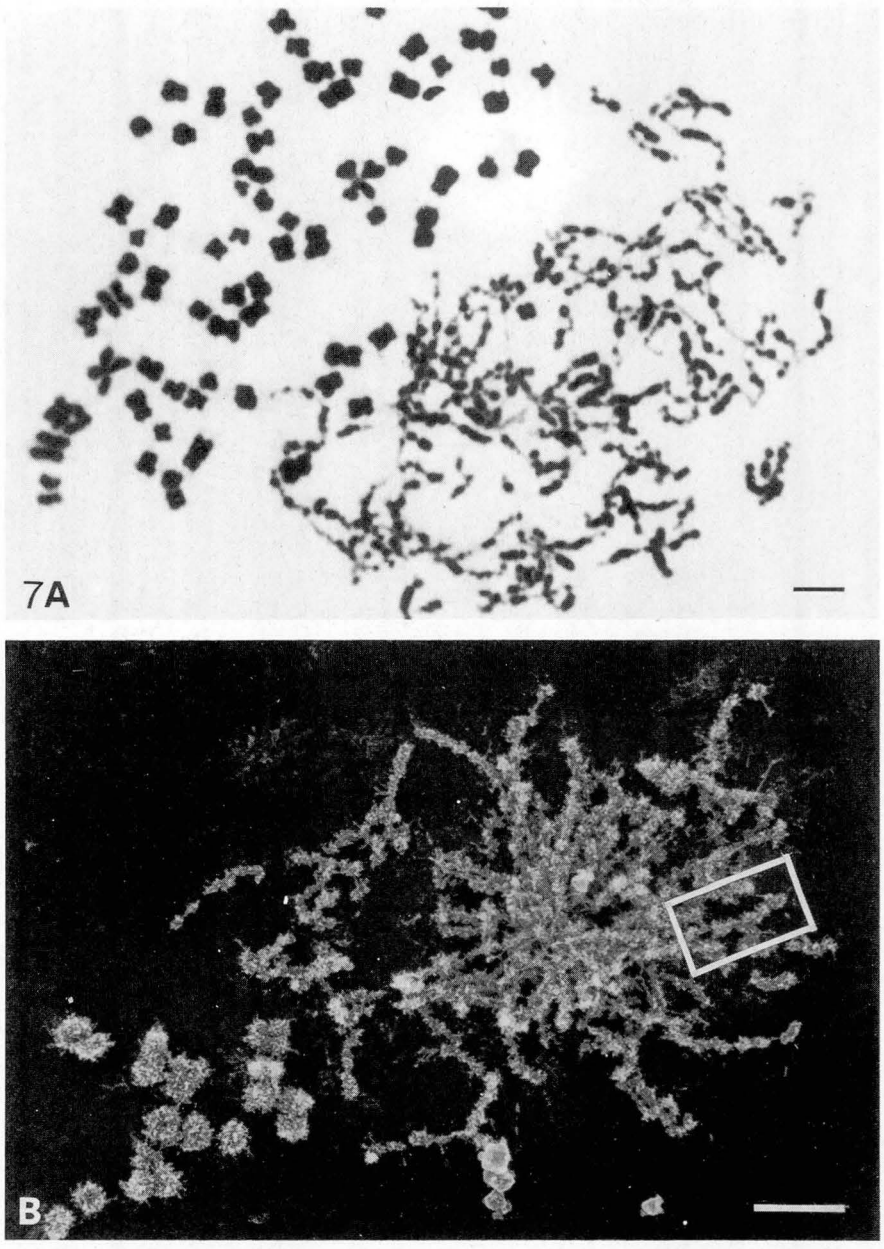

Fig. 7. Light (A) and SEM (B) micrograph of late *S* PCC spreads. Bars, 5 μm.

Metaphase chromosomes appear much more tightly packed than G_2 PCC (Fig. 1F). The width of G_2 PCC chromatids is narrower than metaphase chromatids. The loose packing of G_2 PCC allows a limited view of the internal fibre organization, and longitudinal fibres can be observed running for rather short stretches within the G_2 chromatids.

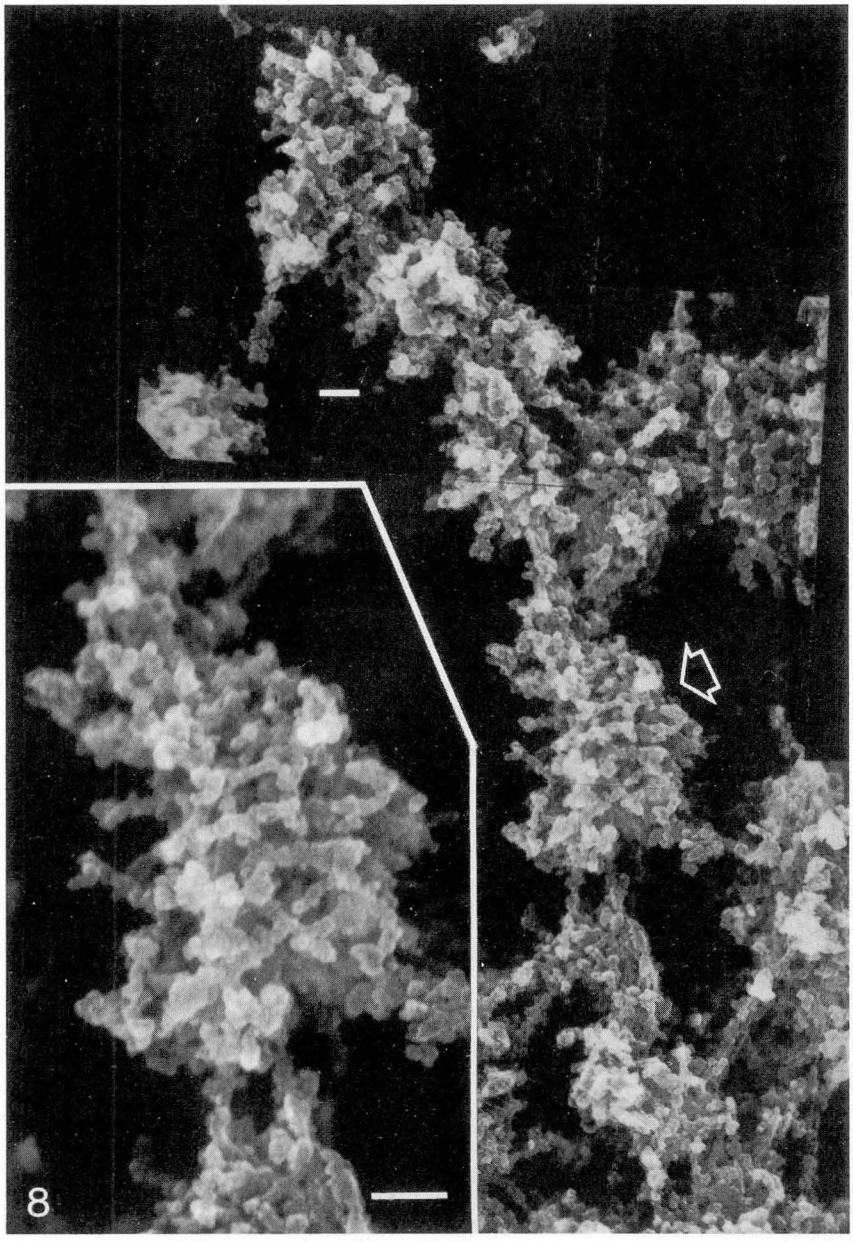

Fig. 8. Ultrastructure of late *S* PCC. The main part of the figure is an enlargement of the boxed region in Fig. 7B. The inset is an enlargement of the segment indicated by the arrow. Bars, 0·2 μm. (From Hanks *et al.* (1983).)

Progressive changes in the arrangement of the chromatin fibre during the cell cycle

The 30 nm chromatin fibre appears to be present in two forms in PCC: (1) looping fibres, and (2) longitudinal fibres. The relative fractions of tightly packed looping fibres and more extended longitudinal fibres vary during the cell cycle. The level of

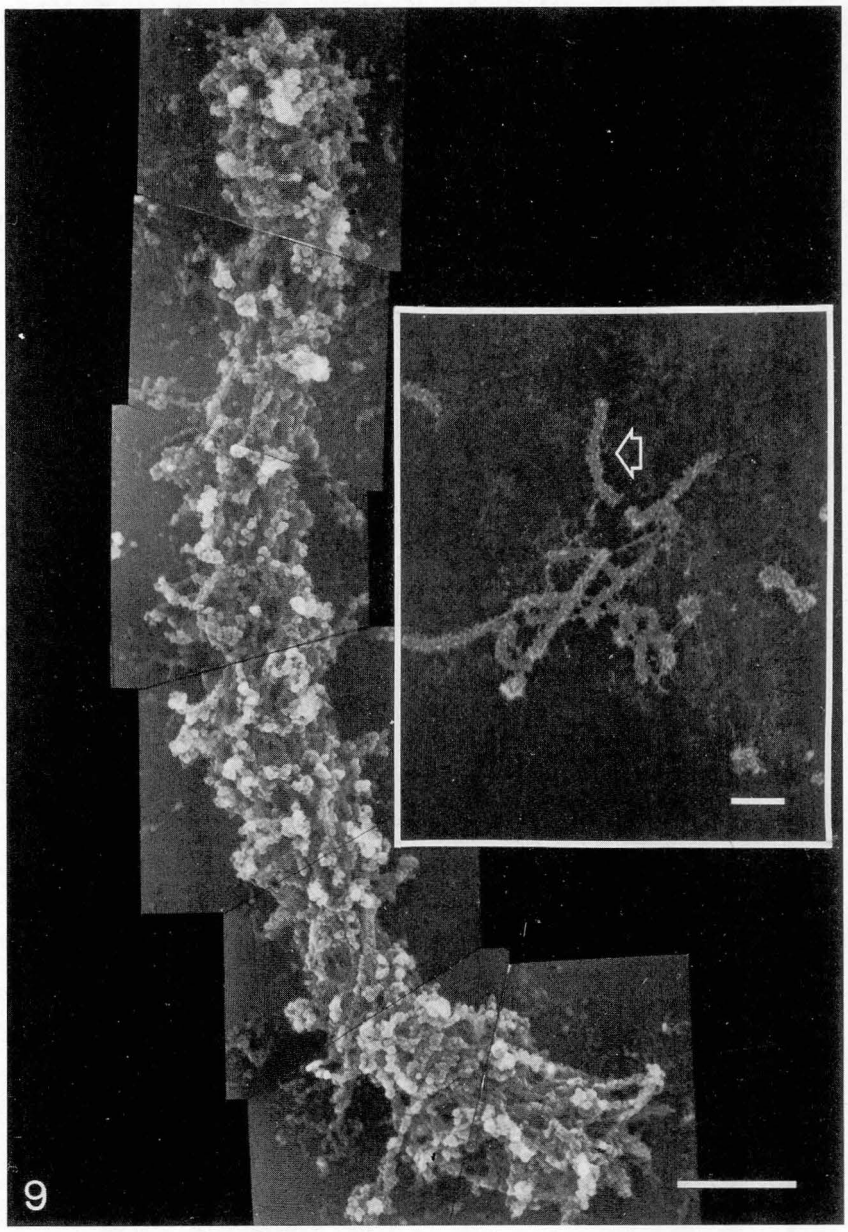

Fig. 9. Ultrastructure of G_2 PCC. The chromosome shown is an enlargement of the chromosome indicated by the arrow in the inset. Bars, 1 μm; inset, 5 μm. (From Hanks *et al.* (1983).)

chromosome extension appears directly related to the ratio of longitudinal to looping fibres within the chromosome. PCC decondensation during G_1 traverse is associated with despiralization of the chromonema, followed by a gradual transition from packed, looping fibres to prominent longitudinal fibres. Autoradiographic studies at

the light-microscopic level have clearly demonstrated that the gaps in *S*-phase PCC are the regions undergoing active DNA replication (Sperling & Rao, 1974; Lau & Arrighi, 1981; Mullinger & Johnson, 1983). We and others have shown that the gaps correspond to sparse, extended longitudinal chromatin fibres (Rohme, 1975; Hanks *et al.* 1983).

Progression through *S* phase is associated with a transition from a highly decondensed to a highly condensed morphology (compare Fig. 1C–F), which corresponds to the reorganization of sparse, extended longitudinal fibres to packed, looping-fibre domains. Lau & Arrighi (1981) have shown that this transition occurs soon after completion of DNA replication within a chromosome segment. Analysis of our electron micrographs of *S*-phase PCC reveals the presence of both single longitudinal fibres (pre-replicative and actively replicating chromatin) and packed looping fibres (post-replicative chromatin). The data suggest that post-replicative chromosome maturation includes the formation of looping-fibre clusters that may arise from a single point of conjunction (Fig. 6, arrow *b*), followed by the coalescence of these clusters into larger looping-fibre domains (Fig. 6, arrow *a*; Fig. 8, insert). The formation of packed looping domains is apparently associated with an increase in the number of longitudinally oriented fibres lying within them (Fig. 6, arrow *a*).

G_2-phase PCC contain numerous looping-fibre domains with longitudinal fibres apparent only in more loosely packed regions of the chromosome. In general, however, the double chromatids of G_2 PCC are organized as packed looping fibres that have a rather uniform density throughout their length (Fig. 9). Longitudinal fibres are not apparent in the highly condensed metaphase chromosomes obtained after Colcemid arrest.

These observations allow us to describe the chromosome condensation cycle in terms of the arrangement of the basic, 30 nm chromatin fibre within PCC. Chromosome decondensation during G_1 is accomplished by uncoiling the spiralized chromonema followed by a gradual transition from packed looping fibres to extended longitudinal fibres. Active sites of DNA replication are thought to be localized within single longitudinal fibres – the maximum level of PCC decondensation. Post-replicative chromosome maturation appears to involve rearrangement of the extended longitudinal fibres into packed looping-fibre clusters. This process begins during *S* phase, soon after replication of a chromosome segment, and appears to be associated with the assembly of a chromosome axis composed of multiple longitudinal fibres. Further compaction of replicated chromosome segments is achieved by coalescence of looping-fibre domains and by spiralization of chromonema, resulting in the formation of a highly compact metaphase chromosome.

PCC versus *metaphase chromosomes*

The results of our studies on the ultrastructural organization of PCC in conjunction with those of numerous investigations on metaphase chromosome structure shed light on the arrangement of chromatin fibres within metaphase chromosomes.

Early ultrastructural studies of whole-mount metaphase chromosomes revealed the presence of loops at the periphery of metaphase chromosomes (Gall, 1963; DuPraw,

1970). Stubblefield & Wray (1971) examined metaphase chromosomes that had been isolated and then treated with distilled water to loosen the packaging of the condensed chromatin and thus reveal the details of fibre arrangement. They observed longitudinally oriented fibres and looping-fibre domains, and proposed the concept of distinct axial and peripheral chromatin components. Golomb & Bahr (1974) used scanning electron microscopy to examine mitotic chromosomes and interphase nuclei. They observed both looping and longitudinal fibres in interphase chromatin and prophase chromosomes; whereas metaphase chromosomes appeared to consist solely of tightly packed looping fibres. Yunis & Bahr (1979) noted that human prophase chromosomes were composed of alternating looping and longitudinal-fibre domains. Laemmli and co-workers examined histone-depleted, surface-spread metaphase chromosomes (Adolph, Cheng & Laemmli, 1977; Paulson & Laemmli, 1977) and observed a 'halo' of looping DNA strands attached to a residual proteinaceous core or scaffold. Further ultrastructural studies of metaphase chromosomes suggested that the chromatin fibre loops are arranged in a radial fashion about the central axis of the chromatid (Marsden & Laemmli, 1979; Adolph, 1980, 1981; Earnshaw & Laemmli, 1983). More recent SEM studies of isolated metaphase chromosomes in conjunction with thin-sectioning studies provide strong evidence for the presence of both radial loops and longitudinal fibres along the chromosome axis (Adolph & Kreisman, 1983). Alternatives, such as the helical-coil model have been proposed on the basis of analysis of chromosomes in intact and disrupted nuclei (Sedat & Manuelidis, 1978). Mullinger & Johnson (1980) studied protein-depleted, Kleinschmidt-spread metaphase chromosomes and proposed an elegant model for chromosome fibre organization. They suggested that the chromatid consists of a chromatin fibre that folds back on itself several times over short regions to form multiple longitudinal fibres. Lateral loops of chromatin emanate from distinct regions (chromomeres) along the chromatid axis. These 'chromomeric blocks' are sites of fibre constraint and are interspersed with regions of the chromonemal axis that contain fewer fibres. They suggested that chromonema compaction at metaphase may occur by shortening the inter-loop axis, by creating new loops or by packing the inter-loop DNA in order to bring looped inserts together. Further shortening would be achieved by spiralization or helical coiling of the chromonema.

In addition to the observations discussed above, the data of Laughlin, Wilkinson-Singley, Olins & Olins (1982), and our data strongly support the Mullinger & Johnson model for chromosome organization. We clearly demonstrate the presence of looping-fibre domains interspersed with regions containing multiple longitudinal fibres. Our observations of late S PCC suggest that compaction of replicated chromosome segments is achieved by coalescence of looping-fibre domains. Our data further imply that spiralization of chromonema may be the final step in mitotic chromosome compaction, since despiralization appears to be the first level of chromosome decondensation observed in G_1-phase PCC.

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