Short Communication

Competence for assembly of sister chromatid cores is progressively acquired during S phase in mammalian cells

Juan F. Giménez-Abián[1,2)a], Duncan J. Clarke[2)b], Consuelo De la Torre[a], Gonzalo Giménez-Martín[a], Ann M. Mullinger[d], C. Stephen Downes[c], Robert T. Johnson[e]

[a] Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid/Spain
[b] Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA/USA
[c] University of Ulster, Coleraine, Co. Londonderry, Northern Ireland/UK
[d] Zoology Department, Cambridge University, Cambridge/UK
[e] Coriell Institute for Medical Research, Camden, NJ/USA

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Condensed sister chromatids possess a protein scaffold or axial core to which loops of chromatin are attached. The sister cores are believed to be dynamic frameworks that function in the organization and condensation of chromatids. Chromosome structural proteins are implicated in the establishment of sister chromatid cohesion and in the maintenance of epigenetic phenomena. Both processes of templating are tightly linked to DNA replication itself. It is a question whether the structural basis of sister chromatid cores is templated during S phase. As cells proceed through the cell cycle, chromatid cores undergo changes in their protein composition. Cytologically, cores are first visualized at the start of prometaphase. Still, core assembly can be induced in G_1 and G_2 when interphase cells are fused with mitotic cells. In this study, we asked if chromatid cores are similarly able to assemble in S-phase cells. We find that the ability to assemble cores is transiently lost during local replication, then regained in chromosome regions shortly after they have been replicated. We propose that core templating occurs coincident with DNA replication and that the competence for the assembly of the sister chromatid cores is acquired shortly after passage of replication forks.

Abbreviations. PCCs Prematurely condensed chromosomes.

[1)] Dr. J. F. Giménez-Abián, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Velázquez 144, E-28006 Madrid/Spain, e-mail: cibtg7a@pinar2.csic.es, Fax: + 34 91 562 75 18.
[2)] The two first authors contributed equally to this work.

Introduction

Metaphase chromosomes consist of two sister chromatids, each one having an axial protein core or scaffold to which loops of chromatin are anchored [2, 8, 9, 11]. Silver impregnation directly stains chromatid cores in mammalian cells at cycle stages where the nuclear envelope is absent, i.e. during the period from prometaphase until mid telophase [5], closely resembling topoisomerase II alpha immunostaining [4]. Still, single chromosomal G_1 cores and double G_2 cores can be stained with silver when interphase chromosomes are made to condense prematurely by mitotic cell fusion (Fig. 1a) [4]. Recent work has demonstrated that CAF-1-dependent chromatin assembly, a process that promotes the maintenance of epigenetically determined chromosomal states, can only occur following DNA replication [15]. Moreover, longitudinal cohesion between sister chromatids is established during DNA replication [17]. Thus, the cohesin Scc1p, in budding yeast, is able to bind to chromatin in S phase and G_2. However, association during ongoing DNA replication is essential for the establishment of cohesion [17]. If the ability to assemble sister chromatid cores is similarly acquired during DNA synthesis, this process should be revealed when S-phase prematurely condensed chromosomes (PCCs) are analysed by silver impregnation.

Material and methods

Cell culture and fusion

SVM 87 cells, a *Muntiacus muntjak* SV 40-transformed fibroblast cell line [12], were grown in monolayer cultures at 37 °C in Eagle's minimal essential medium (MEM) supplemented with 10 % fetal calf serum, nonessential amino acids, penicillin and streptomycin, in an atmosphere of 5 % CO_2.

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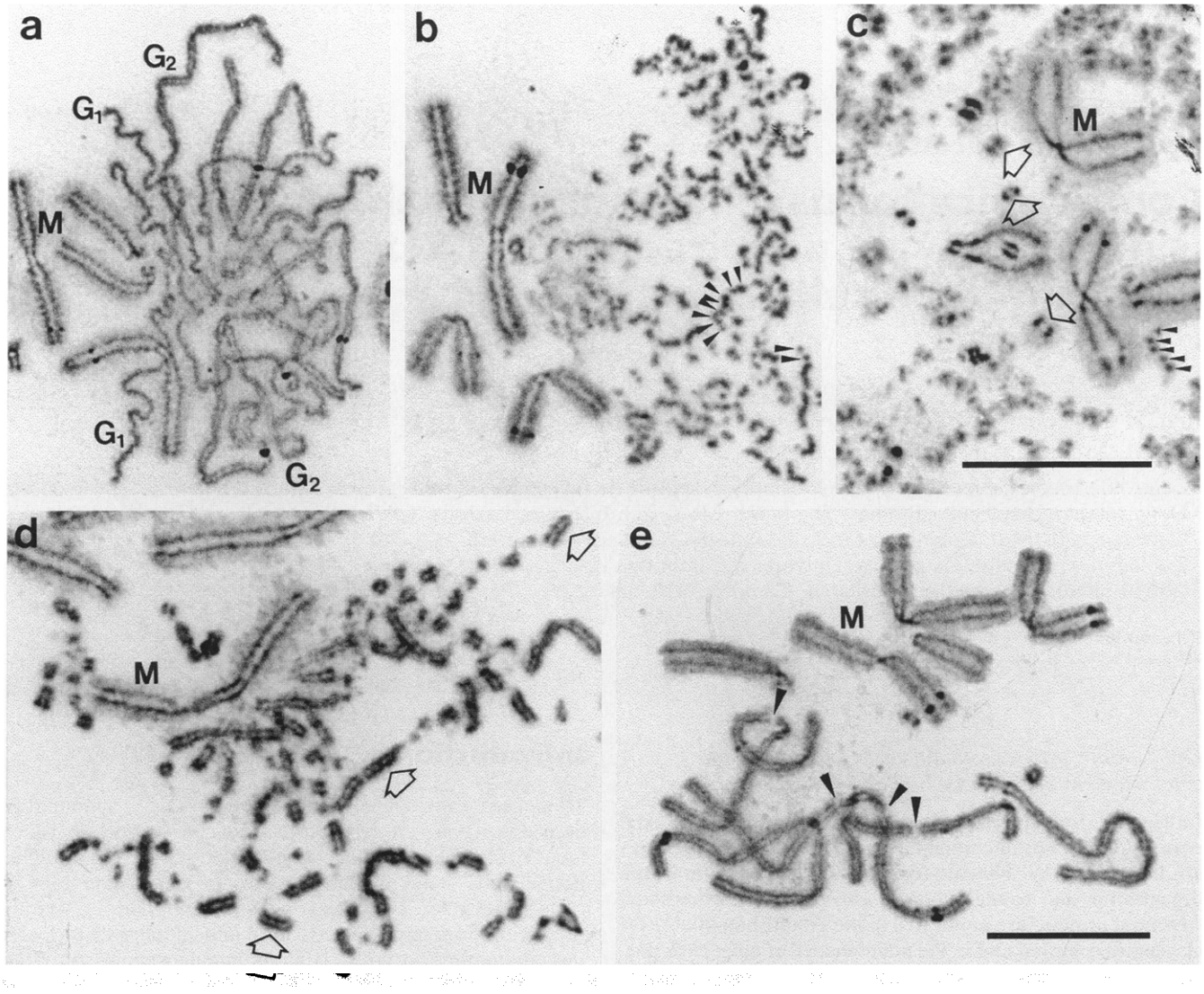

Fig. 1. Prematurely condensed chromosomes of *Muntiacus muntjak* (SVM) cells, impregnated with silver to visualize chromatid cores (methodology as previously described [4, 6]. M = metaphase chromosome, G_1 = G_1 PCC, G_2 = G_2 PCC. (**a**) Fusion of a G_1, a G_2 and a metaphase cell. In G_1 PCCs, cores are continuous and single. G_2 PCCs have continuous double cores. (**b**) Early-S-phase PCC. No double core segments can be seen. Single cores do not stain with silver in a continuous pattern. Rather, the cores are fragmented, appearing as discrete, evenly spaced segments (*small arrowheads*). (**c**) Mid-S-phase PCCs. Some short double core segments are present (*large arrows*), interspersed with regions in which the cores are not detectable. Single core segments (*small arrowheads*) are less abundant than in (**b**). (**d**) Late-S-phase PCCs. No single core segments are detectable. Regions of double cores stained with silver (*large arrows*), interspersed with gaps without a stained core. (**e**) S/G_2-phase PCCs. Chromosomal cores are almost complete. Several core gaps (three of them pericentromeric) remain in this cell (*arrowheads*). Magnification of Fig. a, b and d are similar to that of Fig. e. Bars = 10 μm.

For the induction of premature chromosome condensation, mitotic SVM cells were fused with unsynchronized SVM cultures, according to the standard procedure [6].

Silver impregnation

Silver impregnation was performed according to [4]. Briefly, the material was fixed with Carnoy's, either directly or after a 6 min hypotonic treatment in 50 % culture medium 50 % distilled water. Fixed material was dropped onto slides and air-dried. Dried slides were treated for 23 min with 2 × SSC at 60 °C, then rinsed in distilled water and allowed to dry. The staining solution was prepared immediately before use: 150 μl of 0.05 % formic acid in distilled water was added to 0.1 g of silver nitrate. Each slide was given three drops of the staining solution, then covered with a coverslip. Slides were put in a wet chamber at 75 °C. Usually 3–4 min staining gave optimal staining intensity. Coverslips were then removed with distilled water and slides were air dried and then mounted with Euparal resin.

Results and discussion

In S-phase PCCs, a mixture of unreplicated, replicated and actively replicating DNA coexists [10, 13, 16]. If cores are able to assemble regardless of the cycle stage, S-phase PCCs should reveal segments of double core, correlating with the chromosome segments that have been replicated, interspersed with regions of single core corresponding to segments of DNA that have not yet been replicated [10]. At the other extreme, if cores are only competent to assemble once cells have com-

pleted DNA replication, cores should not be visible in S-phase PCCs. In fact, neither of these extremes proved to be correct. The silver staining pattern of cores in S-phase PCCs revealed that the competence to assemble cores is indeed related to DNA replication.

We examined early-S-phase PCCs to ask whether the ability to assemble cores is lost following G_1. Silver staining revealed a dramatic fragmentation of single chromatid cores into discrete segments, interspersed with regions in which core assembly could not be seen (Fig. 1b). The early S-phase PCCs therefore revealed the localized loss of the competence to assemble a core. These core gaps resemble the chromosome gaps seen in S-phase pulverized PCCs, which were proven to correspond to replicating chromosomal regions by detecting [^{3}H]thymidine incorporation [10, 13, 16]. In these early S-phase PCCs, no double core regions were apparent.

By mid S phase, silver staining of PCCs revealed that single core segments were progressively lost and small regions with double cores appeared (Fig. 1c). These patterns of silver-stained S-phase PCCs closely correspond with the images obtained by scanning electron microscopy of muntjac replicating chromosomes [10].

In late-S-phase PCCs, silver staining revealed double core segments interspersed with regions which were not stained with silver (Fig. 1d). The amount of assembled double core progressively increased as cells were closer to the end of S phase (Fig. 1e), as would be expected if the competence for core assembly were related to the advancement of replication forks. We conclude that sister chromatid cores are able to assemble in chromosome regions shortly after they replicate. Therefore, sister chromatid cores are likely to be templated coincident with DNA replication.

Chromosome scaffolds or cores contain topoisomerase II, ScII (a member of the condensin family of putative ATPases) [2, 3, 7, 14] and other less abundant proteins. Cores are dynamic frameworks that function in the organization and condensation of chromatids. Although premature chromosome condensation can be induced at any stage of the cell cycle, the ability to assemble a core is transiently lost in replicating chromosome regions, then regained. This process probably involves loss or modification of core components prior to the initiation of DNA replication. In fact some core condensins are absent from the nuclear matrix [14] and from the endoreduplicating chromosomes [1]. Such components presumably reassociate with chromosomes following passage of the replication fork. As sister chromatid cohesion also appears to be established at the time of DNA replication [17], it is tempting to propose that the establishment of their cohesion is linked to the gain of competence for assembly of sister chromatid cores.

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