

# STEREO-ELECTRON MICROSCOPY OF DNA-STAINED MITOTIC CHROMOSOMES FROM *DICTYOSTELIUM DISCOIDEUM*

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## INTRODUCTION

The slime mold *Dictyostelium discoideum* is haploid with ca. 0.1 pg DNA divided among 7 chromosomes (Cox et al., 1990). Mitotic chromosomes observed by light microscopy after Giemsa staining (Zada-Hames and Ashworth, 1978; Welker and Williams, 1980), or DAPI staining (Roos, 1987) are  $\leq 2\mu\text{m}$  in length. During mitosis the nuclear envelope remains intact. Mitotic chromosomes stained with uranyl and lead are difficult to visualize by electron microscopy (EM) (Moens, 1976; McIntosh et al, 1985; Roos, 1987); but can be clearly seen with the DNA-specific reagent osmium ammine (Bottini and Roos, 1992). In this study, mitotic chromosomes were stained with a modified reagent osmium ammine-B (OA-B; Olins et al, 1989) and examined by stereo-EM. DNA distribution in mitotic cells revealed unanticipated ultrastructural features: 1) mitotic chromosomes with surface loops of chromatin; 2) plaques of condensed chromatin associated with the nuclear envelope.

## MATERIALS AND METHODS

*D. discoideum* NC4 in exponential growth phase (ca.  $1-2 \times 10^6$  cells/ml) fed *E. coli* B/r in 20mM NaKHPO<sub>4</sub> buffer (pH 6.0) were treated with 10  $\mu\text{g/ml}$  thiabendazole (TBZ) for 3 hr at RT, exactly as described by Welker and Williams (1980). Using a cell preparation procedure modified from Zada-Hames and Ashworth (1978), we obtained 20-40% of total cells in mitosis after TBZ-treatment in DAPI stained preparations.

For EM, TBZ-treated cells were fixed in 2%

paraformaldehyde, 0.5% glutaraldehyde, 0.1M Na cacodylate (pH 7.4) for 1 hr at RT, washed, dehydrated with increasing concentrations of ethanol and embedded in LR White. Specimens were not fixed with OsO<sub>4</sub>. Sections ca. 100 nm thick, mounted on gold grids were stained by a modified Feulgen reaction (Olins et al., 1989) in which osmium ammine-B (OA-B, Polysciences, Inc.) stains DNA specifically. Sections were visualized with a Hitachi H-600 EM operated at 50 KV using a 30  $\mu\text{m}$  objective aperture, to maximize contrast.

## RESULTS AND DISCUSSION

DAPI-stained, TBZ-treated *D. discoideum* exhibited metaphase chromosomes, often in a ring (Figure 1A-C). The diameter of the ring of chromosomes varied, perhaps reflecting the dynamics of metaphase. Speckles of fluorescent DNA were observed outside chromosomes, but still within nuclei (Figure 1D). Although the DAPI images are from air-dried cells, similar chromosomal arrangements were visualized on formaldehyde-fixed non-dried specimens using confocal imaging (collaboration with Y. Usson and M. Robert-Nicoud, Univ. J. Fourier, Grenoble).

Metaphase chromosomes stained with OA-B sometimes showed a ring arrangement as observed with DAPI stained cells in mitosis (Figure 1E, F). Boundaries of chromosomes revealed considerable irregularity. Condensed chromatin plaques were observed apposed to the unstained nuclear envelope. These features are shown in higher magnification stereo-EM images (Figure 2).

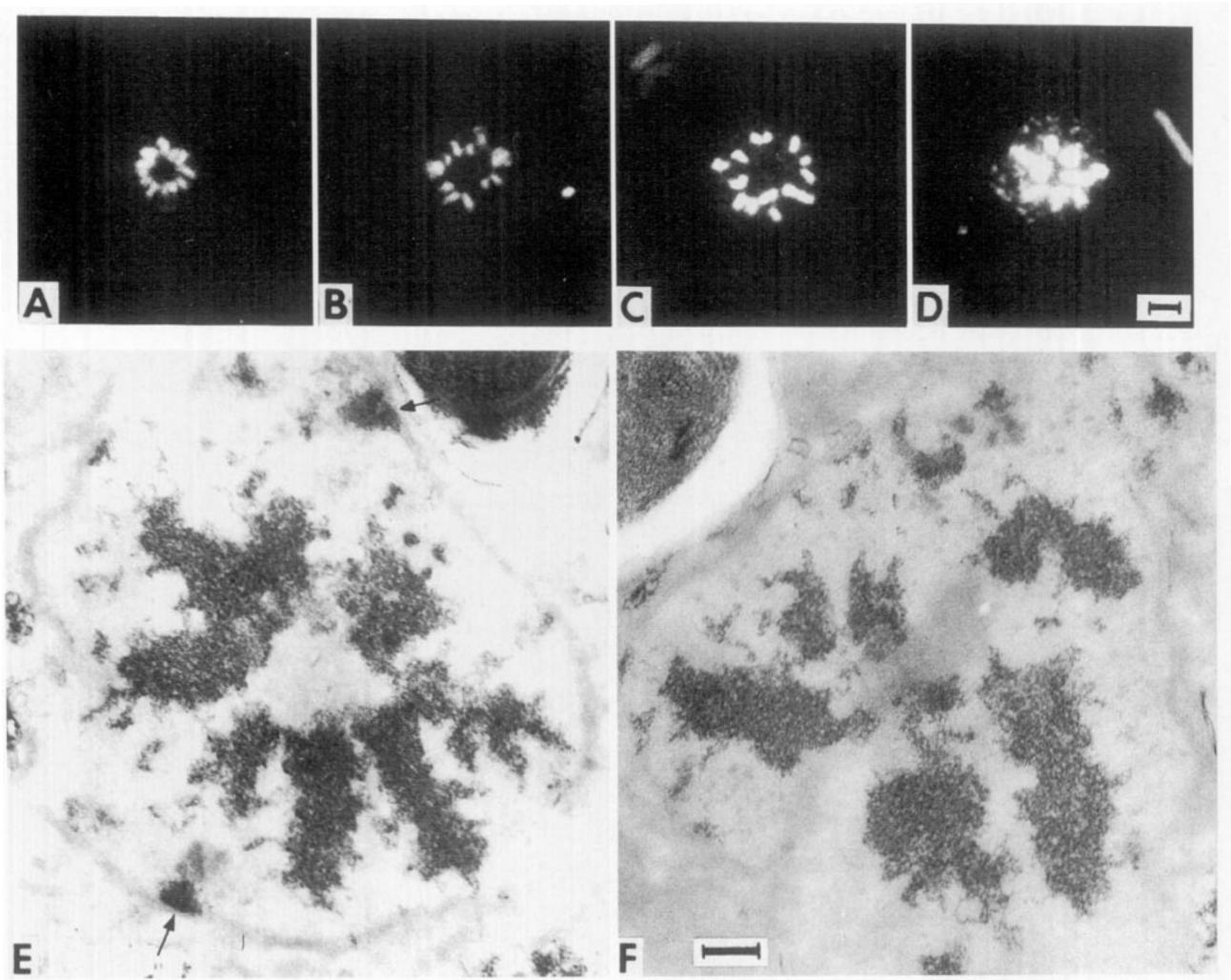


Figure 1. *D. discoideum* mitotic chromosomes stained for DNA. A-D, DAPI-stained acid-fixed air-dried cells. Bar equals 2  $\mu$ m. E and F, EM images of OA-B stained metaphase chromosomes. Arrows point to condensed chromatin plaques. Bar equals 0.2  $\mu$ m.

The measured average diameter of chromatin fibers in the loops was  $20.3 \pm 8.4$  nm (S.D.). Interphase nuclei (not shown) did not appear to contain any condensed chromatin, not even small plaques at the nuclear envelope. The large interphase nucleoli were essentially devoid of OA-B stained DNA. It is conceivable that during interphase, the amplified palindromic extra-chromosomal ribosomal DNA is in an extended conformation; while during metaphase it is condensed at the nuclear envelope and apportioned to daughter cells amitotically, without attachment to the spindle. Future studies will

explore whether mitotic loops correspond to functional chromosomal domains, and attempt to define the mitotic chromatin plaques by *in situ* hybridization.

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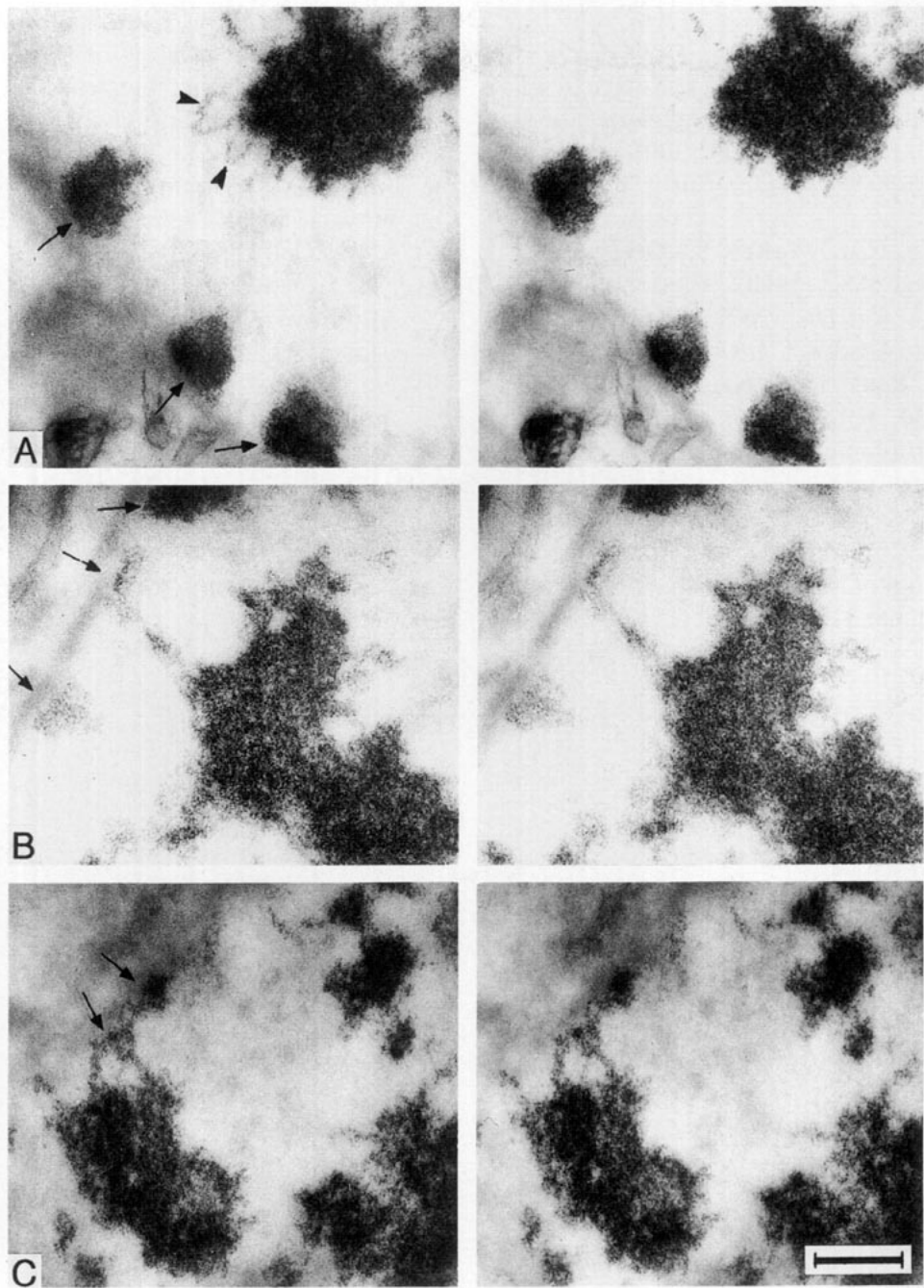


Figure 2. Stereo-EM of OA-B stained mitotic cells. Arrows, condensed chromatin plaques; Arrowheads, chromosome loops. Bar equals 0.2  $\mu\text{m}$ .

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