Ca2+-dependent contractility of isolated and demembranated macronuclei in the hypotrichous ciliate Euplotes aediculatus

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Ca^{2+}-dependent contractility of isolated and demembranated macronuclei in the hypotrichous ciliate *Euplotes aediculatus*

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Abbreviated title: Nuclear contraction in *Euplotes*

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Summary The hypotrichous ciliated protozoan *Euplotes aediculatus* possesses a characteristic C-shaped somatic nucleus (macronucleus) within the cytoplasm, which shows dynamic shape change during the cell cycle. It is shown that isolated macronuclei possess Ca^{2+}-dependent contractility. Macronuclei were isolated, stuck fast on the glass surface, and subjected to different concentrations of Ca^{2+} in a Ca^{2+}-EGTA buffer. The nuclei became expanded at $[Ca^{2+}] < 10^{-7}$ M, and they contracted on subsequent addition of higher concentrations of Ca^{2+}. Cycles of expansion and contraction of the nucleus could be repeated many times by alternate addition of EGTA and Ca^{2+}, indicating that the size of isolated nuclei can be regulated by $[Ca^{2+}]$ alone. The nuclear contraction was observed in all phases of the cell cycle, but contractility was less evident around replication bands in the S phase. In addition to the hypotrichous ciliate *Euplotes*, similar Ca^{2+}-dependent nuclear contractility was found to exist in other cell types, including protozoans of different taxa (a heliozoon *Actinophrys sol* and a peniculine ciliate *Paramecium bursaria*), and also mammalian culture cells (HeLa cells). Our findings suggest a possibility that Ca^{2+}-dependent nuclear contractility may be shared among diverse eukaryotic organisms.

INTRODUCTION

Berezney and Coffey reported in 1974 that a stable structural framework in the nucleus, named “nuclear matrix”, was detected in an isolated rat liver nucleus after chemical extractions [1]. Many investigators explicitly speculated that the nuclear matrix may be a critical and facilitating element in nuclear functions. Although controversies still remain, numerous studies have led to the conclusion that the nuclear matrix is involved in gene expression, replication and transcription of DNA, and also processing and transportation of RNA [2]. Electron microscopic researches have revealed that the interior architecture of the nucleus is constructed by branched 10-nm filaments [3]. In spite of the progress in characterization of nuclear proteins and ultrastructural properties of the nuclear matrix, dynamics and motility of the nucleus have received less attention.

A nuclear protein matrix was detected also in the macronucleus of the ciliate *Tetrahymena* [4]. The nucleus of *Tetrahymena* has been extensively examined as a model system for studying basic structure and function of the eukaryotic nucleus [5]. In 1977, a reversibly contractile nuclear matrix was isolated from *Tetrahymena* macronuclei, and its mechanism of contraction was reported to be different from that of the actin-myosin interaction [6]. Although ultrastructure and essential components of the nucleoskeleton have been well characterized, the biological significance of nuclear contractility remained unclear. Many protozoans possess nuclei unique in size and shape, which sometimes undergo vigorous shape changes [7]. The vegetative cells of hypotrichous ciliates such as *Stylonychia*, *Oxytricha* and *Euplotes* have two types of nuclei, the micro- and the macronucleus, within the cytoplasm. In contrast to the micronucleus, the macronucleus changes in both shape and size during the cell cycle

[7]. In spite of the large number of ultrastructural studies so far carried out [8-14], there is no certain evidence that a contractile system exists in the macronucleus. In this study, we prepared an isolated and demembranated macronucleus of a hypotrichous ciliate *Euplotes aediculatus*, and found that it shows vigorous contraction and relaxation in a Ca^{2+}-dependent manner.

MATERIALS AND METHODS

Euplotes aediculatus cells were purchased from Carolina Biological Supply Company (Burlington, USA), and cultured in Volvic mineral water at 23 ± 1 ºC. *Chlorogonium elongatum* was added twice a week as a food source. Subculturing was carried out at intervals of about 2 weeks.

Isolation of macronuclei was carried out according to Arikawa et al. [8] with slight modifications. Washed cells were put on a hollow slide with a small amount of culture medium, and gently mixed with 250 mM sucrose. A little amount of methyl green dye was added to the sucrose solution for visualization of isolated macronuclei. After freezing at -20 ºC and thawing at room temperature, macronuclei were isolated from the fragile cells by pipetting with a Pasteur pipette. By this procedure, remnants of the nuclear membrane became completely removed from the nuclear surface [8]. After washing with fresh sucrose solution, isolated macronuclei were put on a glass slide coated with poly-L-lysine. To stick the isolated macronuclei on the surface of a glass slide, excess sucrose solution was removed by a thin-tipped Pasteur pipette. After covering with a coverslip, test solutions were introduced from one side of the preparation. For replacement of solutions, the preparation was drained from the other

side using a piece of filter paper. Firmly settled macronuclei were selected for continuous observations under an Olympus BH-2 microscope equipped with Nomarski differential interference optics. Images were recorded on a video cassette recorder (Mitsubishi, HV-S77) for measurement of the approximate area of isolated macronuclei. Free Ca^{2+} concentration was calculated by an iterative procedure according to Suzaki and Williamson [15].

RESULTS AND DISCUSSION

We have recently established a procedure for isolating *Euplotes* macronuclei and examined the three-dimensional surface structure of chromatin bodies and replication bands. By high-resolution scanning and transmission electron microscopy, we showed that the isolation procedure had no destructive effect on the ultrastructure of macronuclei [8]. As shown in Fig. 1a, the characteristic C-shaped appearance of a macronucleus in G1 phase remained unchanged after isolation and demembranation. When "EGTA solution" consisting of 3 mM ethylene glycol bis(β-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA) and 5 mM N-(2-Hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES, pH 7.0) was added, the macronucleus became swollen (Fig. 1b). Then, "Ca^{2+} solution" consisting of 3 mM EGTA, 5 mM $CaCl_2$ and 5 mM HEPES (pH 7.0) was added, and the expanded macronucleus rapidly contracted and returned to its original shape (Fig. 1c). The macronucleus expanded again on subsequent addition of EGTA solution (Fig. 1d). Neither contraction nor relaxation of isolated macronuclei required ATP, and nuclear contraction was not induced by addition of Mg^{2+} or other cations even at concentrations higher than that required for Ca^{2+} (data not shown). The approximate area of an isolated macronucleus was continuously measured and is shown in Fig. 2. Cycles of contraction and relaxation of the nucleus could be repeated several times by alternate addition of Ca^{2+} and EGTA (Fig. 2a). One of the cycles is shown in detail in Fig. 2b, which indicates that both contraction and relaxation responses took place within a few seconds. Although evidence for the presence of tubulin, actin and myosin in the nucleus of various kinds of cells has been shown [16-19], there is no direct evidence to support possible involvement of these molecules in nuclear contractility. In this study, contraction and expansion of the

isolated macronucleus were inhibited by neither colchicine (10 mM) nor cytochalasin B (1 mM), which suggests that the nuclear contractility in *Euplotes* is not mediated by tubulin- or actin-based machineries. The size of an isolated macronucleus at various concentrations of Ca^{2+} was measured and shown in Fig. 3. The relationship between projected area of an isolated macronucleus and free Ca^{2+} concentration showed a sigmoidal dose-response with a threshold [Ca^{2+}] level of 1.1×10^{-7} M.

Considerable evidence shows that cell nuclei in general vary in size and shape during the cell cycle and cell differentiation. In particular, nuclear enlargement precedes the onset of DNA synthesis [20], while nuclear contraction appears to accompany cessation of DNA synthesis [21]. The macronucleus of *Euplotes* also changes in size and shape in the cell cycle. To investigate whether contractility of the macronucleus persists throughout the cell cycle, we applied the same procedure at various phases in the cell cycle. Macronuclei in S phase and G2 or M phase were isolated and shown in Figs. 4 and 5, respectively. The presence of a set of replication bands (arrowheads in Fig. 4) is a characteristic feature of the nucleus in S phase, where DNA replication is taking place [22-26]. When EGTA solution was added to a macronucleus in S phase, the nucleus became expanded within a few seconds (Figs. 4b to c). The nuclear expansion was less evident around the replication bands as compared with other regions in the nucleus. With the subsequent addition of Ca^{2+} solution, the swollen nucleus contracted to almost its original size (Fig. 4d). Ca^{2+}-dependent contraction of the macronucleus was also observed in G2 or M phase (Fig. 5) in which the macronucleus is shorter and thicker compared with those in S or G1 phases. An isolated macronucleus in G2 or M phase (Fig. 5a) became expanded with the addition of EGTA solution (Figs. 5b to c), then contracted on subsequent addition of Ca^{2+} solution

(Fig. 5d). These results indicate that a Ca^{2+}-dependent contractility of the macronucleus exists throughout the cell cycle, except for the region of the replication bands in S phase where contraction also took place but to a lesser degree. Electron microscopic studies on the replication bands which have been carried out by both transmission [10-14, 24, 26-27] and scanning [8] electron microscopy, have shown that chromatin bodies are composed of densely-packed chromatin fibrils which become disintegrated into thin fibrils in the replication band. Such a morphological feature of the replication band may be related to its apparently lower Ca^{2+}-contractility.

In order to examine if Ca^{2+}-dependent nuclear contractility as observed in *Euplotes* is shared with other cell types, other protozoan species and a mammalian culture cell were examined by employing the same procedure. As shown in Fig. 6, contraction of isolated nuclei was observed in protozoans of different taxa (a heliozoon *Actinophrys sol* (Figs. 6a and b) and a peniculine ciliate *Paramecium bursaria* (Figs. 6c and d)), and even in HeLa cells (Figs. 6e and f). These results suggest the possibility that all eukaryotic cells possess a Ca^{2+}-dependent contractile machinery inside their nuclei.

Isolated macronuclei of *Tetrahymena pyriformis* were reported to show contraction by Ca^{2+} in an ATP-independent manner, with contraction of the nuclear membrane postulated as its mechanism [6, 28]. Atomic force microscopic studies have shown that the conformational change of nuclear pore complex components may be responsible for the contraction of the nucleus [29-30]. However, in the present study, nuclear membranes were completely eliminated during preparation [8], which indicates that neither the nuclear membrane nor nuclear pore complexes are involved in the process of nuclear contraction in *Euplotes*. Berezney and Coffey have isolated a

nucleoskeletal framework from rat liver nuclei, which consists of a nuclear protein matrix [1, 31]. The nuclear matrices have also been identified in Chinese hamster cell nuclei [32] and in nuclear ghosts prepared from HeLa cells [33], suggesting that these structures probably contain common components. However, less attention has been paid to their motile properties. In this study, the possibility was raised for the first time that a contractile system is present in many eukaryotic cell nuclei, although its biological significance is presently unclear. The fact that various kinds of cells including protozoans and cultured mammalian cells exhibited similar contraction suggests that this unique property of the nucleus has been conserved during the process of eukaryotic evolution.

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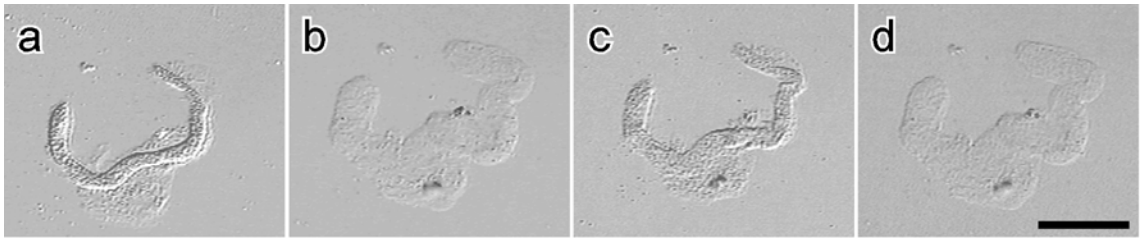

Fig. 1 Sequential light micrographs showing repeated expansion and contraction of an isolated macronucleus of *Euplotes aediculatus*. **a:** A macronucleus just after isolation, showing the characteristic slender C-shape. **b:** The macronucleus expanded remarkably by the addition of 3 mM EGTA. **c:** After subsequent addition of Ca^{2+} at $[Ca^{2+}] = 2 \times 10^{-3}$ M, the relaxed macronucleus became contracted. **d:** After subsequent removal of Ca^{2+} by EGTA, the contracted macronucleus again expanded. Bar = 100 μm.

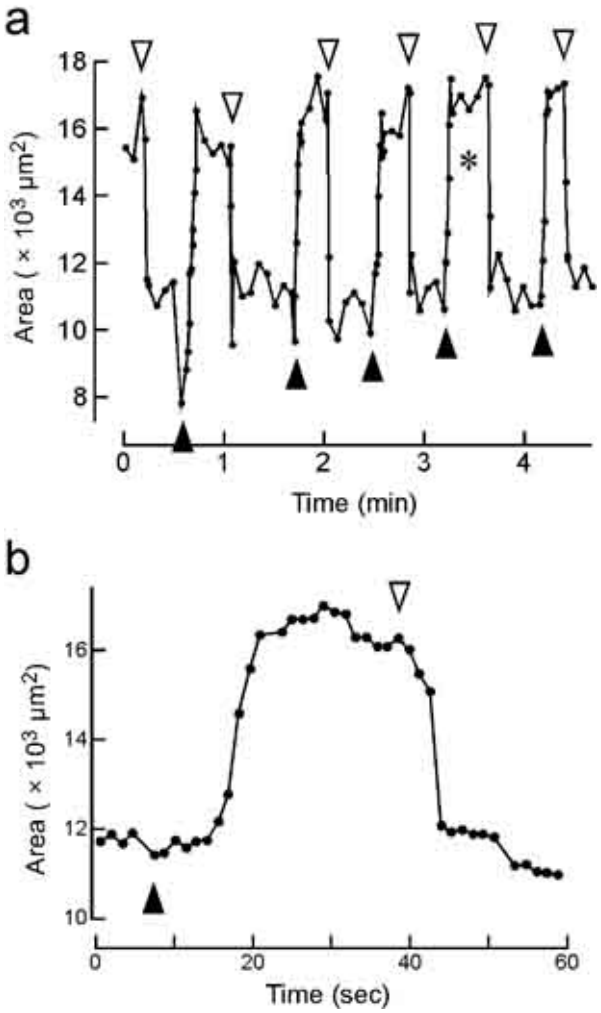

Fig. 2 Trace of the approximate projected area of an isolated macronucleus. Open and filled arrowheads indicate the moments when Ca^{2+} ([Ca^{2+}] = 2 mM) and EGTA (3 mM) were added, respectively. **a:** Cycles of contraction and expansion were observed several times by alternate addition of Ca^{2+} and EGTA. **b:** Enlargement of one of the cycles shown in **a** (marked with an asterisk). Both expansion and contraction occurred within a few seconds after removal and addition of Ca^{2+}, respectively.

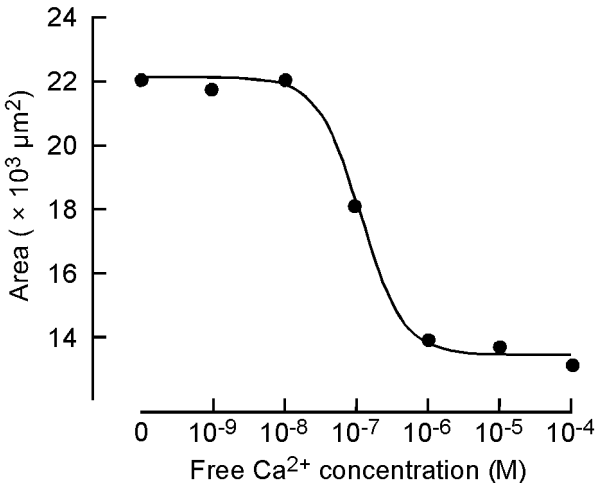

Fig. 3 Relationship between approximate projected area of an isolated macronucleus and free Ca^{2+} concentration. The threshold level of free Ca^{2+} concentration for nuclear contraction was estimated as 1.1×10^{-7} M, by fitting a sigmoidal curve to the data.

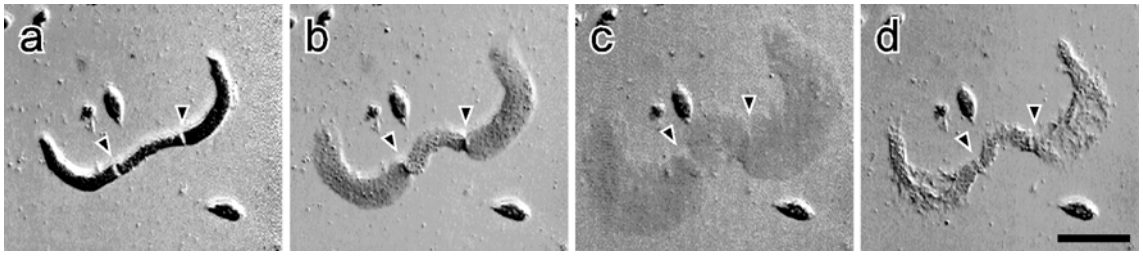

Fig. 4 Sequential light micrographs of an isolated macronucleus in S phase that possessed a pair of replication bands (arrowheads). The macronucleus adhered on a glass surface (**a**) became swollen on addition of EGTA (**b** to **c**). Micrographs in **b** and **c** were taken at 5 and 20 sec. after the addition of EGTA, respectively. The degree of nuclear expansion was restricted around the replication bands. The expanded macronucleus contracted after the addition of Ca^{2+} (**d**). Bar = 100 μm.

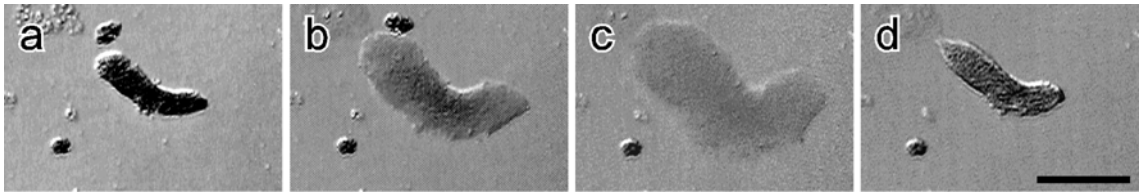

Fig. 5 Light micrographs of an isolated macronucleus in G2 or M phase. Presence of a shorter and thicker macronucleus is characteristic of this phase of the cell cycle. The isolated macronucleus (**a**) became gradually expanded when EGTA solution was added (**b** to **c**), followed by contraction upon subsequent addition of Ca^{2+} solution (**d**). Micrographs in **b** and **c** were taken at 5 and 15 sec. after the addition of EGTA, respectively. Bar = 100 μm.

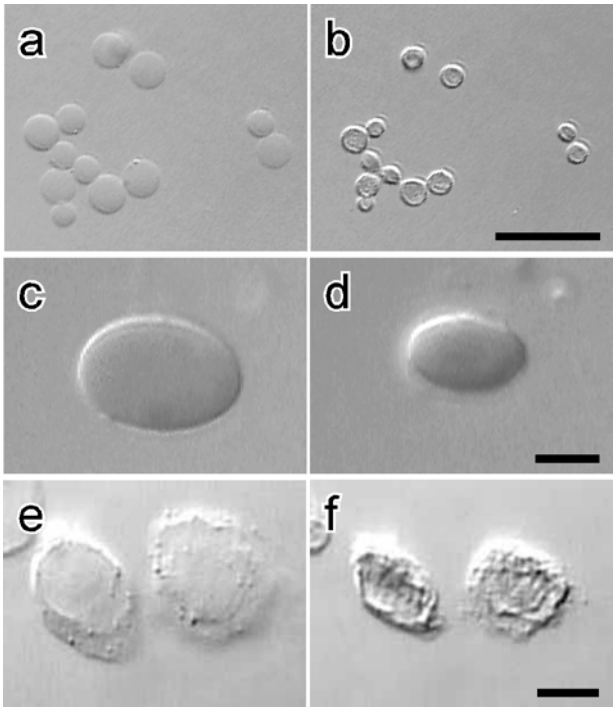

Fig. 6 Light micrographs of isolated nuclei of various cell types. Nuclear contraction was induced by addition of Ca^{2+} ($[Ca^{2+}]$ = 2 mM) in the heliozoon *Actinophrys sol* (**a** and **b**), the ciliate *Paramecium bursaria* (**c** and **d**), and in HeLa cells (**e** and **f**). Nuclei in expanded and contracted states are shown in left (**a**, **c**, **e**) and right (**b**, **d**, **f**) panels, respectively. Bars = 50 (**b**) or 10 (**d** and **f**) μm.