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Cell Calcium 38 (2005) 447–455

cell calcium

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Ca^{2+}-dependent nuclear contraction in the heliozoon *Actinophrys sol*

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Received 10 May 2005; received in revised form 25 June 2005; accepted 27 June 2005
Available online 15 August 2005

Abstract

Ca^{2+}-dependent contractility was found to exist in the nucleus of the heliozoon protozoan *Actinophrys sol*. Upon addition of Ca^{2+} ($[Ca^{2+}]_{free} = 2.0 \times 10^{-3}$ M), diameters of isolated and detergent-extracted nuclei became reduced from 16.5 ± 1.7 μm to 11.0 ± 1.3 μm. The threshold level of $[Ca^{2+}]_{free}$ for the nuclear contraction was 2.9×10^{-7} M. The nuclear contraction was not induced by Mg^{2+}, and was not inhibited by colchicine or cytochalasin B. Contracted nuclei became expanded when Ca^{2+} was removed by EGTA; thus cycles of contraction and expansion could be repeated many times by alternating addition of Ca^{2+} and EGTA. The Ca^{2+}-dependent nuclear contractility remained even after high salt treatment, suggesting a possible involvement of nucleoskeletal components in the nuclear contraction. Electron microscopy showed that, in the relaxed state, filamentous structures were observed to spread in the nucleus to form a network. After addition of Ca^{2+}, they became aggregated and constructed a mass of thicker filaments, followed by re-distribution of the filaments spread around inside of the nucleus when Ca^{2+} was removed. These results suggest that the nuclear contraction is induced by Ca^{2+}-dependent transformation of the filamentous structures in the nucleus.

Keywords: Calcium; Nuclear matrix; Nucleoskeleton; Protozoa

1. Introduction

A large number of investigations have been made on characteristic contractile systems in unicellular organisms. The spasmoneme of vorticellid ciliates [1–6], the myoneme of heterotrichous ciliates [7,8], and the flagellar root of green algae [9–12] are well-known organelles which show Ca^{2+}-dependent contractility. Contraction of these organelles is considered to be generated by proteins such as centrin (caltractin) and spasmin [13,14], which belong to the calmodulin subfamily. Although much progress has been made on characterization of such Ca^{2+}-binding proteins and resulting cytoplasmic contraction at a molecular level, less attention has so far been paid to the dynamics of the nucleus.

In 1974, a stable framework structure termed the "nuclear matrix" was found in an isolated rat liver nucleus by sequential chemical extractions [15,16]. Since then, many investigations have been performed to elucidate possible functions of the nuclear matrix, and it is now clear that the nuclear matrix is involved in the processes of gene expression, replication and transcription of DNA, and also processing and transportation of RNA [17]. The ultrastructure of the nuclear matrix has also been well studied, and branched thin filaments with a diameter of about 10 nm were identified to construct the interior architecture of the nucleus [18]. Despite considerable advances in physiological and morphological investigations, evidence for the existence of contractility of the nuclear matrix has not been reported so far.

Recently, we reported that Ca^{2+}-dependent contractility exists in an isolated and demembranated macronucleus of the hypotrichous ciliate *Euplotes aediculatus* [19]. Furthermore, similar nuclear contraction was observed in several

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doi:10.1016/j.ceca.2005.06.034

species of protozoan cells and even in cultured mammalian cells (HeLa cells). From these results, we have proposed a hypothesis that all eukaryotic cells possess Ca^{2+}-dependent nuclear contractility which has been preserved during the process of eukaryotic evolution. As an example, isolated and detergent-extracted nuclei of the heliozoon *Actinophrys sol* were demonstrated to express the Ca^{2+}-dependent contractility. In the present study, physiological characterization of the contractility and ultrastructural observations of isolated nuclei were carried out, and a possible mechanism of the Ca^{2+}-dependent nuclear contraction is presented.

2. Materials and methods

2.1. Organisms and culture

Actinophryid heliozoon *A. sol* (protozoa) was axenically cultured in a co-culturing condition with *Chlorogonium elongatum* in 10% artificial sea water (47 mM NaCl, 1.1 mM KCl, 1.1 mM $CaCl_2$, 2.5 mM $MgCl_2$, 2.5 mM $MgSO_4$ and 1 mM Tris–HCl, pH 7.8) containing 10% *Chlorogoniumu* medium (0.01% sodium acetate, 0.01% polypepton, 0.02% tryptone peptone, 0.02% yeast extract and 1 μg/ml $CaCl_2$) at $20 \pm 1\,^{\circ}C$ under constant lighting. Subculturing was carried out at intervals of about 2 weeks. Centrifugally collected cells were gently washed with fresh 10% artificial sea water at room temperature before using for experiments.

2.2. Nuclear isolation procedures

Nuclei of *A. sol* were isolated by using a sucrose-Percoll separation technique. At first, a solution A consisting of 2.0 M sucrose, 10% Percoll, 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 overlaid in a test tube with a solution B consisting of 2.0 M sucrose, 3 mM EGTA and 5 mM HEPES (pH 7.0). Washed cells were centrifugally collected and suspended in a solution C consisting of 1.0 M sucrose, 3 mM EGTA and 5 mM HEPES (pH 7.0), homogenized with a Teflon homogenizer, and placed on top of the layered solutions in the test tube. To extract membraneous components from isolated nuclei, 1.0% Triton X-100 was added to the solution C. After centrifugation at $750 \times g$ for 10 min, isolated nuclei were collected from the boundary between solutions A and B. Nuclei were again suspended in solution C, and were centrifuged at $750 \times g$ for 5 min. After removal of the supernatant, the crude preparation of the nuclear pellet was collected from the bottom of the test tube, and subjected to different concentrations of divalent cations for light microscopy or a fixative solution for electron microscopy.

2.3. Light microscopy

Isolated nuclei were placed on a glass slide coated with 0.1% poly-L-lysine. After covered with a coverslip, the nuclei were left for a few minutes until the nuclei became stuck to the glass surface. Test solutions were introduced from one side of the preparation using a Pasteur pipette, and were absorbed from the other side using a piece of filter paper. Although unattached nuclei were flushed away by a stream of test solutions, many nuclei remained on the substratum, which allowed us to observe nuclear responses continuously under an Olympus BX-50 microscope equipped with Nomarski differential interference optics. Images were recorded on a video cassette recorder (Victor, BR-S822) or a high resolution digital camera (Olympus DP11) for measurement of an approximate area of the isolated nuclei using an image analyzing software Scion Image Beta 4.02 (Scion Corporation).

2.4. Electron microscopy

Isolated nuclei were prefixed with 3% glutaraldehyde in an EGTA buffer for 3 min at room temperature. They were then postfixed with a fixative consisting of 1% OsO_4, 0.01 mM $MgSO_4$, 1 mM sucrose, and 50 mM sodium cacodylate (pH 7.0) for 30 min at room temperature. After being washed with 50 mM cacodylate buffer (pH 7.0), fixed nuclei were dehydrated through a graded ethanol series, and embedded in Spurr's epoxy resin [20]. Thin sections were picked up on a Formver-coated copper grid, stained with 3% aqueous uranyl acetate for 15 min and Reynolds' lead citrate [21] for 5 min at room temperature, and then observed under a transmission electron microscope (Hitachi H-7100) operated at 75 kV.

3. Results

3.1. Reduction in diameter of isolated nuclei

A. sol possesses a single nucleus within the cytoplasm. As shown in Fig. 1a, differential interference contrast light microscopy showed that the spherical nucleus (marked "N") was located at the center of the cell body, and cortical nucleolar materials were present at the inner periphery of the nucleus. In this study, Ca^{2+}-dependent contractility was found to exist in isolated and detergent-extracted nuclei. Although nucleolar material could no longer be observed after isolation, the spherical shape of the nucleus was well preserved (Fig. 1b). When nuclear isolation was performed in the presence of calcium ions (2×10^{-3} M free Ca^{2+}), the diameter of the nucleus decreased. Furthermore, the contour of the nucleus became prominent, and the nucleus showed a rigid appearance (Fig. 1c).

Diameters of isolated nuclei were measured under various conditions, and compared with those in living cells (Fig. 2). Nuclear diameters in living cells were in the range of 10.9–19.9 μm, with an average value of 15.1 ± 1.7 μm (Fig. 2a). In the absence of Ca^{2+}, average diameters of the nuclei isolated without (Fig. 2b) and with detergent treatment (Fig. 2d) were slightly larger (15.9 ± 1.5 μm and 16.5 ± 1.7 μm, respectively) than those in the living cells