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CHANGES IN INTRACELLULAR CA^{2+} DISTRIBUTION DURING THE TRANSITION OF FIBROBLASTS FROM THE PROLIFERATING TO THE STATIONARY STATE

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The intracellular distribution of Ca^{2+} was studied in chicken embryo fibroblasts as they progressed from the proliferating to the stationary state in cell culture. Cytoplasmic Ca^{2+} content was approximately six-fold higher in replicating fibroblasts than in confluent, nonproliferating cells. The quantity of Ca^{2+} associated with intracellular organelles (i.e., mitochondria, microsomes, nuclei) did not change during the transition to the stationary state. The ratio of cytoplasmic:organelle Ca^{2+} decreased from approximately 24 in logarithmically growing cells to 1.9 in nonproliferating fibroblasts.

INTRODUCTION

Motility in nonmuscle and muscle cells is ultimately regulated by changes in intracellular Ca^{2+} concentration (1-3). Fibroblasts contain a membranous, energy-dependent Ca^{2+} regulatory system (4) that is likely composed of endoplasmic reticulum, mitochondria and plasma membrane. Although this membranous system qualitatively resembles the sarcoplasmic reticulum of skeletal muscle in its ability to sequester Ca^{2+}, its specific activity for Ca^{2+} uptake is less than 1% of that observed in adult skeletal muscle sarcoplasmic reticulum (4).

The transition of nonmuscle cells from the proliferative to the nonproliferative stage is accompanied by a decrease in the extent of cell motility and by internal changes in the distribution of contractile filaments (2, 5). Because motility is largely regulated by Ca^{2+}, the objective of this study was to determine if the progression of fibroblasts from the proliferative to the stationary state was accompanied by a general decline in the concentration of cytoplasmic Ca^{2+}.

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MATERIALS AND METHODS

Fibroblast Cultures - Preparation of chick embryo fibroblasts, subculture of the cells and enumeration of nuclei in each culture was carried out as previously described (6). Fibroblasts were subcultured at least three times and replicate dishes containing 10^6 cells were plated for each experiment.

Ca^{2+} Distribution - At the start of the labeling period, cells were rinsed once at 37°C with 10 ml of an isotonic, buffered saline solution (137 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1.5 mM NaH_2PO_4, 1.36 mM Na_2HPO_4, 6 mM $NaHCO_3$, 5.5 mM glucose, pH 7.4) to remove residual culture medium. Cells were then equilibrated for 1 hr with 1 μCi/ml of $[^{45}Ca]Cl_2$ in cell culture medium. At the end of the labeling period, cultures were rinsed as quickly as possible four times with ice-cold buffered saline solution to remove extracellular $^{45}Ca^{2+}$. All subsequent steps were carried out at 2°C. Cells were scraped from the surface of the culture dishes with a plastic spatula into 1.0 ml of 150 mM KCl, 20 mM Tris-HCl, pH 7.2. Homogenization was accomplished by twenty strokes of a 7 ml Dounce-type glass homogenizer (Wheaton Scientific, tightly fitting A pestle), and the homogenate was centrifuged at 133,000 x g_{max} for 1 hr. Radioactivity in the supernatant and in the pelleted fraction was attributed to cytoplasmic Ca^{2+} and to organelle-associated Ca^{2+}, respectively. All measurements were made in duplicate. Since cell number changes continuously in proliferating cultures, all data were normalized by dividing the quantity of $^{45}Ca^{2+}$ in each cellular compartment by the number of cells in the culture.

RESULTS

Equilibration with $^{45}Ca^{2+}$ - Relative intracellular Ca^{2+} levels were estimated by measuring the quantity of $^{45}Ca^{2+}$ in the cytoplasmic and organelle compartments. Equilibration of $^{45}Ca^{2+}$ with these two intracellular pools was complete within 20 min in both logarithmically proliferating and stationary cultures (Fig. 1). Thus, a standard 1 hr equilibration period was employed for subsequent experiments.

Fluctuations in Ca^{2+} Levels - The quantity of cytoplasmic $^{45}Ca^{2+}$ dropped dramatically between days 1 and 1.5 after the last subculture, with a gradual decline for the next three days (Fig. 2A). As fibroblasts approached confluency after 4.5 days, the level of cytoplasmic $^{45}Ca^{2+}$ approached a minimum. While the quantity of organelle-associated $^{45}Ca^{2+}$ also dropped between days 1 and 1.5, no major changes were apparent beyond that time (Fig. 2B). The ratio of cytoplasmic to organelle $^{45}Ca^{2+}$ declined steadily from approximately 24 on day 1 to 1.9 in stationary cultures (Fig. 2C). These ratios indicate that 96% of Ca^{2+} is cytoplasmic in proliferating cells, but 65.5% is located within the cytoplasmic compartment in confluent cultures.

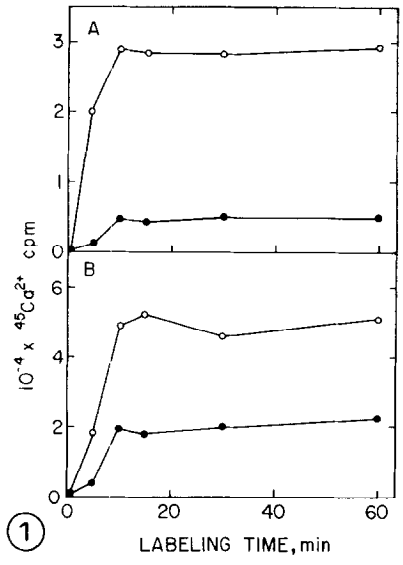

Figure 1. Kinetics of equilibration of exogenous $^{45}Ca^{2+}$ with the cytoplasmic and organelle compartments in logarithmically proliferating fibroblats (A) and in confluent fibroblasts (B). O , cytoplasmic; ● , organelle.

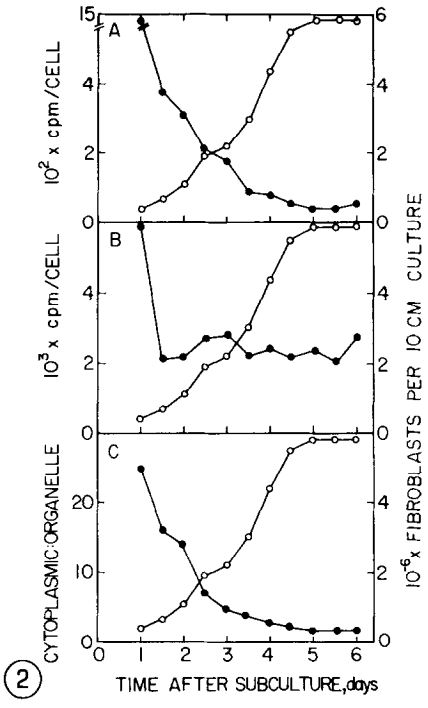

Figure 2. Changes in the intracellular levels of cytoplasmic and organelle-associated $^{45}Ca^{2+}$, and in the ratio of cytoplasmic to organelle-associated $^{45}Ca^{2+}$ in fibroblast cultures as a function of cell density. The number of fibroblasts per dish is shown in all three panels. A) $^{45}Ca^{2+}$ cpm per cell in the cytoplasmic compartment, B) $^{45}Ca^{2+}$ cpm per cell in the organelle compartment, and C) the ratio of $^{45}Ca^{2+}$ cpm in the cytoplasmic and organelle compartments. O , cells/plate; ● , $^{45}Ca^{2+}$ cpm.

Comparison with Muscle Cells - Fibroblasts cease proliferation when cultures approach confluency. Replicating myogenic cells, which may be of a common ancestral lineage as fibroblasts (7) also cease proliferation in cell culture, but for a different reason. Muscle cells withdraw from the cell cycle to undergo terminal differentiation into multinucleated myotubes. Replicating myogenic cells, for all practical purposes, are nonmuscle cells in the sense that they do not accumulate significant quantities of myofibrillar contractile proteins (8). Fully differentiated muscle cultures, on the other hand contain large quantities of these muscle-specific contractile proteins as well as extensive sarcoplasmic reticulum (9). The concentration of cytoplasmic

Ca^{2+} was comparable in replicating myogenic cells and replicating fibroblasts; however, the concentration of cytoplasmic Ca^{2+} was approximately 16-fold lower in postmitotic, multinucleated myotubes than in stationary fibroblasts (Table 1).

DISCUSSION

The proliferative ability of chick embryo fibroblasts is sensitive to alterations in divalent cation concentrations. For example, proliferation rate and DNA synthesis are reduced in proportion to extracellular Mg^{2+} concentration lower than the physiological level (10). Proliferation rate is also reduced by low levels of extracellular Ca^{2+}, but DNA synthesis is only affected at Ca^{2+} levels low enough to cause cell detachment (10). In the present study, the intracellular redistribution of Ca^{2+} was examined as cells withdraw from the proliferative to the stationary stage. Cytoplasmic Ca^{2+} levels decrease several-fold during this transition. It is not clear, however, whether the reduction in Ca^{2+} concentration is a response to the cessation of proliferation, or whether cellular interactions reduce the cytoplasmic Ca^{2+} concentration and this reduction in turn indirectly inhibits initiation of DNA synthesis. In other cell types, a minimum level of Ca^{2+} is essential to trigger DNA synthesis (11).

Table 1. Cytoplasmic Ca^{2+} content of replicating fibroblasts, stationary fibroblasts, replicating myogenic cells and multinucleated muscle cells. Relative Ca^{2+} levels were estimated by equilibration with $^{45}Ca^{2+}$ as described in Materials and Methods.

Cell Type	$10^3 \times {}^{45}Ca^{2+}$ cpm/Nucleus[1]
Replicating fibroblasts	31.20 ± 4.51
Stationary fibroblasts	5.63 ± 0.71
Replicating myogenic cells[2]	28.03 ± 2.61
Multinucleated muscle cells[2]	0.34 ± 0.06

[1]Mean ± SEM of five experiments in which all measurements were in duplicate.

[2]Muscle cell cultures were prepared as described elsewhere (12).

The facts that constitutive contractile proteins are responsible for cell motility and that the activity of these proteins is regulated by Ca^{2+} (2) suggests that these cells possess a mechanism for precise regulation of intracellular Ca^{2+} concentration. An energy-dependent Ca^{2+} regulatory system in fibroblasts has been partially characterized (4). Based on sensitivity to sodium azide (an inhibitor of mitochondrial Ca^{2+} uptake), this membranous system is composed of mitochondria and microsomes (i.e., membranous vesicles of plasma membrane and endoplasmic reticulum). The majority of Ca^{2+} regulatory activity was attributed to the microsomal fraction (4). The activity of the fibroblast Ca^{2+} regulatory system is comparable to the activity of crude sarcoplasmic reticulum preparations from muscle cell cultures (9). However, the fibroblast system may be less efficient than in muscle cell cultures, since relative cytoplasmic Ca^{2+} levels are at least 16-fold lower in muscle cells (Table 1).

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