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STUDIES ON THE SIZE, LOCATION AND TURNOVER OF CALCIUM POOLS ACCESSIBLE TO GROWING *TETRAHYMENA* CELLS

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Summary

Tetrahymena pyriformis cells in the logarithmic phase of growth accumulate 2.5—3.75 times as much calcium per unit volume as is present in the growth medium. It appears that most of this calcium is stored in a non-ionic form, with approximately 30% existing in the cilia, near its site of action in effecting ciliary reversal. The exchange of extracellular $^{45}Ca^{2+}$ with the major internal pools is extremely rapid, exhibiting a $t_{1/2}$ of less than 0.5 h. Sites located on the cilia are responsible for 35—50% of Ca^{2+} influx, with the remainder entering through other positions on the cell surface.

Introduction

It is now widely accepted that Ca^{2+} plays a critical role in the regulation of many important metabolic processes and structural interactions in living cells [1]. The cytoplasmic concentration of Ca^{2+} is generally as much as three orders of magnitude lower than its concentration in the cell's external environment [2]. Thus changes in membrane permeability can effect large increases in the intracellular Ca^{2+} level very quickly. Changes in the cytoplasmic Ca^{2+} concentration can also result through mobilization of calcium stored within the cell in non-ionic forms [1].

Tetrahymena pyriformis and related ciliates possess a variety of Ca^{2+}-regulated processes. The organisms utilize Ca^{2+} for an almost instantaneous control of ciliary reversal [3]. On a much slower time scale, Ca^{2+} also triggers a rise in the intracellular concentration of cyclic AMP [4]. The interaction of intracellular and extracellular Ca^{2+} in regulating these and other reactions is poorly understood.

We have undertaken a study of the size and location of internal calcium pools within growing *Tetrahymena* cells and measured the rate at which they

Abbreviation: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

exchange with extracellular Ca^{2+}. The data indicate that all major pools are in rapid equilibrium with external Ca^{2+} and therefore readily available for modulating the cytosolic Ca^{2+} level.

Materials and Methods

Tetrahymena pyriformis, strain NT-1, was grown at 28°C with shaking in a medium consisting of 2% proteose peptine, 0.5% glucose, 0.2% yeast extract, and a trace of chelated iron [5]. The calcium content of this medium was determined by atomic absorption spectrometry.

$^{45}CaCl_2$ (16 Ci/g Ca) was obtained from ICN, Irvine, CA, and [1-^{14}C]palmitic acid (55 Ci/mol) was purchased from New England Nuclear Corp., Boston, MA. Dibucaine HCl was obtained from the Ciba Pharmaceutical Co., Summit, NJ.

In order to wash *Tetrahymena* cells incubating in $^{45}Ca^{2+}$ free of externally-bound radioisotope and recover them for analysis, they were centrifuged through a gradient of Ficoll (Pharmacia, Piscataway, NJ) in a heat-sealed Pasteur pipette as described by Nandini-Kishore and Thompson [4]. In this technique, an aliquot of cell suspension was layered over 2.0 ml of ice cold 4% Ficoll in inorganic medium [6] containing 1 mM $CaCl_2$ and centrifuged in a clinical centrifuge for 4 min at $150 \times g$. The contents of the Pasteur pipette were then immediately frozen in solid CO_2/acetone, and the tip, containing the cell pellet, was crushed inside a folded filter paper and placed in a scintillation vial for radioassay.

In some experiments cells were deciliated by the use of dibucaine [15]. The method was modified by adding $CaCl_2$ (10 mM, 1 ml) to the cell suspension (10 ml) in growth medium 4 min prior to the addition of dibucaine to final concentration of 3 mM in order to facilitate the deciliation. In other cases a modification of the centrifugation method described above was employed for deciliation. For this, a 4-ml suspension of cells, usually prelabeled by incubation with $^{45}Ca^{2+}$ in proteose peptone medium, was layered over 10 ml 4% Ficoll suspension in a 15-ml centrifuge tube and centrifuged for 4 min at $150 \times g$. The resulting cell pellet was quickly resuspended in proteose peptone medium. (Analysis of control samples showed that only 12% of the remaining ^{45}Ca was residual extracellular radioisotope). Samples (0.25 ml) of the resuspended cells were immediately centrifuged in heat-sealed Pasteur pipettes as described above. But in this case each sample was placed on top of 0.8 ml ice-cold 3.5% Ficoll in inorganic medium [6] 1 mM in $CaCl_2$, layered over 1.2 ml of 4% Ficoll in the same Ca^{2+} supplemented inorganic medium. The 4% Ficoll layer also contained 3.0 mM dibucaine. The gradients, which had been prepared shortly before sample addition to minimize diffusion of dibucaine into the upper Ficoll layer, were then centrifuged for 4 min. at $150 \times g$ in a clinical centrifuge and quickly frozen for recovery of the cell pellet as described above.

Use of the discontinuous gradient and the preliminary larger scale wash to reduce extracellular $^{45}Ca^{2+}$ prevented any dibucaine-induced influx of $^{45}Ca^{2+}$ during the final centrifugation. Concurrent assays for cellular ^{45}Ca by the standard (no dibucaine) Pasteur pipette centrifugation showed that 88% of the original cellular ^{45}Ca was retained throughout the entire procedure. Detached cilia could be recovered from the upper portion of the dibucaine-containing

Ficoll layer by dilution and centrifugation at higher speeds.

The residual amount of dibucaine retained by deciliated or mock-deciliated cells was determined by extracting the cells with chloroform/methanol (1 : 2, v/v) according to the procedure of Bligh and Dyer [7]. The concentration of dibucaine in the resulting extracts, which also contained cellular lipids, was estimated by visual comparison with known quantities of dibucaine on Silica gel G thin-layer chromatographic plates developed in the solvent system chloroform/methanol/water (95 : 35 : 4, v/v) and then visualized by exposure to I_2 vapors.

The volume of *Tetrahymena* cells was calculated from measurements made on cells freshly killed with 1% formaldehyde and examined microscopically with an ocular micrometer. The cells were considered to be prolate spheroids. Calmodulin was quantified by courtesy of J.G. Chafouleas and A.R. Means, using a radioimmunoassay technique [28].

Results

Intracellular calcium levels

The proteose peptone medium utilized for *Tetrahymena* culture was determined to be 0.2 mM in Ca^{2+}. Cells grown for 44 h in 10 ml of this medium supplemented with 167 μCi $^{45}Ca^{2+}$ were assayed for internal ^{45}Ca after rapid centrifugation through a Ficoll gradient (see Materials and Methods). The total calcium content of these logarithmic phase cells was $3.7 \cdot 10^{-7}$ μg Ca/cell. Reassay of the same culture 20 h later, when the cells had entered stationary phase, gave a value of $3.8 \cdot 10^{-7}$ μg Ca/cell. Thus under these culture conditions there was little change in the cellular calcium level during active growth.

Based on a volume measurement of $1.8 \cdot 10^{-8}$ ml/cell, the calcium content of the cells would be equivalent to a $5 \cdot 10^{-4}$ M concentration if the calcium were all evenly distributed in the cell contents. In several other experiments, the values ranged as high as $7.5 \cdot 10^{-4}$ M Ca. This amounts to a 2.5—3.75-fold enrichment over the level in the medium. However, it is clear from previous work [8—10] that much of the cellular calcium is sequestered in various storage sites throughout the cell. Evidence has been presented that the free Ca^{2+} concentration in cytoplasm of the larger but closely related ciliate *Paramecium* is 10^{-6} M or lower [11].

Intracellular distribution of calcium

In addition to calcium deposits in mitochondria and dense cytoplasmic granules [8,9], ultrastructural observations [10,12,13] have revealed the presence of major Ca^{2+} binding sites on the cytoplasmic surface of the ciliary membranes at a point just above their union with the cell body. Because these sites have been invoked as potential regulatory centers for rapid changes in ciliary movement, we wished to determine the percentage of whole cell calcium in detached cilia. This is not a straightforward measurement to make because the concentration of calcium is unlikely to remain at its in vivo value in cilia and deciliated cells recovered after deciliation by the rather lengthy procedures normally employed [14]. For a more rapid analysis we utilized a modification

of the dibucaine deciliation of Thompson et al. [15].

In order to reduce the time needed for recovery of cilia and deciliated cells, treatment of the cells with dibucaine was carried out by layering cells prelabeled in $^{45}Ca^{2+}$-medium directly into a sealed-tip Pasteur pipette containing a Ficoll solution. As described in Materials and Methods, the lower portion of the Ficoll was 3 mM in dibucaine. The ^{45}Ca-containing cells were then centrifuged through the gradient, first freeing them from extracellular $^{45}Ca^{2+}$ in the upper part of the gradient and then removing the cilia before pelleting the deciliated cells, all in an elapsed time of less than 4 min. Failure to displace all extracellular $^{45}Ca^{2+}$ prior to exposure of the cells to dibucaine was demonstrated to cause $^{45}Ca^{2+}$ influx.

The efficiency of deciliation using this modification was 75%, as measured in a separate experiment by comparing the recovery of ^{14}C-labeled lipids from cilia of long-term [^{14}C]palmitate-labeled cells with the recovery (2% of total lipid) achieved using the standard dibucaine technique [15], observed by phase microscopy to remove virtually all somatic cilia. When the rapid deciliation procedure was applied in two separate experiments to cells grown for 18 h (six cell generations) in the presence of $^{45}Ca^{2+}$, loss of cilia was accompanied by a 25% reduction in cell-associated ^{45}Ca. Considering that the deciliation process used for this analysis is only 75% effective, the real proportion of the cellular calcium contained in cilia is nearer to 30%.

Dynamics of Ca^{2+} flux

As indicated above, the intracellular calcium of *Tetrahymena* is apparently present in a small, ionic pool and in one or more non-ionic pools, probably consisting of insoluble salts. The extracellular pool of Ca^{2+} is linked to the large, presumably non-ionic intracellular calcium pool(s) by the small cytosolic pool of ionic calcium. The accessibility of the two former reservoirs for modulating the Ca^{2+} level in the latter, physiologically active Ca^{2+} pool, has been measured.

Fig. 1 shows a typical pattern of $^{45}Ca^{2+}$ uptake by cells growing in enriched proteose peptone medium. There is an extremely rapid uptake of $^{45}Ca^{2+}$ within the first 5 min, followed by a slower rise to plateau at $3—4 \cdot 10^{-7}$ μg Ca/cell. It has not been possible to discern whether uptake during the initial 5-min period is due solely to equilibration with a readily available cytoplasmic Ca^{2+} pool or whether it represents $^{45}Ca^{2+}$ binding to the cell surface. Because the ^{45}Ca measurement is not reduced by centrifugation through unlabeled Ca^{2+}- or La^{3+}-containing gradients [4] and because sufficient monovalent cations are present in the medium to discourage appreciable surface binding of $^{45}Ca^{2+}$ [16], we favor the former explanation.

A different type of experiment strengthens the above conclusion. Cells were centrifuged through a Ficoll gradient containing dibucaine in its lower segment, as described in an earlier paragraph. But in this case prior exposure of the cells to $^{45}Ca^{2+}$ was limited to a period of 10 min. The deciliated cells retained 91% of the total $^{45}Ca^{2+}$ measured in non-deciliated cells centrifuged through a dibucaine-free Ficoll at the same time. Thus after its entry, much of the Ca^{2+} moves directly into the cell body without being initially detained at the high affinity sites near the base of the cilia.

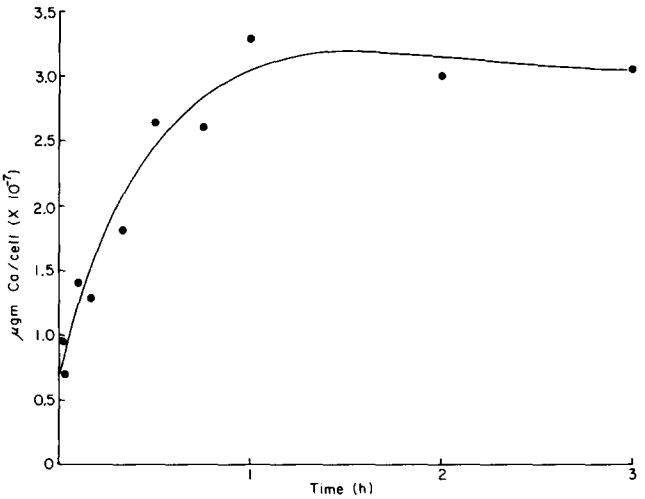

Fig. 1. The uptake of $^{45}Ca^{2+}$ by *Tetrahymena* cells in the logarithmic phase of growth. At time 0 $^{45}Ca^{2+}$ (5 μCi/ml) was added to enriched proteose peptone cultures having a density of approx. 10^5 cells/ml. At the indicated times, aliquots were assayed by the rapid centrifugation technique described in Materials and Methods. The results are averages of three experiments.

The dynamics of cellular calcium was also investigated by measuring the efflux of $^{45}Ca^{2+}$ from the prelabeled cells transferred to unlabeled proteose peptone medium (Fig. 2). In these cells, the efflux was rapid for the first half hour and then slowly approached an equilibrium with the surrounding medium. As in the $^{45}Ca^{2+}$ influx experiments (Fig. 1), some uncertainty surrounds the quantification of rapid Ca^{2+} loss at the early time intervals because an unknown amount of Ca^{2+} was removed from the cells during the wash in unlabeled proteose peptone prior to resuspension and sampling. However, it seems clear from the efflux studies as well as the influx experiments that the half-time ($t_{½}$) for Ca exchange is less than 0.5 h.

A number of variations of the above experiments were carried out. For example, ^{45}Ca-prelabeled cells washed and resuspended in a calcium-free inorganic medium [6] sustained a rapid loss of $^{45}Ca^{2+}$ as in Fig. 2 but reached equilibrium with the medium after extruding approx. 50% of the original cellular calcium. In the calcium-free inorganic medium diluted 1 : 99 with H_2O and supplemented with 1 mM EGTA and 0.25 mM $MgCl_2$, the rate of ^{45}Ca loss over a 6-h period was intermediate between that observed in EGTA-free inorganic medium and proteose peptone.

The cellular site of Ca^{2+} uptake

Various authors have presented evidence that the major and perhaps sole site for Ca^{2+} entry in ciliates is located in the ciliary membranes [17,18]. Using the Ficoll centrifugation technique described above, we have reinvestigated this problem.

Cells in the late logarithmic phase of growth were deciliated in enriched proteose peptone medium by the unmodified method of Thompson et al.

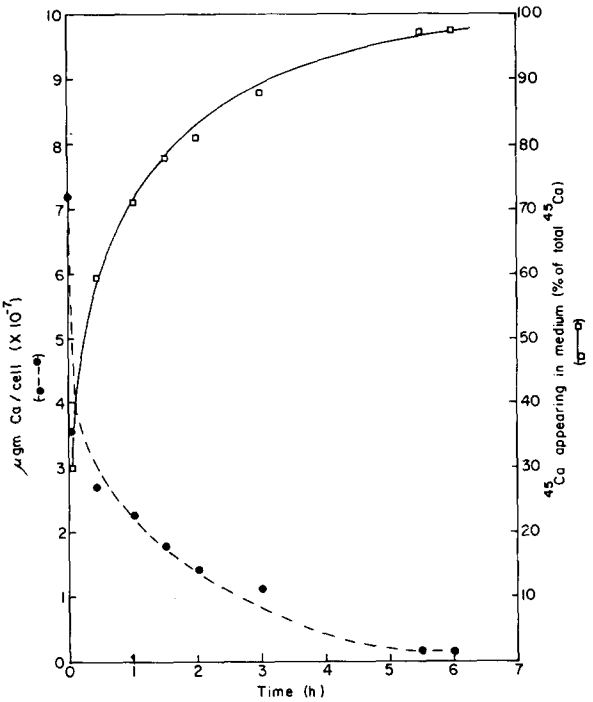

Fig. 2. The efflux of $^{45}Ca^{2+}$ from *Tetrahymena* cells in the logarithmic phase of growth. Cells were prelabeled by growth for 12—18 h in enriched proteose peptone containing 5 μCi $^{45}Ca^{2+}$/ml. When the cells had reached a density of approx. $1.2 \cdot 10^5$ cells/ml, they were quickly washed and resuspended in non-radioactive proteose peptone. At the indicated intervals after resuspension (0 time), aliquots were assayed as in Fig. 1 for whole-cell ^{45}Ca. Aliquots of cell suspension were also centrifuged at 150 × *g* for 4 min followed by sampling of the supernatant for ^{45}Ca analysis. Points are the averages of two experiments.

[15]. The deciliated cells were washed two times with fresh medium to remove most of the dibucaine and then resuspended in enriched proteose peptone medium at a density of 10^6 cells/ml.

After incubating the resuspended cells for 5 min, measurement of $^{45}Ca^{2+}$ uptake was begun. A 0.75-ml aliquot of cell suspension was treated with 15 μl (55 μCi) of $^{45}Ca^{2+}$. At 5 and 10 min after the $^{45}Ca^{2+}$ addition, triplicate 0.1-ml samples were centrifuged through cold Ficoll gradients in Pasteur pipettes. Additional aliquots were tested for $^{45}Ca^{2+}$ uptake at later times as indicated in Fig. 3. Concurrently, freshly killed cells were compared by phase microscopy with non-deciliated controls in order to estimate the extent of cilia regrowth.

At the beginning of the sampling of $^{45}Ca^{2+}$ uptake (0 min, Fig. 3) virtually all cilia, including oral cilia, had been removed from the cells, in agreement with an earlier report [15]. However, the cells were still capable of taking up $^{45}Ca^{2+}$ to the extent of $3.8 \cdot 10^{-8}$ μg/cell by 5 min and $5.5 \cdot 10^{-8}$ μg/cell by 10 min. After the first half hour of reciliation, during which the average length of the growing cilia increased to 30—40% of its normal value (Fig. 3, top) $^{45}Ca^{2+}$ uptake had risen significantly, but continued ciliary regrowth was accompanied by no further enhancement of $^{45}Ca^{2+}$ influx.

Two types of control experiments were performed to confirm the correla-

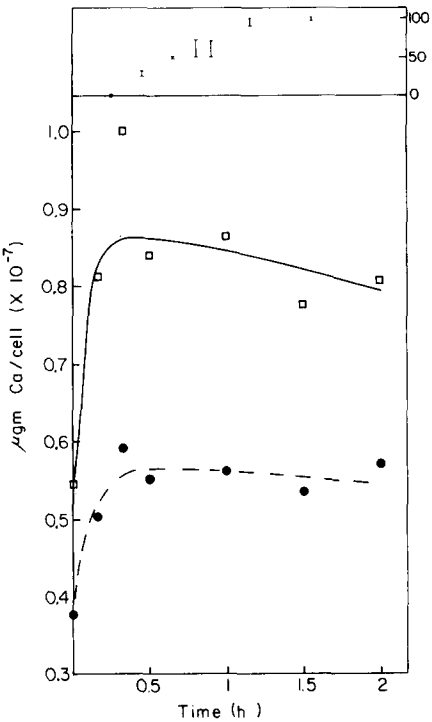

Fig. 3. The uptake of Ca^{2+} by deciliated *Tetrahymena* cells (averaged from two experiments). Cells in the logarithmic phase of growth (2.5—3.0 · 10^5 cells/ml) were deciliated in growth medium by the use of 3 mM dibucaine. The deciliated cells were washed twice in ice-cold proteose peptone (2 X 45 ml) and finally resuspended in the same medium at ambient temperature to give the final cell density of approx. 1 · 10^6 cells/ml. At the indicated intervals, $^{45}Ca^{2+}$ (55 μCi) was added to a 0.75-ml sample of cell suspension. Triplicate 0.1-ml aliquots were assayed 5 (•) and 10 (□) min after radioisotope addition by the rapid centrifugation technique described in Materials and Methods. The upper part of the figure shows the rate of cilia regrowth as gauged visually by phase microscopic examination of cells freshly killed with formaldehyde.

tion between ciliary regrowth and increasing $^{45}Ca^{2+}$ uptake. In one instance, cells were washed with organic medium as described above except that dibucaine was omitted. Uptake of $^{45}Ca^{2+}$ by these fully ciliated cells did not exhibit low initial values followed by a rising trend during the first hour of testing.

The second type of control involved treatment of the cells with a low level of dibucaine, calculated to simulate the effects of any dibucaine remaining in the cells after the post-deciliation washing procedure. Analysis of chloroform/methanol extracts of the deciliated, washed cells had indicated a residual dibucaine content of 30 μg (in approx. 4.5 · 10^7 cells). It was found that an equivalent number of control cells put through the complete washing procedure without dibucaine and then treated with 0.06 mM dibucaine (2% of the level required for efficient deciliation) retained 54 μg dibucaine in their chloroform/methanol extract but exhibited no significant deciliation. This level of 0.06 mM dibucaine was chosen to test whether dibucaine has a direct effect on the cell's permeability for Ca^{2+} that is unrelated to its deciliating action. Cells

placed in the low dibucaine concentration swam normally without the motions indicative of depolarization. The $^{45}Ca^{2+}$ uptake in this control experiment was also constant over the first hour of testing, indicating that the results in Fig. 3 are not simply a response to the trace of residual dibucaine.

The entry of Ca^{2+} through the ciliary membrane and through the plasma membrane could alter a variety of metabolic pathways, both within the cilia proper and in the main body of cellular cytoplasm. Jamieson et al. [26] have recently identified calmodulin, the calcium-dependent regulatory protein, in both the above-mentioned compartments of *Tetrahymena*. By use of a radioimmunoassay technique [28] kindly performed for us by J.G. Chafouleas and A.R. Means, we have been able to confirm the presence of calmodulin in the NT-1 strain of *Tetrahymena* utilized in the present study. Deciliated cell bodies were found to contain 0.1% of their total protein as calmodulin, and cilia isolated by dibucaine treatment contained an equal or somewhat greater level of this protein.

Discussion

There is increasing recognition that Ca^{2+} plays a crucial role in coordinating diverse aspects of cellular metabolism [1]. Ciliated protozoa offer an interesting and not atypical example of Ca^{2+}-mediated control. Ca^{2+} has been shown to be the principal cation responsible for the depolarization-induced reversal of the ciliary beat in *Paramecium* [3], and Ca^{2+} is also known to initiate the secretion of epinephrine by *Tetrahymena*, leading to an elevated cyclic-AMP level [4]. A number of other physiological processes in ciliates, ranging from food vacuole formation [19] to sexual conjugation [20], appear to be controlled at least to some extent by Ca^{2+}.

Although the ciliated protozoan is a simple organism, it is at the same time a very complex cell. With respect to its calcium metabolism, there have been reported to be multiple intracellular pools and very specifically localized Ca^{2+} entry sites at the cell surface. In order to proceed logically towards an understanding of calcium as a regulatory factor in these organisms, it has become necessary to obtain more information regarding the dynamics of cytoplasmic Ca^{2+} as it interacts with pools of storage calcium within the cell and available calcium outside the cell.

Much is already known regarding Ca^{2+} fluxes, particularly in *Paramecium*. Since a great deal of the basic experimentation has been electrophysiological in approach, we have considerable data regarding Ca^{2+} fluxes over a time scale of seconds during and after membrane depolarization [3,21]. Less information is available for intervals of minutes to a few hours. One very interesting study covering this time range was published by Browning and Nelson [22]. These workers confirmed that Ca^{2+} efflux in *Paramecium* is mediated by an active process while influx is passive. They also found rather large intracellular stores of calcium, in agreement with our observations in *Tetrahymena*.

The principal aim of the present investigation was to determine the size and the accessibility of the pools of calcium available for utilization by *Tetrahymena* over the period of approximately an hour, during which most stimuli which affect Ca^{2+}-mediated control over metabolism exert their action. We first

examined the size of the intracellular calcium storage depots. These have been identified by Munk and Rosenberg [8] as being aggregates of calcium and magnesium pyrophosphates. The salts form as large granules in stationary phase cells or cells grown under nutritionally adverse conditions. However, in logarithmic or early stationary phase cell granules are less important, and the major intracellular deposits of calcium salts probably reside in mitochondria [9]. Apart from these deposits, several other cellular sites having strong affinities for Ca^{2+} have been located near the basal region of ciliary membranes and at other points in the cell cortex [10,12,13]. We found a corrected value of approx. 30% of cellular calcium to be removed from the cell by a rapid deciliation. Based on the known distribution of calcium binding sites in the cortex, it would seem that most of this was present in cilia, although the discharge of some Ca^{2+} from mucocysts cannot be rigorously excluded.

Our findings with respect to the equilibrium of extracellular $^{45}Ca^{2+}$ with intracellular calcium (Figs. 1 and 2) prove that at least all the quantitatively significant intracellular pools of calcium equilibrate quite rapidly with external Ca^{2+} and, by inference, with the small intracellular ionic pool of calcium. It is clear that some type of sensitive control must be in effect so as to maintain most of the intracellular calcium in a readily available yet non-ionic form.

The other major source of calcium for use in modulating the low ($<10^{-6}$ M) cytoplasmic Ca^{2+} concentration is that located in the extracellular environment. The standard medium used for *Tetrahymena* culture is $2 \cdot 10^{-4}$ M in Ca^{2+}. Dunlap [18] and Ogura and Takahashi [17] have provided evidence that in *Paramecium* membrane depolarization triggers an influx of Ca^{2+} through gates situated just above the base of the cilia. Cells deciliated by a lengthy incubation in chloral hydrate did not exhibit normal Ca^{2+}-dependent electrical and behavioral properties. These properties reappeared in parallel with the regrowth of cilia. Based on a different experimental approach, Satir and Oberg [23] proposed that Ca^{2+} enters the *Paramecium* cell at yet another site on the cortex, namely at the position where a 'rosette' of integral membrane proteins interacts with maturing trichocysts prior to their discharge.

Our experiments confirm and extend the finding of ciliary involvement in Ca^{2+} uptake. But they establish that Ca^{2+} influx by this pathway accounts for no more than 35—40% of Ca^{2+} entering the cell under non-depolarizing conditions. This cilia-mediated entry is restored when cilia regrowth is only partially complete, confirming that the basal structures are the only portions of the cilia essential for this Ca^{2+} influx.

Depolarization of the plasma membrane would be expected to enhance the influx of Ca^{2+} through the ciliary gates [17,18]. In findings published elsewhere [4], our laboratory has shown that depolarization triggered by the sudden uptake of glucose actually stimulates Ca^{2+} uptake by only about 25%. We have not yet tested other depolarizing stimuli.

Eckert [3] calculated that the amount of Ca^{2+} entering a *Paramecium* cell during a depolarization-induced calcium response would be sufficient to raise the Ca^{2+} concentration in cilia by an increment of at least 10^{-6} M. While it may well be true that depolarization results in a dramatic and almost instantaneous rise in ciliary Ca^{2+}, the deciliation experiments described above show that the equilibration of ciliary $^{45}Ca^{2+}$ with the bulk of calcium in the cell is complete

by as few as 10 min. Thus while it might seem feasible for the mechanism of Ca^{2+}-mediated ciliary reversal to involve a virtually closed system in which extracellular Ca^{2+} and intraciliary, non-ionic calcium stores are utilized for controlling the Ca^{2+} concentration in the cilia, the evidence suggests that the entire cellular pool of calcium may be intimately involved in events lasting longer than a few milliseconds.

Relatively little firm evidence has been found that would link Ca^{2+} to ciliate physiological processes other than those involving depolarization of the surface membranes. However, the calcium-dependent regulator protein (calmodulin) [24], recently identified in *Tetrahymena* by Suzuki et al. [25] has now been confirmed by Jamieson et al. [26] and also in the present report as a significant component of both isolated *Tetrahymena* cilia and deciliated cell bodies. Through the action of calmodulin, even a modest rise in cytoplasmic Ca^{2+} might trigger a variety of intracellular processes.

As the importance of metabolic pathways subject to control by Ca^{2+} becomes more widely appreciated, the need for information regarding the distribution of cellular calcium increases. The ready availability of the non-ionic calcium stores within *Tetrahymena* indicates that this large reservoir may in some cases play a more important regulatory role than Ca^{2+} entering the cell from its surroundings. The participation of these calcium reserves could be especially important in relatively slow processes in ciliates, such as chemotaxis [27] and the secretion and action of hormones [4]. By selectively depleting either the internal or the external calcium supply, it may be possible to establish whether these pools serve different purposes.

Acknowledgments

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