

Regulation of Anaphase Chromosome Motion in *Tradescantia* Stamen Hair Cells by Calcium and Related Signaling Agents

D. H. Zhang, D. A. Callaham, and P. K. Hepler

Department of Botany, University of Massachusetts, Amherst, Massachusetts 01003

Abstract. Several lines of evidence support the idea that increases in the intracellular free calcium concentration ($[Ca^{2+}]_i$) regulate chromosome motion. To directly test this we have iontophoretically injected Ca^{2+} or related signaling agents into *Tradescantia* stamen hair cells during anaphase and measured their effect on chromosome motion and on the Ca^{2+} levels. Ca^{2+} at (+)1 nA for 10 s ($\sim 1 \mu M$) causes a transient (20 s) twofold increase in the rate of chromosome motion, while at higher levels it slows or completely stops motion. Ca^{2+} buffers, EGTA, and 5,5'-dibromo-1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid, which transiently suppress the ion level, also momentarily stop motion. Injection of K^+ , Cl^- , or Mg^{2+} , as controls, have no effect on motion. The injection of $GTP\gamma S$, and to a lesser extent GTP, enhances motion similarly to a low level of Ca^{2+} . However, inositol 1,4,5-trisphosphate, $ATP\gamma S$, ATP, and $GDP\beta S$ have no effect.

Measurement of the $[Ca^{2+}]_i$ with indo-1 reveals that the direct injections of Ca^{2+} produce the expected increases. $GTP\gamma S$, on the other hand, causes only a small $[Ca^{2+}]_i$ rise, which by itself is insufficient to increase the rate of chromosome motion. Further studies reveal that any negative ion injection, presumably through hyperpolarization of the membrane potential, generates a similar small pulse of Ca^{2+} , yet these agents have no effect on motion. Two major conclusions from these studies are as follows. (a) Increased $[Ca^{2+}]_i$ can enhance the rate of motion, if administered in a narrow physiological window around $1 \mu M$; concentrations above $1 \mu M$ or below the physiological resting level will slow or stop chromosomes. (b) $GTP\gamma S$ enhances motion by a mechanism that does not cause a sustained uniform rise of $[Ca^{2+}]_i$ in the spindle; this effect may be mediated through very localized $[Ca^{2+}]_i$ changes or Ca^{2+} -independent effectors.

RECENT evidence indicates that the intracellular free calcium concentration ($[Ca^{2+}]_i$)¹ increases during anaphase in dividing stamen hair cells of *Tradescantia* (Hepler and Callaham, 1987; Hepler, 1989b). The increase, which begins after the initial separation of the sister chromosomes marking the onset of anaphase, attains a peak 10–15 min later when the chromosomes reach the pole and then returns to basal levels at the onset of cytokinesis. The rising phase shows a remarkably close temporal correlation with the time when the chromosomes are moving to the poles and therefore supports the idea that the ion might regulate chromosome motion during anaphase.

One likely way in which Ca^{2+} could modulate the rate of chromosome motion would be through a facilitation of spindle microtubule (MT) depolymerization (Weisenberg, 1972;

Olmsted and Borisy, 1975; Salmon and Segall, 1980; Kiehart, 1981; Cande, 1981). Most agree that the breakdown of MTs, if not the motor for motion, is at least the governor (Salmon, 1975, 1989; Nicklas, 1975, 1987). Thus physiological processes that enhance the depolymerization of the spindle MTs during anaphase A, i.e., movement of chromosomes to the poles, would be expected to increase the rate of motion. But it is also quite possible that increases in $[Ca^{2+}]_i$ could affect chromosome motion by processes other than facilitated MT disassembly; for example, Ca^{2+} could activate a mechanochemical motor such as dynein (Otter, 1989; McIntosh, 1989; McIntosh and Porter, 1989; Hirano-Ohnishi and Watanabe, 1989) or kinesin. Alternatively, Ca^{2+} may facilitate the solation of the spindle cytoplasm through the activation of a Ca^{2+} -dependent actin binding protein (Stossel et al., 1985; Yin, 1988) and thereby enhance the movement of chromosomes.

Despite the general acceptance of the idea that Ca^{2+} might modulate the motion of chromosomes during anaphase, there is surprisingly little direct information on this problem. It is well known that an elevated $[Ca^{2+}]_i$ facilitates the depolymerization of spindle MTs both in vitro (Salmon and Segall, 1980) and in vivo (Kiehart, 1981). However, there

The results herein were presented in preliminary form at the Joint Meeting of the American Society for Cell Biology and American Society for Biochemistry and Molecular Biology in January, 1989.

1. *Abbreviations used in this paper:* BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid; $[Ca^{2+}]_i$, intracellular free calcium concentration; DAG, 1,2-diacylglycerol; G-protein, guanine nucleotide binding protein; IP₃, inositol 1,4,5-trisphosphate; MT, microtubule.

appears to be only one study in which changes in the level of Ca^{2+} have been reported to modulate the rate of chromosome motion. Using permeabilized PtK cells, Cande (1981) increased the $[\text{Ca}^{2+}]_i$ from 0.1 to 0.5 μM in the lysis buffer and observed a brief acceleration in chromosome-to-pole motion followed by an immediate cessation in movement.

To expand our understanding of the control of chromosome motion in living cells, we have microinjected the ion or related signaling agents into stamen hair cells of *Tradescantia* during anaphase. We have carefully correlated the time and magnitude of the ion injection with the change in rate of motion. In parallel studies we have independently measured the level of Ca^{2+} . The results reported herein show that increases in $[\text{Ca}^{2+}]_i$, within a limited physiological window, can stimulate chromosome motion twofold. If the $[\text{Ca}^{2+}]_i$ is raised above this range or lowered below resting levels, chromosome motion will slow or stop. The signaling agent $\text{GTP}\gamma\text{S}$, on the other hand, accelerates the rate of motion without elevating the detected $[\text{Ca}^{2+}]_i$ into this physiological window, thus providing evidence for either a very localized $[\text{Ca}^{2+}]_i$ increase that is below the limit of our detection or a Ca^{2+} -independent pathway for the stimulation of chromosome motion.

Materials and Methods

Preparations

Stamens of the flowering plant *Tradescantia virginiana* were isolated from immature flower buds by squeezing them out from the excised base of the buds into a few drops of culture medium containing 5 mM KCl, 0.1 mM CaCl_2 , and 5 mM HEPES at pH 7.0. The anthers were removed and the stamen filaments with attached hairs were transferred into a warm (35°C) thin layer of 1% low temperature gelling agarose (type VII; Sigma Chemical Co., St. Louis, MO) containing 0.02% Triton X-100 in a simple microscope chamber. Stamen hairs were excised from the filaments with a razor and immobilized by hardening the agarose at low temperature (4°C) for 15 s. The preparation was then flooded with the culture medium. For measurements of the rate of chromosome motion, long cylindrical tip cells that had just entered anaphase were selected for microinjection and video microscopy (see Hepler and Callaham, 1987 for more detail). For measurements of $[\text{Ca}^{2+}]_i$, changes associated with injections, tip cells in interphase were largely used, although independent tests were made on dividing cells to confirm the efficacy of using interphase cells (see Measurements of $[\text{Ca}^{2+}]_i$).

Microinjections

Microneedles were pulled from borosilicate glass capillaries on a vertical pipette puller (model 700; David Kopf Instruments, Tujunga, CA) to a tip diameter of $\sim 0.1 \mu\text{m}$. The needle tip was back filled with 20 mM experimental solution by capillarity, and the stem of the needle was loaded by syringe with either the same solution or 100 mM KCl to provide electrical conductivity. Microiontophoresis was conducted using an amplifier (Microprobe System M 707A; World Precision Instruments, Inc., New Haven, CT) with the aid of a micromanipulator (model MO-103R; Narishige Scientific Instrument Laboratories, Tokyo, Japan) for precise microneedle maneuvering. After the onset of anaphase, cells were impaled with an injection needle, usually at the region of the previous metaphase plate (Fig. 1 a) but occasionally at the spindle pole (Fig. 1 b). 5 min after the onset of anaphase, iontophoretic current was initiated. Normally we applied 1–4 nA for 10 s, but other levels were used and are indicated on the graphs.

Measurements of Chromosome Motion

Anaphase cells were observed on a Zeiss IM-35 inverted microscope equipped with Nomarski differential interference contrast optics. Images obtained via a video camera were recorded on a VCR. Normally, 4 min after the onset of anaphase, or 1 min before the initiation of iontophoretic current,

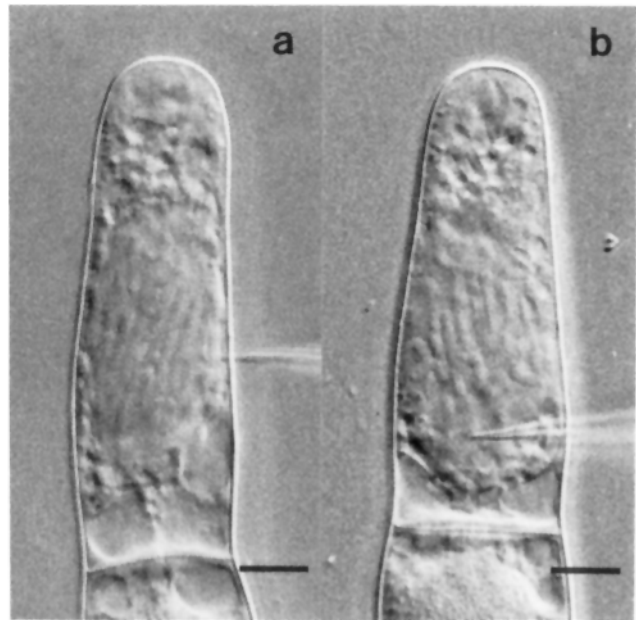


Figure 1. Nomarski DIC image of *Tradescantia* stamen hair cells. After the onset of anaphase, long cylindrical tip cells were impaled with an injection needle for microiontophoresis, usually at the region of the previous metaphase plate (a) but occasionally at the spindle pole (b). Bars, 10 μm .

it is possible to clearly observe and measure the oppositely moving sets of sister chromosomes and for that reason video recordings were begun at that time and were continued for the next 4–5 min. Measurements of chromosome motion were made using a Colorado Video Analyzer 321 (Colorado Video Inc., Boulder, CO) by placing a cursor on the screen and tracing the distance between the two kinetochores of the oppositely moving chromosomes with an average time interval of 3.3 s between readings. The voltage output from the video analyzer upon moving the cursor was recorded by a chart recorder (Microscribe 4500 Recorder; Fisher Scientific, Pittsburgh, PA) and converted into the displacement in micrometers.

Measurements of $[\text{Ca}^{2+}]_i$

To measure the $[\text{Ca}^{2+}]_i$, cells were loaded iontophoretically (Hepler and Callaham, 1987; Callaham and Hepler, 1990) with indo-1 free anion, an $[\text{Ca}^{2+}]_i$ indicator (Grynkiewicz et al., 1985), to a final concentration of 50–100 μM . Unfortunately, the dye tends to accumulate in the vacuole (Hepler and Callaham, 1987; Callaham and Hepler, 1990) and then becomes useless as an indicator of $[\text{Ca}^{2+}]_i$. However, evidence shows that after injection there is a 20–30-min time period during which useful data can be obtained (data not shown). Because of problems with dye sequestration (Hepler and Callaham, 1987; Callaham and Hepler, 1990) we were greatly impeded in our efforts to make the Ca^{2+} measurements in dividing cells, specifically at the 4–5 min point after the onset of anaphase. Since it takes ~ 3 min to complete the injection of indo-1 and another few minutes to align and introduce a pipette containing Ca^{2+} or a signaling agent we were compelled to dye load a late metaphase cell and hope that it would proceed into anaphase without any delay. However, due to variable metaphase transit times, and also because the dye loading process tends to slow metaphase (but not anaphase) progression, we only seldom succeeded in arriving at the 5-min point of anaphase while still within the 20–30-min window post dye loading. For these reasons we normally made these measurements of $[\text{Ca}^{2+}]_i$ on interphase cells wherein we could inject indo-1 and then immediately proceed with the measurement of Ca^{2+} transients that correlate with the subsequent injection of Ca^{2+} or related signaling agents. We have, however, tested our protocol on a few dividing cells and have obtained the same results. We are confident therefore that the Ca^{2+} transients shown herein apply to dividing cells.

In vitro calibration was accomplished by using a small microcuvette (Vitro Microslides; Vitro Dynamics, Inc., Rockaway, NJ) with a fixed path

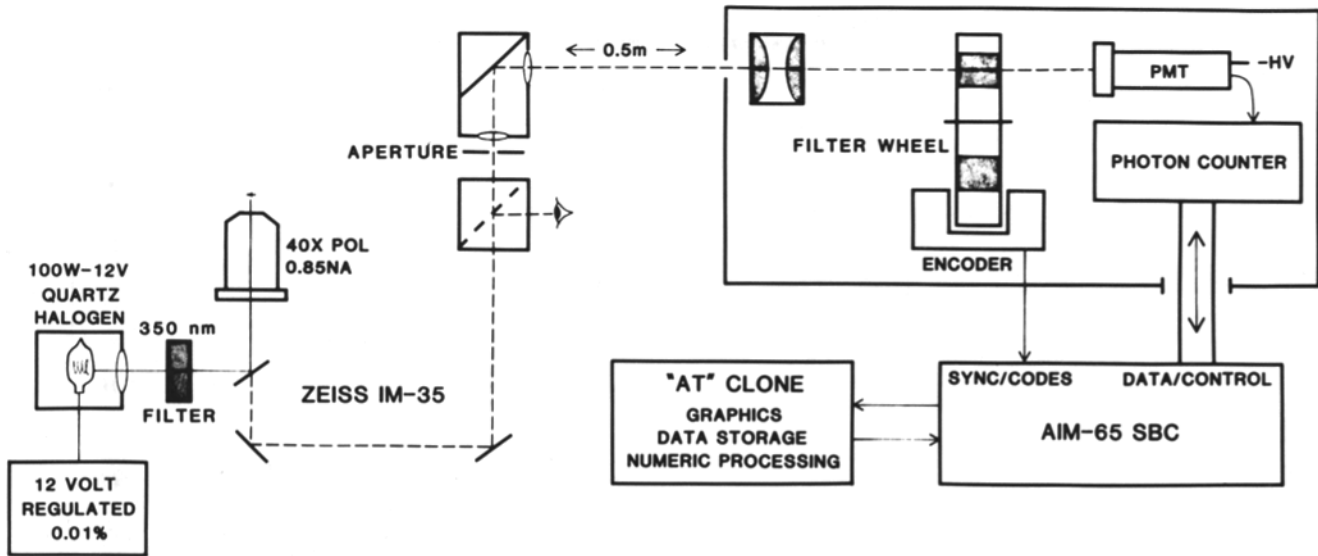


Figure 2. A diagram of the fluorescence microspectrophotometer. 350 nm light from a quartz-halogen lamp (left) passes through a 40 \times pol lens to the object. Fluorescence emission, collected by the lens, travels back through the Zeiss IM-35 microscope and light passing through the field limiting aperture is directed by mirrors to a photon-counting photomultiplier tube (PMT) (right). A spinning wheel in the emission light path alternates between 405 and 490 nm filters. The computer system stores the photon counts and calculates ratios.

length of 50 μm , which is similar to the 25–30- μm path length of a stamen hair cell. Stock solutions of 60 μM indo-1 in a Ca^{2+} -BAPTA buffer, which contain a predetermined free $[\text{Ca}^{2+}]$ and 60% (by weight) sucrose (to adjust for viscosity; Tsien et al., 1985; Poenie, 1990) were perfused through the microcuvettes and the emission change due to the free $[\text{Ca}^{2+}]$ recorded. The measuring device (Fig. 2) consists of a photon-counting photomultiplier to detect fluorescence emission at 405 and 490 nm. Excitation is achieved with a 100 W tungsten-halogen lamp together with a 350-nm (10 nm full width at half max) narrow band filter producing $70 \mu\text{W}\cdot\text{mm}^{-2}$ at the specimen. A spinning filter wheel (1,800 rpm) positioned in the emission light path allows for sampling of the high and low Ca^{2+} wavelengths at 30 cycles per second. The fluorescence ratios reveal the changes in free $[\text{Ca}^{2+}]$.

Results

Anaphase in dividing stamen hair cells of *Tradescantia virginiana* consists almost entirely (>90%) of chromosome-to-pole motion, or anaphase A (Hepler and Palevitz, 1986). In cultured cells, the chromosomes move at a relatively constant rate of 1.0–1.5 $\mu\text{m}/\text{min}$ until they approach the poles, at which point their motion slows for 1–2 min before stopping altogether. The time required for chromosomes to reach the poles is ~ 10 –15 min depending on the rate of motion and the size of the cell. For the best linearity and ease of measurement of chromosome movement, the experiments were performed within the time period of 4–9 min after the onset of anaphase. Fig. 3 shows the rate of chromosome motion plotted out by the displacement of chromosomes vs. time from a typical control cell with no impalement or injection. During the time period of our measurement the chromosomes moved at a constant rate of 1.1 $\mu\text{m}/\text{min}$.

Control Injections

During the course of these studies iontophoretic injections of both positive (+) and negative (–) current have been used to introduce the ions and signaling agents into the cells. Therefore to control against a resulting modulation in chro-

mosome motion being due to a nonspecific effect such as the act of impalement or to the hyperpolarization or depolarization of the membrane potential we conducted a series of injections using K^+ and Cl^- ions at different levels. These studies reveal that injections of either K^+ (Fig. 4) ($[+]\text{I}-4$ nA for 10 s, with 100 mM KCl in the injection needle) or Cl^- (data not shown) ($[-]\text{I}-4$ nA for 10 s, with 100 mM KCl in the injection needle) have no effect on the rate of motion. Similarly, neither K^+ nor Cl^- injections tested at these levels exhibited any inhibition of cytoplasmic streaming, a sen-

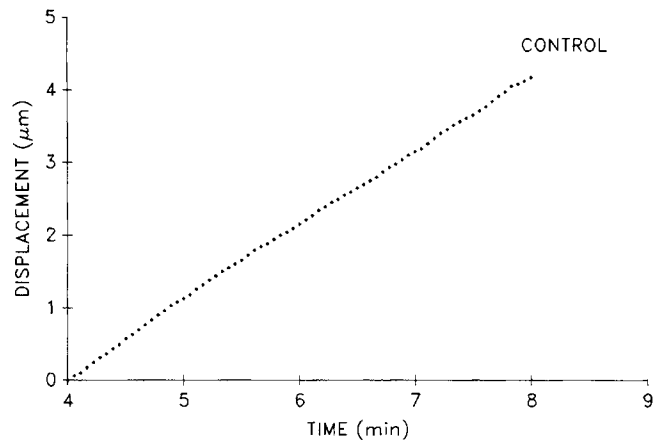


Figure 3. The rate of chromosome motion of an isolated *Tradescantia* stamen hair cell. Chromosome movement was observed on a Nomarski DIC microscope and recorded on a VCR. Measurements of chromosome motion were made using a video analyzer. For the best linearity and ease of observation, the time period of 4–9 min after onset of anaphase was selected for measurement of chromosome movement. In this control cell chromosomes move at a relatively constant rate of 1.1 $\mu\text{m}/\text{min}$ during anaphase.

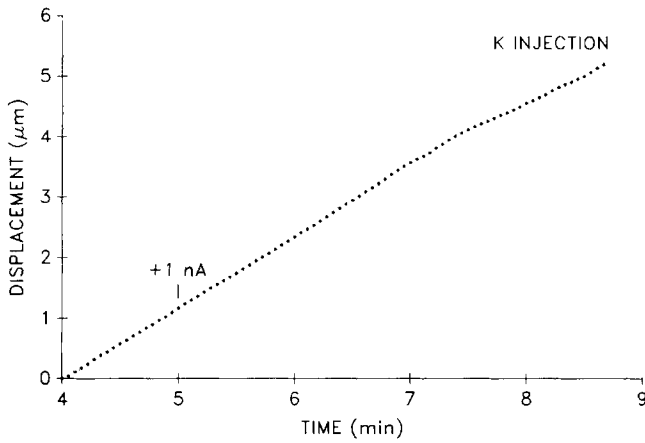


Figure 4. Injection of K^+ has no effect on the rate of chromosome motion. After the onset of anaphase, the cell was impaled with an injection needle containing 100 mM KCl at the region of the previous metaphase plate. 5 min after the onset of anaphase, iontophoretic current was introduced (vertical line) for 10 s (unless specified, all impalements and injections were carried out in this way). Chromosomes move at $1.2 \mu\text{m}/\text{min}$ before and after injection. Up to (+)4 nA (10 s) have been tested and have no effect on the rate of motion.

sitive indicator of $[Ca^{2+}]_i$ in plant cells (Hepler and Wayne, 1985), including nondividing *Tradescantia* stamen hair cells.

Ca^{2+} Injections

A Ca^{2+} injection of (+)1 nA for 10 s with 20 mM $CaCl_2$ and 100 mM KCl in the injection needle, which is just sufficient to inhibit cytoplasmic streaming in nondividing cells and is thus taken to be $\sim 1 \mu\text{M}$ (Shimmen and Tazawa, 1982; Woods et al., 1984), causes a transient increase in the rate of motion from 1.1 to $2.1 \mu\text{m}/\text{min}$ that lasts ~ 20 s before returning to the basal rate (Fig. 5). If the injection is less, for example, (+)0.5 nA for 10 s, then there is no resulting change in the rate of chromosome motion, nor does a similar injection into a nondividing cell cause inhibition of cytoplasmic streaming.

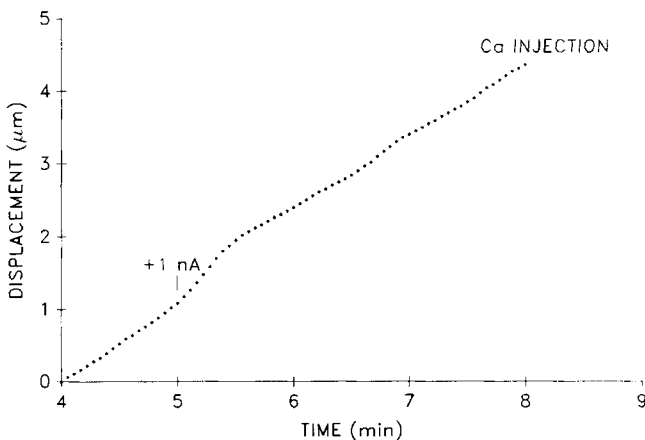


Figure 5. Ca^{2+} at a low level [(+)1 nA, 10 s with 20 mM $CaCl_2$ and 100 mM KCl in the injection needle), ($\sim 1 \mu\text{M}$) causes a transient increase in the rate of motion from 1.1 to $2.1 \mu\text{m}/\text{min}$ that lasts ~ 20 s before returning to the basal rate.

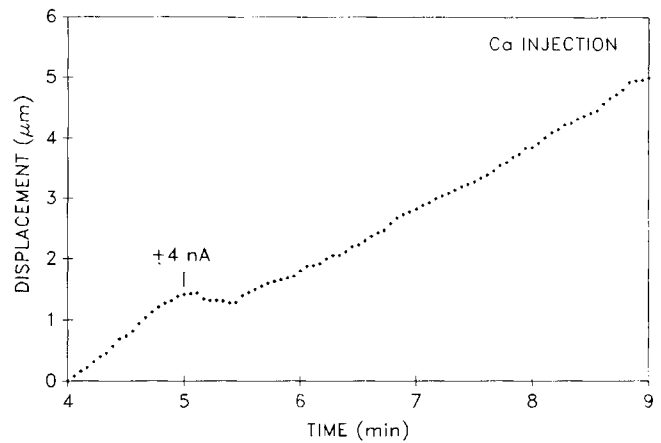


Figure 6. Ca^{2+} at a high level [(+)4 nA, 10 s with 100 mM $CaCl_2$ in the injection needle), ($\sim 10 \mu\text{M}$) causes chromosomes to transiently stop and even regress slightly before resuming normal motion. The inhibition lasts ~ 30 s without noticeable disorganization of the mitotic apparatus.

On the other hand, if a high level of Ca^{2+} [(+)4 nA, 10 s with 100 mM $CaCl_2$ in the injection needle) is injected into the cell, producing $\sim 10 \mu\text{M}$ $[Ca^{2+}]_i$, then the chromosomes transiently stop and even regress slightly before resuming motion (Fig. 6). A careful inspection of cells with the highly elevated levels of Ca^{2+} indicates no particular disorganization of the mitotic apparatus or chromosome pattern. An intermediate level of Ca^{2+} [(+)2 nA, 10 s with 20 mM $CaCl_2$ and 100 mM KCl in the injection needle), $\sim 2 \mu\text{M}$, produces a brief slowing of anaphase motion (Fig. 7). That the modulation of chromosome motion caused by Ca^{2+} injection is not simply due to a nonspecific divalent cation effect is ruled against by control injections of Mg^{2+} [(+)1–4 nA, 10 s with 20 mM $MgCl_2$ and 100 mM KCl in the injection needle); these have no effect on motion (data not shown), nor on cytoplasmic streaming.

The injections described above have all been localized at

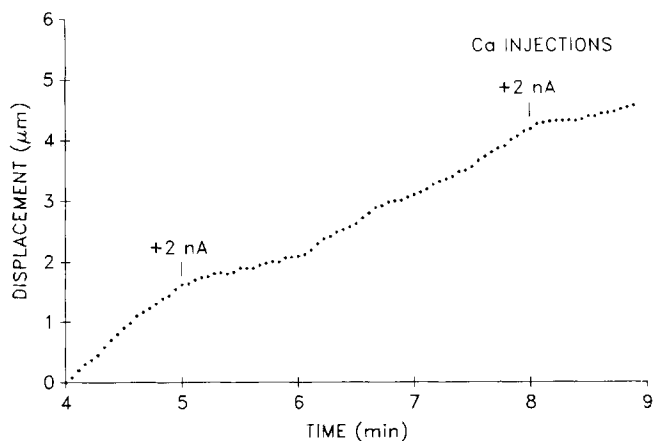


Figure 7. Ca^{2+} at an intermediate level [(+)2 nA, 10 s with 20 mM $CaCl_2$ and 100 mM KCl in the injection needle), ($\sim 2 \mu\text{M}$) causes a brief slowing of chromosome motion. Two injections were made at different times and caused a similar inhibitory effect on anaphase motion.

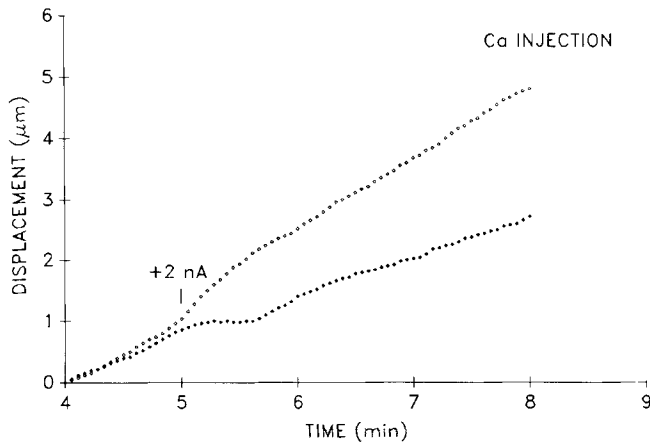


Figure 8. Ca^{2+} at an intermediate level ($[+2]$ nA, 10 s with 20 mM CaCl_2 and 100 mM KCl in the injection needle), ($\sim 2 \mu\text{M}$) injected into a spindle pole region causes an inhibition of motion to the proximal pole (solid diamonds) and a stimulation of motion to the distal pole (open diamonds).

the mid plane of the cell, essentially the zone occupied by the plate of chromosomes at late metaphase. However, if the injection is directed to one of the spindle pole regions, then an intermediate level of Ca^{2+} ($[+2]$ nA, 10 s with 20 mM CaCl_2 and 100 mM KCl in the injection needle) generates a slowing of motion to the proximal pole while simultaneously accelerating the motion to the distal pole (Fig. 8). Presumably due to a diffusion gradient, the proximal pole experiences a high, inhibitory level of Ca^{2+} , whereas the distal pole experiences a concentration that yields an effect similar to a low level of Ca^{2+} injection. Again, in parallel studies on nondividing cells that exhibit active cytoplasmic streaming, we normally find that the injection of Ca^{2+} first causes inhibition of streaming in that region of the cytoplasm close to the pipette tip and only later do we observe these effects on distant parts of the cell, indicating the existence of a concentration gradient across the cell.

To further probe the effect of Ca^{2+} on chromosome mo-

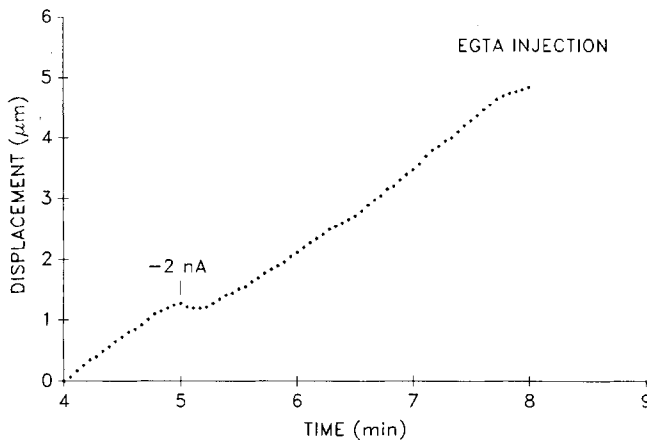


Figure 9. Ca^{2+} -free EGTA ($[-2]$ nA, 10 s with 20 mM EGTA and 100 mM KCl in the injection needle) causes a stoppage and even a slight regression of chromosome motion. The inhibition is transient and the chromosomes return to normal rates in 20–30 s.

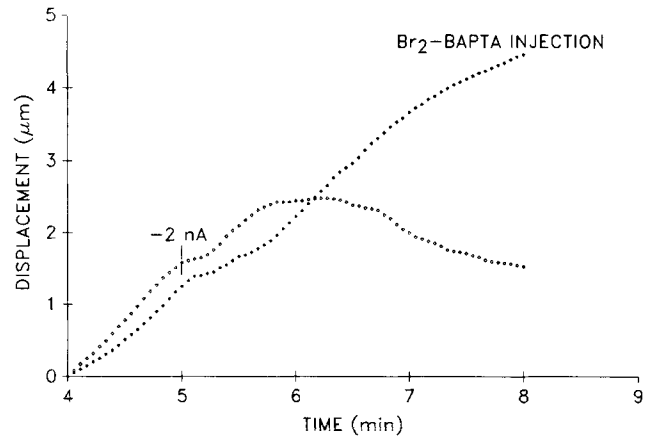


Figure 10. Ca^{2+} -free Br_2 -BAPTA ($[-2]$ nA, 10 s with 20 mM Br_2 -BAPTA and 100 mM KCl in the injection needle) either causes a brief inhibition of chromosome motion (solid diamonds, four out of eight cells studied) or an inhibition of the entire process of anaphase (open diamonds).

tion we have tested the Ca^{2+} buffers, EGTA and 5,5'-dibromo-1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). Injections of Ca^{2+} -free EGTA and Br_2 -BAPTA cause a stoppage of chromosome motion. With EGTA ($[-2]$ nA, 10 s with 20 mM EGTA and 100 mM KCl in the injection needle) the effect is transient and the chromosomes return to normal rates in 20–30 s (Fig. 9); but with Br_2 -BAPTA ($[-2]$ nA, 10 s with 20 mM Br_2 -BAPTA and 100 mM KCl in the injection needle) the inhibition may last for one to several minutes and even prevent cells from completing anaphase (Fig. 10). Br_2 -BAPTA, an EGTA derivative with an estimated intracellular K_d of $4 \mu\text{M}$ (Speksnijder et al., 1989), carries four negative charges in its Ca^{2+} -free form. When bound to Ca^{2+} in a one-to-one molar ratio it still carries two

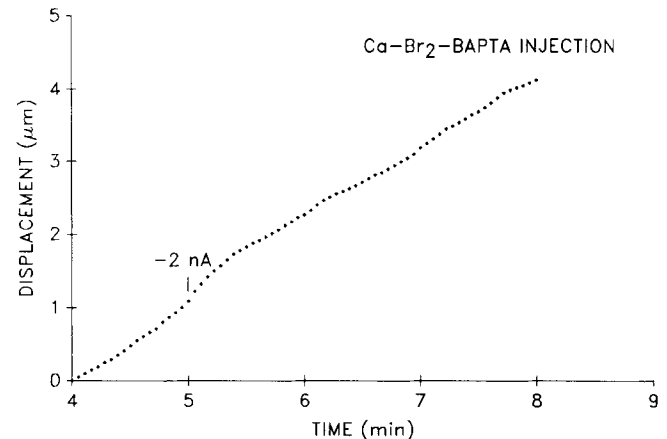


Figure 11. Ca^{2+} -saturated Br_2 -BAPTA ($[-2]$ nA, 10 s with 20–40 mM CaCl_2 , 20 mM Br_2 -BAPTA and 100 mM KCl in the injection needle) causes an increase in chromosome motion similar to that produced by a low level Ca^{2+} injection. Br_2 -BAPTA when bound to Ca^{2+} in a 1:1 molar ratio carries two negative charges and thus can be introduced into the cell with a negative current. Inside the cell the Ca^{2+} dissociates from Br_2 -BAPTA and causes the acceleration of motion.

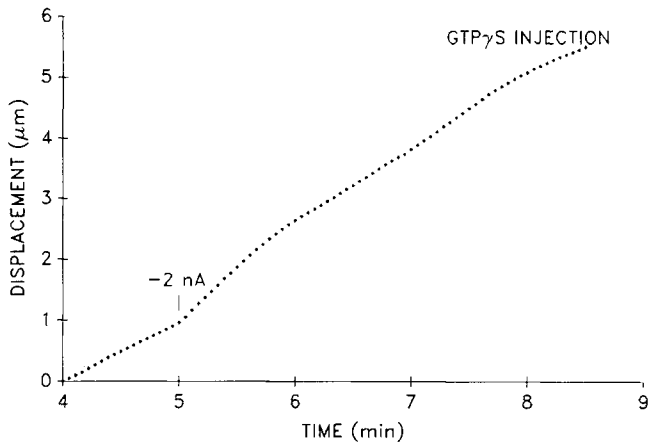


Figure 12. GTP γ S ($[-]2$ nA, 10 s with 20 mM GTP γ S and 100 mM KCl in the injection needle) causes an increase in chromosome motion that is usually longer lasting than that produced by Ca $^{2+}$.

negative charges and thus can be introduced into the cell with a negative current. Once inside the cell, in an environment of low Ca $^{2+}$, the ion dissociates from the buffer, causing a transient increase in the [Ca $^{2+}$]. Fig. 11 shows that injection of Ca $^{2+}$ -saturated Br $_2$ -BAPTA ($[-]2$ nA, 10 s with 20–40 mM CaCl $_2$, 20 mM Br $_2$ -BAPTA, and 100 mM KCl in the injection needle) produces a transient acceleration of motion similar to a low level Ca $^{2+}$ injection. Since only two negative charges are present on each Ca $^{2+}$ -Br $_2$ -BAPTA molecule, roughly twice as much is delivered during the course of the ($-$)2 nA, 10-s injection as when free Br $_2$ -BAPTA is injected. Although the transient rate increase and [Ca $^{2+}$] $_i$ rise are the expected results of Ca $^{2+}$ release from the Ca $^{2+}$ -Br $_2$ -BAPTA, it is not clear why the Br $_2$ -BAPTA left in the cell does not cause a subsequent slowing or even arrest as occurs when free Br $_2$ -BAPTA is injected.

Signaling Agents

There are different signaling agents which may or may not achieve their effect through an ability to mobilize Ca $^{2+}$. Inositol 1,4,5-triphosphate (IP $_3$) has been the focus of much recent attention, and many studies show that it mediates the

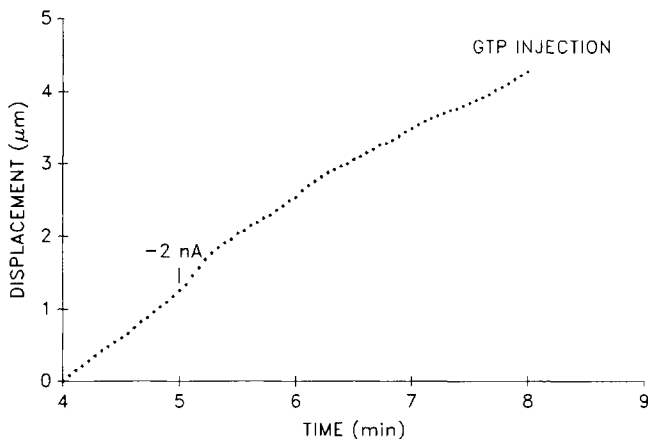


Figure 13. GTP ($[-]2$ nA, 10 s with 20 mM GTP and 100 mM KCl in the injection needle) causes only a brief increase in motion.

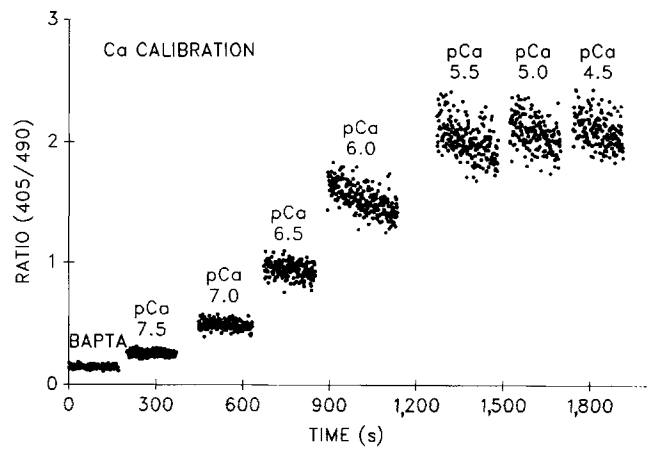


Figure 14. An *in vitro* calibration curve of [Ca $^{2+}$]. Stock solutions of 60 μ M indo-1 in a Ca $^{2+}$ -BAPTA buffer, which contains a predetermined free [Ca $^{2+}$] and 60% sucrose, were perfused through the microcuvettes with a fixed path length of 50 μ m and the emission change due to the free [Ca $^{2+}$] recorded by the fluorescence microspectrophotometer. The curve reveals that the dye responds to changes in [Ca $^{2+}$] between 30 and 3,000 nM [Ca $^{2+}$]. Above 3,000 nM or 3 μ M the response to increases in [Ca $^{2+}$] is much less pronounced and the dye appears to saturate at 10 μ M.

release of Ca $^{2+}$ from an internal store believed to be endoplasmic reticulum (Berridge, 1987; Berridge and Irvine, 1989; Joseph et al., 1984; Gill et al., 1986; Sekar and Hokin, 1986; Majerus et al., 1986). Microinjection of IP $_3$ ($[-]2$ nA, 10 s with 20 mM IP $_3$, 5 mM KCl, and 0.1 mM Hepes pH 7.8 in the injection needle) into anaphase cells however has essentially no effect on the rate of chromosome motion (data not shown). Since we have been puzzled by these results we have tried a variety of different currents and times of injection. However, with the exception of two positives in a total group of 50 cells we have repeatedly failed to cause either an increase or decrease in the rate of motion. We also note that parallel injections on nondividing cells similarly fail to inhibit cytoplasmic streaming. It seems possible that the injected IP $_3$ is rapidly inactivated through dephosphorylation and is thus unable to generate a sufficient [Ca $^{2+}$] $_i$ increase.

Other signaling agents that we have tested are GTP γ S and GTP, the rationale being that they might generate an increase in [Ca $^{2+}$] $_i$ through a reaction cascade that involves the stimulation of a guanine nucleotide binding protein (G-protein), the activation of phospholipase C, and the subsequent hydrolysis of phosphatidyl inositol 4,5-bisphosphate to 1,2-diacylglycerol (DAG) and IP $_3$ (Gilman, 1987). Alternatively Ca $^{2+}$ levels could be modulated by GTP γ S and GTP through the activation of another type of G-protein (Gi) that may directly regulate plasma membrane Ca $^{2+}$ channels (Gomperts, 1983; Yatani et al., 1987; Brown and Birnbaumer, 1988). However, one must realize that GTP γ S and GTP could stimulate processes by mechanisms quite independent of their effect on Ca $^{2+}$ mobilization, for example, through a DAG-dependent activation of protein kinase C and the consequent phosphorylation of key proteins. Regardless of the mechanism of action, the results show clearly that the injection of GTP γ S ($[-]2$ nA, 10 s with 20 mM GTP γ S and 100 mM KCl in the injection needle) produces a prolonged stimulation on the rate of motion (Fig. 12), whereas GTP ($[-]2$ nA,

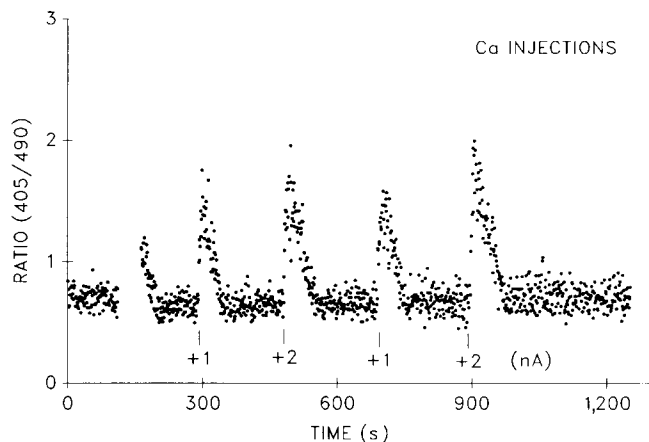


Figure 15. Fluorescence ratio measurements in response to iontophoretic injections of Ca^{2+} . Cells were first loaded iontophoretically with 50–100 μM indo-1, and allowed to settle for ~ 2 min. Subsequently they were impaled with a Ca^{2+} injection needle (20 mM CaCl_2 , 100 mM KCl). The first upward deflection is a wound response due to the influx of Ca^{2+} at the moment of impalement and/or diffusion of Ca^{2+} from the micropipette. Cells usually return to the resting $[\text{Ca}^{2+}]_i$ level within ~ 2 min. Based on the calibration curve (Fig. 14) and the comparisons of different levels of injections as well as their effect on cytoplasmic streaming, a Ca^{2+} injection at (+)1 nA for 10 s (low level) appears sufficient to cause the $[\text{Ca}^{2+}]_i$ to increase from the resting level ($\sim 0.2 \mu\text{M}$) to about 1 μM ; at (+)2 nA for 10 s (intermediate level) the $[\text{Ca}^{2+}]_i$ would be expected to reach 2 μM .

10 s with 20 mM GTP and 100 mM KCl in the injection needle) yields only a brief increase in motion (Fig. 13), possibly due to its rapid metabolic inactivation. Control injections include ATP γ S, ATP, and GDP β S, none of which alters the rate of motion (data not shown).

$[\text{Ca}^{2+}]_i$ Measurements

Changes of $[\text{Ca}^{2+}]_i$ associated with injections were mea-

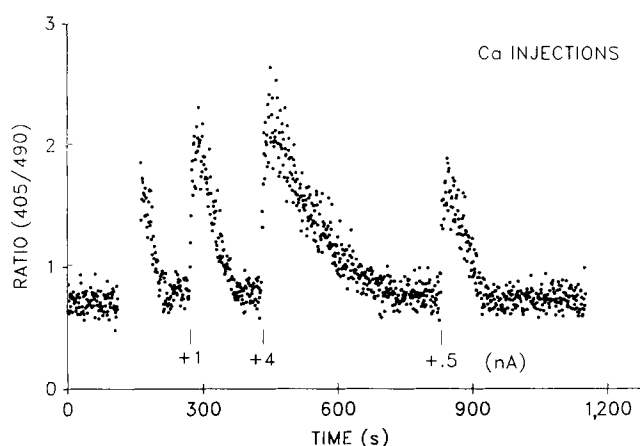


Figure 16. Ca^{2+} injection at the high level [(+)4 nA, 10 s with 100 mM CaCl_2 in the injection needle) has reached $\sim 10 \mu\text{M}$ and takes substantially longer to return to basal level when compared to lower current injections. The higher concentration of CaCl_2 in the absence of KCl in the delivery pipette, and the higher iontophoretic current deliver more Ca^{2+} to the cell.

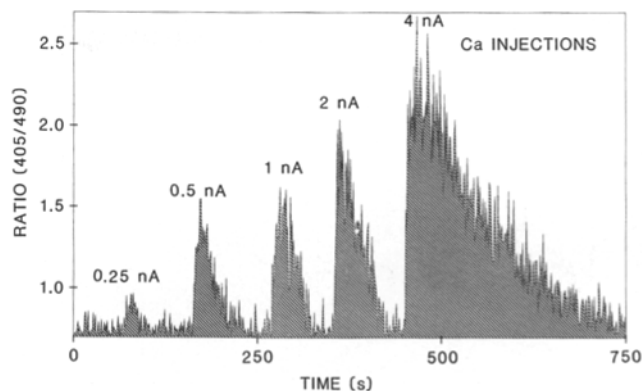


Figure 17. A comparison of different levels of Ca^{2+} injections (with 20 mM CaCl_2 and 100 mM KCl in the injection needle for [+] 0.25 – 2.0 nA injections and 100 mM CaCl_2 in the injection needle for [+] 4.0 nA injection). The graph compares the area of each spike above the resting level. From left to right the relative area of each spike is 1, 4.5, 5.8, 9.5 and 73.9, respectively.

sured using the fluorescent $[\text{Ca}^{2+}]_i$ dye, indo-1. In an effort to assess the sensitivity of the dye and our instrumentation we have calibrated the *in vitro* response of indo-1 under conditions of dye concentration (60 μM), path length (50 μm), and viscosity similar to that encountered in the cells. The resulting measurements reveal clear differences in the fluorescence ratio when the $[\text{Ca}^{2+}]_i$ is modulated with buffers between 30–3,000 nM. Above 3,000 nM or 3 μM the response to increases in $[\text{Ca}^{2+}]_i$ is much less pronounced and the dye appears to saturate at 10 μM (Fig. 14). Thus the instrument and dye levels employed are capable of detecting $[\text{Ca}^{2+}]_i$ changes in the physiological range. The importance of adjusting for cytoplasmic viscosity has been borne out by the work of Tsien et al. (1985), and more recently by Poenie (1990). Moreover, the use of sucrose is recommended because it introduces relatively little Ca^{2+} contamination, and has no apparent effect on the Ca^{2+} -dye affinity (Poenie, 1990).

Fluorescence measurements of cells loaded with 50–100 μM indo-1 reveal pronounced changes in the ratio (405:490

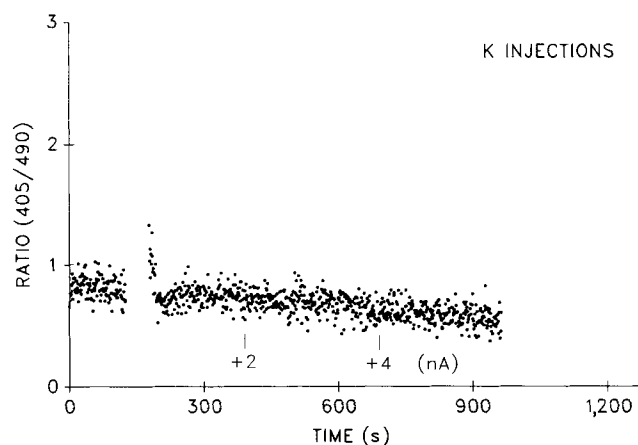


Figure 18. Injection of K^+ [(+)2 and [+] 4 nA, 10 s with 100 mM KCl in the injection needle) has no effect on the fluorescence ratio.

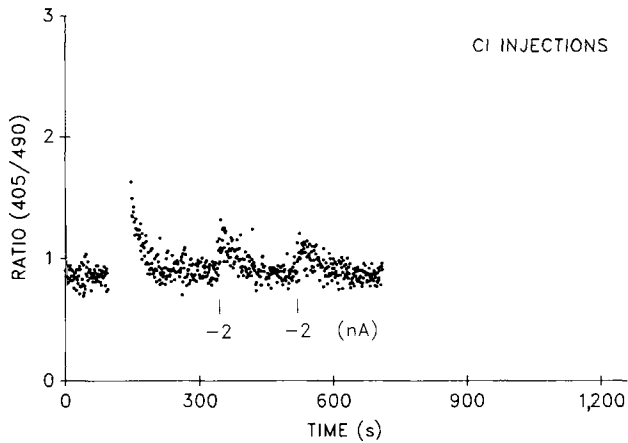


Figure 19. Injection of Cl^- ($[-]2$ nA, 10 s with 100 mM KCl in the injection needle) or any negative ion always causes a small ratio change, indicating that the $[\text{Ca}^{2+}]_i$ has increased. The negative ion effect is presumably due to a hyperpolarization of the membrane potential, which would tend to drive any positive ion, including Ca^{2+} , into the cell. However, these changes are substantially smaller in magnitude than those that have an effect on chromosome motion or on cytoplasmic streaming. They also may be localized in the peripheral cytoplasm at a distance from the Ca^{2+} -sensitive site that modulates chromosome motion.

nm) in response to iontophoretic injections of Ca^{2+} (Figs. 15 and 16). Clear and repeatable differences in the fluorescence ratio result from injections at the various current levels between (+)0.25–4.0 nA (for $[+]$ 0.25–2.0 nA injections, the needles were loaded with 20 mM CaCl_2 and 100 mM KCl; for $[+]$ 4.0 nA injection, the needle was loaded with 100 mM CaCl_2). However, due to a variety of factors that pertain to the performance of the dye in the living cells including dye compartmentation (Poenie et al., 1986; Hepler and Callahan, 1987; Malgaroli et al., 1987; Ratan et al., 1988; Callahan and Hepler, 1990), protein binding (Konishi et al., 1988), dye bleaching (Becker and Fay, 1987), cytoplasmic

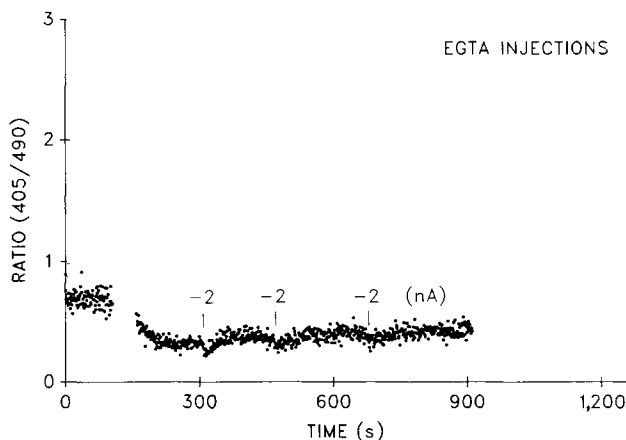


Figure 20. Ca^{2+} -free EGTA ($[-]2$ nA, 10 s with 20 mM EGTA in the injection needle) causes a downward deflection in the fluorescence ratio. Due to the diffusion of the EGTA, the $[\text{Ca}^{2+}]_i$ is buffered to a new resting level (~ 50 nM) even before the initiation of iontophoretic currents.

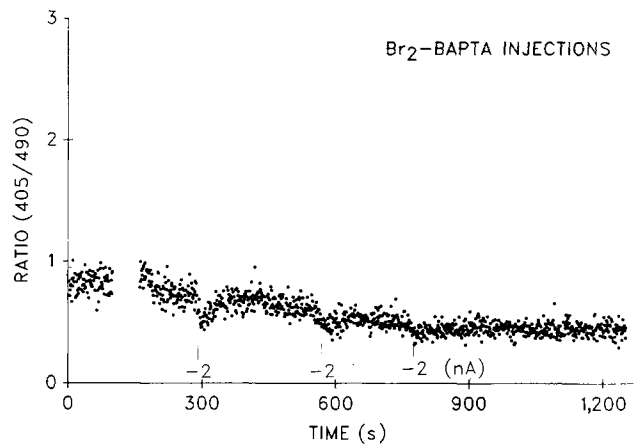


Figure 21. Ca^{2+} -free Br_2 -BAPTA ($[-]2$ nA, 10 s with 20 mM Br_2 -BAPTA in the injection needle) produces a downward deflection in the fluorescence ratio. Like EGTA it also causes the basal level of Ca^{2+} to decline.

viscosity (Tsien et al., 1985), etc., we do not believe that an absolute value for the $[\text{Ca}^{2+}]_i$, based on the calibration obtained from the glass microcuvette, can be determined. Thus we present herein only approximations of the $[\text{Ca}^{2+}]_i$ that are based on comparisons between different peak heights and roughly calibrated on the degree to which a given height $[\text{Ca}^{2+}]_i$ blocks cytoplasmic streaming, which is inhibited in *Nitella* and tomato protoplasts at ~ 1 μM (Shimmen and Tazawa, 1982; Woods et al., 1984). Since a Ca^{2+} injection at $(+)$ 1 nA for 10 s (Fig. 15) is just sufficient to cause incipient inhibition of streaming in nondividing cells, we infer that the $[\text{Ca}^{2+}]_i$ has reached ~ 1 μM . Also when we compare these low level injections with the calibration curve, they yield a value of ~ 0.8 – 1.0 μM ; $(+)$ 2 nA for 10 s would be expected to approach 2 μM . The injections at $(+)$ 4 nA for 10 s (Fig. 16) are thought to be substantially higher (~ 10 μM) because in contrast to the delivery pipette, which in the previous two instances contained 20 mM CaCl_2 and 100

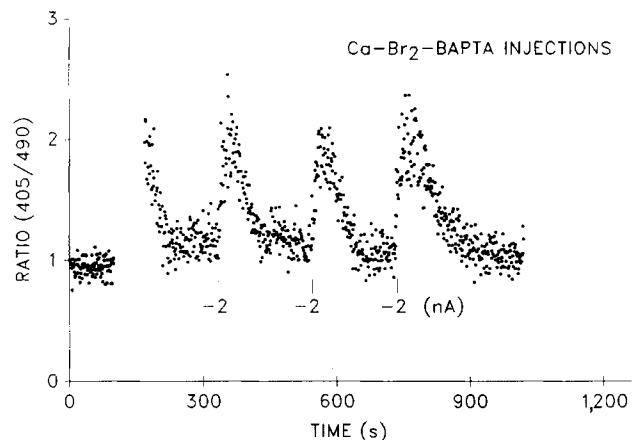


Figure 22. Ca^{2+} -saturated Br_2 -BAPTA ($[-]2$ nA, 10 s with 40 mM CaCl_2 and 20 mM Br_2 -BAPTA in the injection needle) causes an increase in $[\text{Ca}^{2+}]_i$ that appears similar to stimulatory low level Ca^{2+} injection.

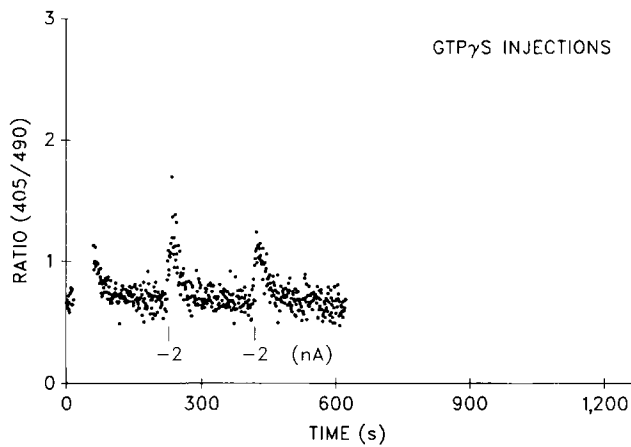


Figure 23. $\text{GTP}\gamma\text{S}$ ($[-]2$ nA, 10 s with 20 mM $\text{GTP}\gamma\text{S}$ in the injection needle) under the same conditions that cause a marked increase in chromosome motion only produces a small increase in the $[\text{Ca}^{2+}]_i$ (~ 0.3 – 0.5 μM).

mM KCl, the (+)4 nA pipette contained 100 mM CaCl_2 . A comparison of different levels of Ca^{2+} injections is also shown in Fig. 17, which compares the area of each spike above the resting level.

Control injections of either K^+ (Fig. 18) or Mg^{2+} (data not shown) have no effect on the fluorescence ratio. However, injection of Cl^- or any negative ion always causes a small but repeatable ratio change, indicating that the $[\text{Ca}^{2+}]_i$ has increased (Fig. 19). The negative ion effect we believe is due to a hyperpolarization of the membrane potential that would increase the electrical charge gradient, tending to drive any positive ion, including Ca^{2+} , into the cell. These changes are substantially smaller in magnitude than those caused by injections of Ca^{2+} that are sufficient to stimulate chromosome motion. In addition it seems likely that these Ca^{2+} increases are localized in the peripheral cytoplasm and are not able to alter the activity in the spindle apparatus. The large upward deflections observed in the initial part of this graph and in several other instances are wound responses due to the influx of Ca^{2+} at the moment of impalement.

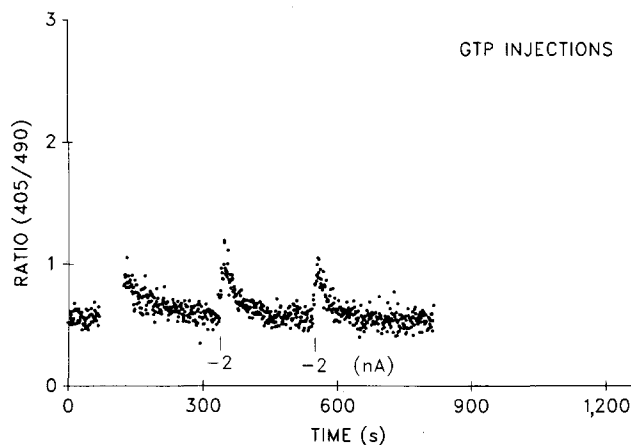


Figure 24. GTP, at a level which stimulates chromosome motion ($[-]2$ nA, 10 s with 20 mM in the injection needle) causes only a small increase in $[\text{Ca}^{2+}]_i$.

Injection of Ca^{2+} -free EGTA and Br_2 -BAPTA causes a downward deflection in the fluorescence ratio (Figs. 20 and 21), while Ca^{2+} -saturated Br_2 -BAPTA causes a brief upward shift in the ratio (Fig. 22). With Ca^{2+} -saturated Br_2 -BAPTA, the increase in $[\text{Ca}^{2+}]_i$ appears similar to a stimulatory low level Ca^{2+} injection. It is pertinent to note that the stimulation of chromosome motion can be achieved by increasing the $[\text{Ca}^{2+}]_i$ to the proper level regardless of whether the ion is introduced through either a positive or negative current injection. These observations weigh against changes in the membrane potential as a controlling factor for chromosome motion. Also notice that with both Ca^{2+} -free EGTA and Br_2 -BAPTA, the $[\text{Ca}^{2+}]_i$ is buffered to a new resting level (~ 50 nM) even before the initiation of iontophoretic currents. Presumably there is diffusion of the buffer into the cell, which lowers the basal level of Ca^{2+} . Iontophoresis of additional buffer into the cell further reduces the $[\text{Ca}^{2+}]_i$, but this is only transient and it recovers within 1 min.

$\text{GTP}\gamma\text{S}$ and GTP, at a level which stimulates chromosome motion, cause only a small $[\text{Ca}^{2+}]_i$ rise (Figs. 23 and 24), which is in the same range as is produced by the injection of any nonactinic anion. Further studies reveal that $\text{ATP}\gamma\text{S}$, ATP , $\text{GDP}\beta\text{S}$, and IP_3 generate similar small $[\text{Ca}^{2+}]_i$ increases, yet these agents have no effect on chromosome motion (data not shown). Interestingly, $\text{GTP}\gamma\text{S}$ does have an effect on cytoplasmic streaming; after injection there appears to be an inhibition of the movement of large particles, with smaller ones continuing motion. By contrast, a level of Ca^{2+} just sufficient to inhibit streaming causes all particles to stop and cytoplasmic clumps to form around the pipette tip. Finally, this level of Ca^{2+} causes a fairly rapid disintegration of the transvacuolar strands near the micropipette tip, whereas $\text{GTP}\gamma\text{S}$ does not.

Table I presents a summary of the effect that the different agents have on chromosome motion, $[\text{Ca}^{2+}]_i$, and cytoplasmic streaming.

Discussion

Calcium

The results show that modulation of the $[\text{Ca}^{2+}]_i$ in the physiological range markedly affects the rate of chromosome motion during anaphase A in dividing stamen hair cells of *Tradescantia*. If the $[\text{Ca}^{2+}]_i$ is increased to the micromolar level, then chromosome motion can be accelerated two-fold above the control rate. If, however, the $[\text{Ca}^{2+}]_i$ is increased beyond a few micromolar then the chromosomes will slow their motion or stop. Similarly, if the $[\text{Ca}^{2+}]_i$ is lowered below the physiological resting level with EGTA or Br_2 -BAPTA chromosome motion can be inhibited. There appears, therefore, to be a good correlation between the movement of chromosomes and the level of Ca^{2+} , with the maximum enhancement being achieved at ~ 1 μM (Table I).

The mechanism by which elevated $[\text{Ca}^{2+}]_i$ facilitates the motion of chromosomes is not known, but one likely way would be through the stimulation of MT depolymerization. Since the process being observed in the stamen hair cells is movement of the chromosomes to the poles, it follows that the chromosomal spindle fibers must shorten. It is well known that increased levels of Ca^{2+} , presumably acting through calmodulin (Vantard et al., 1985; Keith, 1987; Allan

Table I. Summary of the Effect of Different Agents on $[Ca^{2+}]_i$, Chromosome Motion, and Cytoplasmic Streaming

Agent	Concentration in pipette	Current	Approximate $[Ca^{2+}]_i$	Cell number	Effect on motion	Cell number	Effect on streaming	Figure
	<i>mM</i>	<i>nA</i>	<i>μM</i>					
Control	—	—	$\sim .2$	193	—	5	—	15–24
K ⁺	100	(+)1–4	$\sim .2$	15	None	6	None	4, 18
Cl ⁻	100	(-)1–4	$\sim .5$	17	None	6	None	19
Mg ²⁺	20 + 100 KCl	(+)1–4	$\sim .2$	3	None	8	None	—
Ca ²⁺	20 + 100 KCl	(+)1	~ 1	32	Increased	9	Incipient inhibition	5, 15, 17
Ca ²⁺	20 + 100 KCl	(+)2	~ 2	15	Decreased	4	Stopped for ~ 20 s	7, 15, 17
Ca ²⁺	100	(+)4	~ 10	14	Stopped	5	Stopped for ~ 1.5 min	6, 16, 17
EGTA	20 + 100 KCl	(-)2	$\sim .05$	2	Stopped	5	None	9, 20
Br ₂ -B*	20 + 100 KCl	(-)2	$\sim .05$	17	Stopped	8	None	10, 21
Ca ²⁺ -Br ₂ -B*	20 + 100 KCl	(+)2	~ 1	9	Increased	5	Incipient inhibition	11, 22
IP ₃	20 + 5 KCl	(-)1–4	$\sim .5$	10	None	50	None	—
GTP γ S	20 + 100 KCl	(-)2	$\sim .5$	29	Increased	6	Partially stopped	12, 23
GTP	20 + 100 KCl	(-)2	$\sim .5$	7	Increased	6	Incipient partial inhibition	13, 24
ATP γ S	20 + 100 KCl	(-)2	$\sim .5$	17	None	6	None	—
ATP	20 + 100 KCl	(-)2	$\sim .5$	3	None	6	None	—
GDP β S	20 + 100 KCl	(-)2	$\sim .5$	3	None	6	None	—

* B, BAPTA.

and Hepler, 1989; Welsh and Sweet, 1989), stimulate the depolymerization of spindle MTs (Salmon and Segall, 1980; Kiehart, 1981), and thus it seems reasonable that their facilitated breakdown could increase the rate of motion. In making these statements we are unable to specify whether the MT depolymerization is the motor or simply the governor of chromosome motion since our data are consistent with either process. The measured $[Ca^{2+}]_i$ changes correlated with chromosome motion changes also cannot provide information as to the site of calcium action, i.e., the spindle poles or the kinetochores.

Although MT depolymerization is the most obvious way in which Ca²⁺ might act, it is not the only possible mechanism. An elevated $[Ca^{2+}]_i$ could simulate a mechanochemical motor responsible for chromosome motion. The well known ability of Ca²⁺ to activate or modulate a host of motile processes, including those that are caused by MTs as well as microfilaments (Campbell, 1983), provides ample reason for entertaining the idea that the ion has a direct effect on the spindle motor, whatever it is. Alternatively, Ca²⁺ might indirectly control the rate of motion by modulating the gel structure of the spindle apparatus. For example, through the activation of a protein such as gelsolin, a spindle-associated actin network could be fragmented and reduced in viscosity (Stossel et al., 1985; Yin, 1988), thus permitting more rapid movement of chromosomes embedded therein.

An important but somewhat unexpected aspect of our results has been the finding that elevations in the $[Ca^{2+}]_i$ beyond the 1 μM level cause a slowing or complete stoppage of chromosome motion. If elevated $[Ca^{2+}]_i$ facilitates the depolymerization of MTs then one might have predicted either an even faster rate of motion, or a complete disruption of the spindle structure followed by disorganization of the chromosomes. Although we expect that the high $[Ca^{2+}]_i$ depolymerizes the spindle MTs, we nevertheless fail to observe any gross disruption of the mitotic apparatus such as a dispersal or rearrangement of the chromosomes. The elevated $[Ca^{2+}]_i$ thus affects spindle structure quite differently from colchicine (Hepler and Palevitz, 1986) and other anti-MT agents

(Cassimeris et al., 1986; Keifer, A. Q., D. A. Callaham, and P. K. Hepler, manuscript in preparation) that cause noticeable disorganization of the chromosomes.

The inhibition of chromosome motion caused by high $[Ca^{2+}]_i$ has also forced us to rethink our conclusions concerning the effect of energy depletion on anaphase. Previously we argued that it seemed unlikely that either DNP or azide inhibited chromosome motion through Ca²⁺ modulation since the mitotic apparatus did not become disorganized as we had expected it would if the $[Ca^{2+}]_i$ had been sharply elevated (Hepler and Palevitz, 1986). Two observations now show that that conclusion is in error. First, the demonstration that high intracellular levels of Ca²⁺ do not cause the spindle apparatus to become disorganized; indeed it freezes in place just as with azide treatment. Second, recent direct measurements reveal that azide (2–2.5 mM) causes the intracellular level of Ca²⁺ to rise to a level that would inhibit chromosome motion (Keifer, A. Q., D. A. Callaham, and P. K. Hepler, manuscript in preparation). The entire story concerning the role of energy during anaphase will have to be reanalyzed in light of the ability of elevated $[Ca^{2+}]_i$ to inhibit chromosome motion.

Another interesting aspect of the results is the ability of an intermediate level of Ca²⁺, when injected into one spindle pole region, to retard chromosome motion to the proximal pole, while at the same time accelerating motion to the opposite pole. Presumably due to formation of a concentration gradient stemming from the pipette tip, the proximal half spindle experiences a sufficient Ca²⁺ level to inhibit motion while the distal half spindle experiences a lower stimulatory level. These observations underscore the necessity of raising the $[Ca^{2+}]_i$ within only a very limited physiological window in order to achieve acceleration of motion. They also show that it is possible to uncouple chromosome movement in the two half spindles. Previously, modulating agents such as heat (Nicklas, 1979) or UV irradiation (Forer, 1974), when applied to one half spindle also modulated chromosome motion in the other half spindle. Results from these studies gave rise to the idea that the motility processes are coupled. Here

we show that with Ca^{2+} they can be uncoupled, with each half spindle responding independently to the local ion level.

Finally, the results clearly show that briefly lowering the $[\text{Ca}^{2+}]_i$ below the normal physiological level causes an equally brief inhibition in chromosome motion. With EGTA the chromosomes soon resume normal rates of motion and complete anaphase. However, with Br_2 -BAPTA 50% of the cells (four out of eight cells studied) do not complete anaphase, and become permanently arrested. The separating sets of chromosomes may even come together to form a single restitution nucleus. Why do these two Ca^{2+} chelators yield different results, especially when their effects on the $[\text{Ca}^{2+}]_i$ appears to be the same? For example, both agents cause a brief depression in the resting level that quickly returns to normal. It seems possible that part of their difference may be due to the higher molecular mass of Br_2 -BAPTA (950 vs. 350 for EGTA), which would impede its movement between cells (Tucker, 1982), thus causing it to remain at a relatively high concentration in the cell into which it was injected. Using fluorescent dyes of differing molecular masses, for example, Tucker (1982) has shown in stamen hairs of *Setcreasea* that the plasmodesmata exclude molecules >800 – $1,000$ D. Even though Br_2 -BAPTA may only briefly lower the overall $[\text{Ca}^{2+}]_i$, it would still be free to act as a shuttle and dissipate local Ca^{2+} gradients within the cell. These ideas are derived from the recent work of Speksnijder et al. (1989) who find that BAPTA buffers effectively block cell division and polar development in fucoid eggs without killing the cells. Since the most effective buffers are those whose K_d rests approximately in the mid-point of the Ca^{2+} gradient, they argue that the buffer achieves its effect by binding the ion at the high point of the gradient and quickly diffusing to the low point where the ion is released. If this explanation holds for the *Tradescantia* stamen hair system then our results with Br_2 -BAPTA might provide evidence, in a preliminary way, that local gradients in the $[\text{Ca}^{2+}]_i$ are required for anaphase chromosome motion. Indeed, it is attractive to imagine that a specific invaginating ER complex, through controlled ion release, might create conditions of locally high $[\text{Ca}^{2+}]_i$ along the chromosomal spindle fiber or specifically at the kinetochore itself and thus locally regulate the depolymerization of the MTs or the activation of a spindle motor (Hepler, 1989a). These ideas await further experimentation.

Signaling Agents

The observations that $\text{GTP}\gamma\text{S}$ and to a lesser extent GTP enhance chromosome motion, whereas $\text{ATP}\gamma\text{S}$, ATP , and $\text{GDP}\beta\text{S}$ have no effect (Table I), support the idea that G-binding proteins may participate in the regulation of chromosome motion. For example, the activation of a G-protein by $\text{GTP}\gamma\text{S}$ might stimulate a phospholipase C and the subsequent breakdown of phosphatidyl inositol 4,5-bisphosphate to IP_3 and DAG. IP_3 might then cause the release of Ca^{2+} from the ER and thereby stimulate the motile events. However, the injection of IP_3 has no effect on chromosome motion. Moreover, injection of $\text{GTP}\gamma\text{S}$ does not cause a change in $[\text{Ca}^{2+}]_i$, which by itself is sufficient to stimulate chromosome motion. It is a question therefore how $\text{GTP}\gamma\text{S}$ and GTP stimulate motion. Perhaps, in contrast to the other factors tested, they cause large but highly localized changes in $[\text{Ca}^{2+}]_i$, for example along kinetochore fibers. However, because of our measuring system which integrates the signal over the entire

mitotic apparatus these hypothetical large but local transients appear small. Unfortunately, attempts to image these events have not provided definitive answers (Gross, D. J., P. K. Hepler, D. H. Zhang, and D. A. Callaham, unpublished observations).

Another possible explanation may be related to the different chemical/biochemical forces that affect IP_3 , GTP , $\text{GTP}\gamma\text{S}$, and Ca^{2+} when injected into the cell. IP_3 , for instance, may be rapidly dephosphorylated when injected just beneath the plasma membrane and never reach its relevant target sites deep within the spindle apparatus. $\text{GTP}\gamma\text{S}$, in contrast, would not be rapidly inactivated, and furthermore would be free to diffuse to target sites where it could give sustained effects, possibly through localized $[\text{Ca}^{2+}]_i$ changes.

Despite these explanations, the mode of action of GTP and $\text{GTP}\gamma\text{S}$ may be independent of Ca^{2+} . Indeed, $\text{GTP}\gamma\text{S}$ at the level that stimulates chromosome motion does have an inhibitory effect on streaming, but its action is quite different from that associated with Ca^{2+} injection. The latter, when introduced to the level that stimulates chromosome motion, causes a rapid cessation of all motion, together with an extensive clumping of the cytoplasm around the pipette tip, and the loss of structure such as the transvacuolar strands. $\text{GTP}\gamma\text{S}$, on the other hand, affects streaming much more slowly than Ca^{2+} ; it causes the larger cytoplasmic particles to slow and stop while the smaller particles continue to move. Thus streaming is modified but not totally inhibited, and moreover the cytoplasmic structures such as the transvacuolar strands are not destroyed. Because of the bifurcating nature of the phosphatidyl inositol pathway it is possible that the effects caused by $\text{GTP}\gamma\text{S}$ result from the production of DAG and the activation of protein kinase C. Phosphorylation of certain key proteins, such as a MT motor, might stimulate the rate of chromosome motion. These ideas may provide fertile ground for future experiments.

Conclusions

In summary our results show that when the $[\text{Ca}^{2+}]_i$ is elevated within a limited range (to $\sim 1 \mu\text{M}$) the rate of chromosome to pole motion can be increased by as much as twofold. Levels of Ca^{2+} above that or below the resting physiological level cause slowing or inhibition of motion. The signaling agent $\text{GTP}\gamma\text{S}$ also generates a twofold increase in the rate of chromosome motion, without causing a substantial change in the detectable $[\text{Ca}^{2+}]_i$. Its action, therefore, may be through a mechanism that is partially if not entirely independent of Ca^{2+} . If, however, $[\text{Ca}^{2+}]_i$ changes are indeed involved in the mediation of the $\text{GTP}\gamma\text{S}$ response, then the changes must occur in very restricted domains, a mechanism that would provide the tightest spatial control and most efficient signaling.

We thank our colleagues at University of Massachusetts for helpful comments.

This work has been supported by grants from the National Science Foundation (DCB-88-01750) and the United States Department of Agriculture (88-37261-3727).

Received for publication 13 November 1989 and in revised form 23 March 1990.

References

Allan, E., and P. K. Hepler. 1989. Calmodulin and calcium-binding proteins. In *The Biochemistry of Plants: A Comprehensive Treatise*. Vol. 15. Molecu-

- lar Biology. A. Marcus, editor. Academic Press, Inc., San Diego. 455-484.
- Becker, P. L., and F. S. Fay. 1987. Photobleaching of fura-2 and its effect on determination of calcium concentrations. *Am. J. Physiol.* 253:C613-C618.
- Berridge, M. J. 1987. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* 56:159-193.
- Berridge, M. J., and R. F. Irvine. 1989. Inositol phosphates and cell signalling. *Nature (Lond.)*. 341:197-205.
- Brown, A. M., and L. Birnbaumer. 1988. Direct G protein gating of ion channels. *Am. J. Physiol.* 254:H401-H410.
- Callahan, D. A., and P. K. Hepler. 1990. Measurement of free calcium in plant cells. In *Cellular Calcium: A Practical Approach*. J. G. McCormack and P. H. Cobbold, editors. IRL Press, Oxford. In press.
- Campbell, A. K. 1983. *Intracellular Calcium: Its Universal Role as Regulator*. John Wiley & Sons, Inc., New York. 556 pp.
- Cande, W. Z. 1981. Physiology of chromosome movement in lysed cell models. In *International Cell Biology 1980-1981*. H. G. Schweiger, editor. Springer Verlag, Berlin. 382-391.
- Cassimeris, L. U., Wadsworth, P., and E. D. Salmon. 1986. Dynamics of microtubule depolymerization in monocytes. *J. Cell Biol.* 102:2023-2032.
- Forer, A. 1974. Possible roles of microtubules and actin-like filaments during cell-division. In *Cell Cycle Controls*. G. M. Padilla, I. L. Cameron, and A. Zimmerman, editors. Academic Press, Inc., New York. 319-336.
- Gill, D. L., T. Ueda, S.-H. Chueh, and M. W. Noel. 1986. Ca^{2+} release from endoplasmic reticulum is mediated by a guanine nucleotide regulatory mechanism. *Nature (Lond.)*. 320:461-464.
- Gillman, A. G. 1987. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615-649.
- Gomperts, B. D. 1983. Involvement of guanine nucleotide-binding protein in the gating of Ca^{2+} by receptors. *Nature (Lond.)*. 306:64-66.
- Grynkiewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440-3450.
- Hepler, P. K. 1989a. Membranes in the mitotic apparatus. In *Mitosis: Molecules and Mechanisms*. J. S. Hyams and B. R. Brinkley, editors. Academic Press, Inc., San Diego. 241-271.
- Hepler, P. K. 1989b. Calcium transients during mitosis: observations in flux. *J. Cell Biol.* 109:2567-2573.
- Hepler, P. K., and D. A. Callahan. 1987. Free calcium increases during anaphase in stamen hair cells of *Tradescantia*. *J. Cell Biol.* 105:2137-2143.
- Hepler, P. K., and B. A. Palevitz. 1986. Metabolic inhibitors block anaphase A in vivo. *J. Cell Biol.* 102:1995-2005.
- Hepler, P. K., and R. O. Wayne. 1985. Calcium and plant development. *Annu. Rev. Plant Physiol.* 36:397-439.
- Hirano-Ohnishi, J., and Y. Watanabe. 1989. Ca^{2+} /calmodulin-dependent phosphorylation of ciliary β -tubulin in *Tetrahymena*. *J. Biol. Chem.* 105:858-860.
- Joseph, S. K., A. P. Thomas, R. J. Williams, R. F. Irvine, and J. R. Williamson. 1984. Myo-inositol 1,4,5-trisphosphate: a second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. *J. Biol. Chem.* 259:3077-3081.
- Keith, C. H. 1987. Effect of microinjected calcium-calmodulin on mitosis in PtK2 cells. *Cell Motil. Cytoskeleton.* 7:1-9.
- Kiehart, D. P. 1981. Studies on the in vivo sensitivity of spindle microtubules to calcium ions and evidence for a vesicular calcium-sequestering system. *J. Cell Biol.* 88:604-617.
- Konishi, M., A. Olson, S. Hollingworth, and S. M. Baylor. 1988. Myoplasmic binding of fura-2 investigated by steady-state fluorescence and absorbance measurements. *Biophys. J.* 54:1089-1104.
- Malgaroli, A., D. Milani, J. Meldolesi, and T. Pozzan. 1987. Fura-2 measurement of cytosolic free Ca^{2+} in monolayers and suspensions of various types of animal cells. *J. Cell Biol.* 105:2145-2155.
- Majerus, P. W., T. M. Connolly, H. Deckmyn, T. S. Ross, T. E. Bross, H. Ishii, V. S. Bansal, and D. B. Wilson. 1986. The metabolism of phosphoinositide-derived messenger molecules. *Science (Wash. DC)*. 234:1519-1526.
- McIntosh, J. R. 1989. Assembly and disassembly of mitotic spindle microtubules. In *Cell Movement. II. Kinesin, Dynein, and Microtubule Dynamics*. F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc., New York. 403-419.
- McIntosh, J. R., and M. E. Porter. 1989. Enzymes for microtubule-dependent motility. *J. Biol. Chem.* 264:6001-6004.
- Nicklas, R. B. 1975. Chromosome movement: current models and experiments on living cells. In *Molecules and Cell Movement*. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 97-117.
- Nicklas, R. B. 1979. Chromosome movement and spindle birefringence in locally heated cells: interaction versus local control. *Chromosoma (Berl.)*. 74:1-37.
- Nicklas, R. B. 1987. Chromosomes and kinetochores do more in mitosis than previously thought. In *Chromosome Structure and Function: The Impact of New Concepts*. J. P. Gustafson, R. Appels, and R. J. Kaufman, editors. Plenum Publishing Corp., New York. 53-74.
- Olmsted, J. B., and G. G. Borisy. 1975. Ionic and nucleotide requirements for microtubule polymerization in vitro. *Biochemistry.* 14:2996-3005.
- Otter, T. 1989. Calmodulin and the control of flagellar movement. In *Cell Movement. I. The Dynein ATPase*. F. D. Warner, P. Satir, and I. R. Gibbons, editors. Alan R. Liss, Inc., New York. 281-298.
- Poenie, M. 1990. Alteration of intracellular fura-2 fluorescence ratios by viscosity and a simple correction. *Cell Calcium.* 11:85-91.
- Poenie, M., J. Alderton, R. Steinhardt, and R. Tsien. 1986. Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. *Science (Wash. DC)*. 233:886-889.
- Ratan, R. R., F. R. Maxfield, and M. L. Shelanski. 1988. Long-lasting and rapid calcium changes during mitosis. *J. Cell Biol.* 107:993-999.
- Salmon, E. D. 1975. Spindle microtubules: thermodynamics of in vivo assembly and role in chromosome movement. *Ann. NY Acad. Sci.* 253:383-406.
- Salmon, E. D. 1989. Microtubule dynamics and chromosome movement. In *Mitosis: Molecules and Mechanisms*. J. S. Hyams and B. R. Brinkley, editors. Academic Press, Inc., San Diego. 119-181.
- Salmon, E. D., and R. R. Segall. 1980. Calcium-labile mitotic spindles isolated from sea urchin egg (*Lytechinus variegatus*). *J. Cell Biol.* 86:355-356.
- Sekar, M. C., and L. E. Hokin. 1986. The role of phosphoinositides in signal transduction. *J. Membr. Biol.* 89:193-210.
- Shimmen, T., and M. Tazawa. 1982. Cytoplasmic streaming in the cell model of *Nitella*. *Protoplasma.* 112:101-106.
- Speksnijder, J. E., A. L. Miller, M. H. Weisenfeld, T.-H. Chen, and L. F. Jaffe. 1989. Calcium buffer injections block fucoid egg development by facilitating calcium diffusion. *Proc. Natl. Acad. Sci. USA.* 86:6607-6611.
- Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner. 1985. Nonmuscle actin-binding proteins. *Annu. Rev. Cell Biol.* 1:353-402.
- Tsien, R. Y., T. J. Rink, and M. Poenie. 1985. Measurement of cytosolic free Ca^{2+} in individual small cells using fluorescence microscopy with dual excitation wavelengths. *Cell Calcium.* 6:145-157.
- Tucker, E. B. 1982. Translocation in the staminal hairs of *Setcreasea purpurea*. I. A study of cell ultrastructure and cell-to-cell passage of molecular probes. *Protoplasma.* 113:193-201.
- Vantard, M., A. M. Lambert, J. DeMey, P. Picquot, and L. J. Van Eldik. 1985. Characterization and immunocytochemical distribution of calmodulin in higher plant endosperm cells: localization in the mitotic apparatus. *J. Cell Biol.* 101:488-499.
- Weisenberg, R. C. 1972. Microtubule formation in vitro in solutions containing low calcium concentrations. *Science (Wash. DC)*. 177:1104-1105.
- Welsh, M. J., and S. C. Sweet. 1989. Calmodulin regulation of spindle function. In *Mitosis: Molecules and Mechanisms*. J. S. Hyams and B. R. Brinkley, editors. Academic Press, Inc., San Diego. 203-240.
- Woods, C. M., V. S. Polito, and M. S. Reid. 1984. Response to chilling stress in plant cells. II. Redistribution of intracellular calcium. *Protoplasma.* 121:17-24.
- Yatani, A., J. Codina, Y. Imoto, J. P. Reeves, L. Birnbaumer, and A. M. Brown. 1987. A G protein directly regulates mammalian cardiac calcium channels. *Science (Wash. DC)*. 238:1288-1292.
- Yin, H. L. 1988. Gelsolin: calcium- and polyphosphoinositide-regulated actin-modulating protein. *BioEssays.* 7:176-178.