Table 5: Effect of actin and myosin inhibitors on backwards movement in CalA-treated spermatocytes

Treatment	# of cells	# of half-bivalent pairs followed			
		total	backwards	Slowed or stopped after BDM or LatB	no change after BDM or LatB
1st – CalA in anaphase 2nd – BDM or LatB in late anaphase	7	15	12	0	12

nonetheless favour the interpretation that acceleration is due to increased myosin activity because it is consistent with other experiments in which myosin and actin inhibitors block chromosome movement [1,15]. Whereas the interpretation of each individual study or of the action of each individual drug might be debated, the overall fact that inhibitors of actin and myosin generally inhibit movement and a myosin enhancer speeds up movement makes a strong case for involvement of myosin in anaphase chromosome movements and supports our interpretation that the acceleration is caused by myosin hyperactivation. Thus, while we cannot definitively rule out interpretations in which other PPase1 enzymes are involved, we think it likely that chromosome acceleration is due to enhanced myosin activity.

Our interpretation that CalA effects are due to blocking MLCPase would seem to be negated by our experiments using Y27632. Y27632, a specific inhibitor of Rho-K, slowed chromosome movement in anaphase. This effect presumably is due to reduced myosin phosphorylation, and therefore myosin is less active after Y27632 addition. CalA was not expected to accelerate these chromosomes, since myosin was not phosphorylated, but it did. The interpretation of these results is ambiguous, however, because the same result was obtained in studying smooth muscle contraction, which is known to be due to myosin activity [92]. It seems that inhibition of MLCPase by CalA unmasks another phosphorylation pathway, separate from Rho-K pathway and therefore not inhibited by Y-27632 [68]. This is confirmed in other experiments in which inhibition of smooth muscle Rho-K by Y27632 or H-1152 unmasked an integrin-linked kinase which then phosphorylated myosin [55]. Thus, because of such potential redundant phosphorylation pathways, this particular experiment is ambiguous and is not a clear test of how CalA causes chromosome acceleration.

The only previous data on CalA effects on mitosis that we know of is by Hamaguchi and Kuriyama [89]. The authors concluded that anaphase chromosome movements in sand dollar eggs were blocked by okadaic acid and CalA. With respect to CalA, they state in the text that chromosome movements were inhibited by CalA at concentrations ≥ 1–2 μM. We generally used concentrations 20 times lower than that, but we found that concentrations of 0.5 μM caused chromosomes to accelerate. Thus there appears to be a discrepancy between the two sets of results. However, from the description in Figure 10 of [89], it would seem that the authors derived their conclusion from fluorescence micrographs of chromosomes positions after injection of CalA. Their description in Figure 10 that "chromosome movement did not occur, although the chromosomes aggregated into two clusters", does not necessarily have to be due to blocked movements, though; the clusters could have arisen from backwards movements of the kind we described. With respect to the apparent discrepancy between our "no effect" of okadaic acid (Table 1) and the blockage of movement by okadaic acid reported by Hamaguchi and Kuriyama [89], their blockage of movement occurred at concentrations of 1–2 μM, which is 20–40 times higher than the concentration we used (50 nM). Indeed, they found that 50 nM okadaic acid had little or no effect on anaphase in their cells, so there is no discrepancy between the two sets of results.

CalA alters the distribution of various spindle proteins in crane-fly spermatocytes. P-squash staining relocates in the cell, from being associated with the kinetochore microtubules to being at the poles, at the kinetochores, and around the chromosomes. Similar results occur in other systems. For example, there are increased levels of phosphorylated myosin after CalA treatment in various cells [68,69,72,74,75,84] and CalA causes relocation of actin, myosin and other cytoskeletal components [83,98,99]. In crane-fly spermatocytes the microtubule bundles become thinner, presumably due to splitting, narrowing and disappearance of microtubules from the bundles. This is consistent with results in other studies that showed that microtubules are disassembled after CalA [98,100]. In crane-fly spermatocytes actin filaments become more visible in spindle fibres possibly due to actin filament stabilization by inhibition of MLCPase, similar to results in sea urchin eggs [69,76].

When CalA is added in prometaphase, chromosomes in crane-fly spermatocytes lose their attachment to microtubules (Fig. 6C). In our experiments, the unattached chromosomes rotated and moved rapidly up and down in the spindle, similar to the rapid movements displayed by the