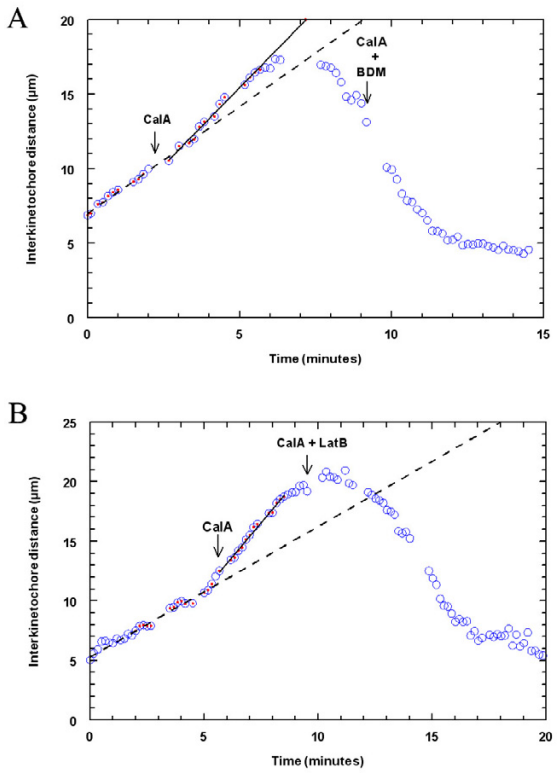

Figure 8
Backwards chromosome movements in crane-fly spermatocytes treated with CalA are not actin or myosin dependent. **(A)** Chromosome movements in a cell treated with CalA in anaphase (left arrow), and with BDM in CalA added after the chromosomes started to move backwards (right arrow). Backwards movements are not affected by the myosin inhibitor BDM. The dashed line represents linear regression through the circles with dots before addition of CalA and the solid line through the circles with dots after addition of CalA. **(B)** Chromosome movements in a cell treated with CalA in anaphase (left arrow), and with LatB in CalA added after the chromosomes reached the poles, at the time when they started to move backwards (right arrow). Backwards movements are not affected by actin inhibitor LatB. The dashed line represents linear regression through the circles with dots before addition of CalA and the solid line through the circles with dots after addition of CalA.

sex chromosomes after CalA treatment during anaphase. The longitudinal movements might be due to chromosomes capturing and sliding along remnant microtubules, but we can only speculate on forces that might be producing the rotations. All these extraordinary movements, though, seem to indicate that there is loss of equilibrium and lack of coordination between different force producers.

CalA caused late anaphase chromosomes to move backwards. Backward chromosome movements in anaphase have been seen previously in crane-fly spermatocytes, but only rarely and only when the poleward force was blocked by UV microbeam irradiation [101,102]. However, chromosome arms, severed with a laser beam, regularly moved backwards [95]. Backward movements were seen also in silkworm spermatocytes after UV irradiation of a spindle pole [103] : the chromosomes associated with the irradiated pole moved across the equator to the opposite pole. In grasshopper spermatocytes [104] there are fast backwards movements after UV irradiation of the kinetochore in early anaphase. Ilagan and Forer [101], LaFountain et al. [95] and Wong and Forer [102] all considered that there are mechanical connections between the arms of separating half-bivalents in crane-fly spermatocytes and that these connections, or "tethers"[95], elastically cause the backwards movements. Even though the tethers act on both partner half-bivalents, the backwards movements need not be symmetric, with both partners moving equally, because in all previous observations only one chromosome (or arm) moved, the one no longer attached to the pole. We think the same applies to CalA treated cells: when the connection to the pole is lost, then that chromosome moves to the equator independent of the partner at the other pole. We assume that the accelerated movements to the pole are associated (for some chromosomes) with pre-mature release of attachment to the pole, which allows the backward movements to be driven by the elastic tethers between partners. In our experiments, backwards movements in CalA treated spermatocytes were not altered by LatB or BDM, suggesting that the movements are not dependent on actin or myosin. It is possible that actin filaments remaining in the interzone after CalA treatment (e.g., Fig. 6E) are resistant to LatB by virtue of the bundling induced by Cal A, and that the backwards movements might require these actin filaments. Two experiments speak against this possibility, however. For one, we know that LatB removes actin filaments from normal cells [1] yet backwards movements still take place when cells pre-treated with LatB are treated with CalA (Table 3, 4). This indicates that actin filaments are not necessary for backwards movements to take place. For another, BDM has no effect on backwards movements, indicating that those movements do not require myosin or actomyosin. Thus our results indicate that backwards movements require neither actin nor myosin. On the other hand, titin, the protein responsible for muscle elasticity [105-107] is present between the arms of separating half-bivalents in control crane-fly spermatocytes as well as in CalA treated spermatocytes and could provide the necessary elasticity. Thus, our interpretation is that backwards movements are due to titin filaments which pull the chromosomes back together. As a corollary, we suggest that the regularly organized spindle matrix and