Possible roles of actin and myosin during anaphase chromosome movements in locust spermatocytes

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Summary. We tested whether the mechanisms of chromosome movement during anaphase in locust (Locusta migratoria L.) spermatocytes might be similar to those described for crane-fly spermatocytes. Actin and myosin have been implicated in anaphase chromosome movements in crane-fly spermatocytes, as indicated by the effects of inhibitors and by the localisations of actin and myosin in spindles. In this study, we tested whether locust spermatocyte spindles also utilise actin and myosin, and whether actin is involved in microtubule flux. Living locust spermatocytes were treated with inhibitors of actin (latrunculin B and cytochalasin D), myosin (BDM), or myosin phosphorylation (Y-27632 and ML-7). We added drugs (individually) during anaphase. Actin inhibitors alter anaphase: chromosomes either completely stop moving, slow, or sometimes accelerate. The myosin inhibitor, BDM, also alters anaphase: in most cases, the chromosomes drastically slow or stop. ML-7, an inhibitor of MLCK, causes chromosomes to stop, slow, or sometimes accelerate, similar to actin inhibitors. Y-27632, an inhibitor of Rho-kinase, drastically slows or stops anaphase chromosome movements. The effects of the drugs on anaphase movement are reversible: most of the half-bivalents resumed movement at normal speed after these drugs were washed out. Actin and myosin were present in the spindles in locations consistent with their possible involvement in force production. Microtubule flux along kinetochore fibres is an actin-dependent process, since LatB completely removes or drastically reduces the gap in microtubule acetylation at the kinetochore. These results suggest that actin and myosin are involved in anaphase chromosome movements in locust spermatocytes.

Keywords: Chromosome movement; Mitosis; Actin; Myosin; Latrunculin B; 2,3-Butanedione monoxime; Myosin phosphorylation inhibitor.

Introduction

Nuclear division (karyokinesis or mitosis) includes reorganisation of different cell structures. As cell division begins, a bipolar spindle forms that ensures accurate segregation of the condensed chromosomes between daughter cells during anaphase. Most models proposed for chromosome movement consider anaphase as a microtubule-based mechanism, in which spindle microtubules and their motors are the key, if not the only, spindle components involved in the functioning of the spindle (reviewed in McIntosh et al. 2002) and in producing tubulin flux in kinetochore microtubules (Sawin and Mitchison 1991). Some models describe anaphase as an actin-based mechanism in which actin and myosin are involved in chromosome movement (Foter and Pickett-Heaps 1998, Silverman-Gavrila and Foter 2001, Silverman-Gavrila and Foter 2003) and work together in a spindle matrix (Fabian et al. 2007).

Actin and myosin have been found in a variety of spindles (Foter et al. 2003: table I, Margolin 2005) and various functional tests using inhibitors indicate that actin and myosin are needed for attachment of prometaphase chromosomes to the spindle in crane-fly spermatocytes (Foter and Pickett-Heaps 1998, Silverman-Gavrila and Foter 2000a), PtK cells (Sanger et al. 1989) and algae (Sampson et al. 1996), and that actin and myosin are required for anaphase movements in these and other systems (Schmit and Lambert 1990, LaFountain et al. 1992, Sampson et al. 1996, Foter and Pickett-Heaps 1998, Silverman-Gavrila and Foter 2001, Margolin 2005). Treatments of crane-fly spermatocytes in anaphase with actin or myosin inhibitors generally cause slowing or stopping of the movements of partner half-bivalents, though sometimes they have no effect (Silverman-Gavrila and Foter 2001, Fabian and Foter 2005). The chromosomes often recover in the continued presence of the drug. While these data implicate actin and myosin in anaphase chromosome movement, other components also may be utilised, since movements are normal...
in actin-free spindles formed by removing actin in prometaphase and keeping cells continuously in an antiaxon drug (Fabian and Forer 2005). Thus, redundant mechanisms may be present and available to move chromosomes during anaphase.

The localisations of various cytoskeletal proteins in spindles also suggest functional roles for actin and myosin. Confocal microscope studies of crane-fly spermatocytes, using immunocytochemistry, indicate that actin and myosin are located in spindles in positions where they would be expected to be, were they involved in force production (Silverman-Gavrila and Forer 2003). Another muscle protein, titin, responsible for muscle elasticity, also is present in crane-fly spermatocytes (Fabian et al. 2007), in association with the matrix proteins scaffold (Walker et al. 2000), megator (Qi et al. 2004), and chromator (Rath et al. 2004), consistent with the involvement of all these proteins with a spindle matrix (Fabian et al. 2007).

Actin and myosin also are required for tubulin flux within kinetochore microtubules in crane-fly spermatocytes (Silverman-Gavrila and Forer 2000b). Flux, a steady poleward movement of tubulin subunits in the kinetochore microtubules (Sawin and Mitchison 1991), is due to incorporation of tubulin at the kinetochore (polymerisation) coupled to depolymerisation of the microtubules at their minus ends at the pole (Mitchison 1989, Maddox et al. 2003). Tubulin flux is considered to be important for the poleward movement of chromosomes during anaphase (reviewed in Rogers et al. [2005]). Flux usually is measured after intracellular injection of fluorescent tubulin or photoactivatable tubulin (e.g. Mitchison 1989, LaFountain et al. 2004). Other methods also have been used, e.g., staining with antibodies against total tubulin and against acetylated (stable) tubulin (Wilson et al. 1994, Wilson and Forer 1997, Forer and Wilson 2000). Kinetochore microtubules are more stable than the polar microtubules (discussed in Wilson and Forer [1989]) and these stable microtubules are acetylated (Palazzo et al. 2003). Staining with antibody against acetylated tubulin demonstrated acetylation only in the kinetochore microtubules. A “gap in acetylation” at the kinetochore (Wilson and Forer 1989) represents newly incorporated tubulin that has not had time to be acetylated. Thus, this gap is a measure of tubulin flux along kinetochore microtubules (Wilson et al. 1994, Wilson and Forer 1997). We studied flux in locust spermatocytes by measuring the gap in tubulin acetylation at the kinetochore.

Most of the data implicating actin and myosin in anaphase movements were obtained from crane-fly spermatocytes, which are unique in many ways. For example, sex chromosomes have fibres to both poles; they enter anaphase only after the autosomes reach the poles (Forer 1969); and there are signals between sex chromosomes (Ilagan and Forer 1997), between partner autosomes (Wong and Forer 2003), and between autosomes and sex chromosomes (Sillers and Forer 1981). Thus, we studied locust spermatocytes to see how general the conclusions drawn from crane-fly spermatocytes were. Locusts were available to us, and in many aspects of division, locust spermatocytes are similar to the better studied grasshopper spermatocytes, which have been used for a variety of experiments related to chromosome orientation in the spindle (Ault 1984, 1986; Rebollo and Arana 1995), microtubule attachment and role of tension in checkpoint signals (Nicklas et al. 2001), and anaphase movements of chromosomes (Ris 1949, Ault and Nicklas 1989, Paliulis and Nicklas 2000, Chen and Zhang 2004). We are aware of only a few papers that describe locust (Locusta spp.) spermatocytes. McClung (1902) and Mohr (1914) gave detailed descriptions of stages of division in spermatocytes of members of the family Locustidae on the basis of fixed and stained preparations. Rees and Jamieson (1954) described the supernumerary chromosome in Locusta migratoria. Moens (1969) described the fine structure of meiotic chromosome polarisation and pairing in L. migratoria spermatocytes. White (1935) studied the effects of X-rays on mitosis in L. migratoria. Using electron micrographs, Gawadi (1974) showed that actin filaments are present in locust spermatocyte spindles, that these filaments are parallel to the microtubules and that they occasionally attach to the chromosomes, most of them with the pointed end facing the equator. Other studies have shown that intracellular Ca2+ might have a role in controlling anaphase movements in locust spermatocytes by regulating microtubule assembly and disassembly, activation of actin filaments, or stimulation of a dynein-like ATPase (Beier and Hauser 1981). The role of actin in anaphase chromosome movements in locust spermatocytes was emphasised by studies using inhibitors (vanadate and taxol) which disrupt the spindle framework (Daub and Hauser 1986, 1988). These studies also support the model that considers microtubules not as the force producers in anaphase, but rather as the rate-limiting factor in anaphase motion, as originally proposed by Forer (1974). Since there have been few detailed descriptions of living spermatocytes, part of our study is a basic description of meiosis in living locust spermatocytes.

In addition to studying live control cells, we localised actin, myosin, and other potentially functional proteins in...