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Characterization and phosphorylation of an MPM-2 reactive sperm protein complex involved in zygotic aster formation

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Abstract

The zygotic centrosome, contributed by the sperm, forms an array of microtubules that spread throughout the ooplasm. These microtubules are used by the female pronucleus to come into juxtaposition with the male pronucleus in order to form the zygotic nucleus. Zygotic centrosome regulation has been shown to involve an MPM-2 phosphoepitope that is dephosphorylated prior to aster formation. The MPM-2 reactive candidate appears as a triplet of proteins with MWapp of 85, 81, and 77 kDa on SDS-PAGE. The objectives of this work were to investigate the relationship among the triplet proteins, determine physical characteristics of the MPM-2 reactive protein, and investigate potential kinases that may act on the MPM-2 target epitope. Amino acid analysis and peptide mapping show that the triplet proteins are nearly identical with all of the peptide fragments of the smaller proteins contained in the largest of the triplet. Physical properties, immunoreactivity, and isolation characteristics demonstrate that the reactive protein is part of the outer dense fiber/segmented column complex. The protein is highly insoluble in aqueous solutions and requires a reducing agent and a chaotropic agent, such as urea, to remain in solution. Kinase studies reveal that the MPM-2 reactive protein is maintained in a phosphorylated state by a kinase that is Maturation Promoting Factor (MPF) or dependent on MPF. Further, the epitope is dephosphorylated coincident with a drop in MPF in activated oocyte extract while MAP kinase levels remain high. This information

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indicates that the MPM-2 reactive sperm protein is part of the segmented columns or dense fibers and likely exerts its influence on the zygotic centrosome indirectly, perhaps through blocking. The epitope on the protein appears to be regulated through the action of kinases that maintain the phosphorylation of the protein. Dephosphorylation is thought to occur by ubiquitous phosphatases that are favored upon deactivation of MPF and downstream kinases. ^

Subject Area

Biology, Cell

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