Metaphase I Arrest of Starfish Oocytes Induced via the MAP Kinase Pathway is Released by an Increase of Intracellular pH

Kazuyoshi Chiba

Department of Biology, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan

Reinitiation of meiosis in oocytes usually occurs as a two-step process during which release from the prophase block is followed by an arrest in metaphase of the first or second meiotic division (MI or MII). The mechanism of MI arrest in meiosis is poorly understood, although it is a widely observed phenomenon in invertebrates. The blockage of fully grown starfish oocytes in prophase of meiosis I is released by the hormone 1-methyladenine. It has been believed that meiosis of starfish oocytes proceeds completely without MI or MII arrest, even when fertilization does not occur.

In this study we found that MI arrest of starfish oocytes occurs in the ovary after germinal vesicle breakdown. This arrest is maintained both by the Mos/MEK/MAP kinase pathway and the blockage of an increase of intracellular pH in the ovary before spawning. Immediately after spawning into seawater, activation of Na^+/H^+ antiporters via a heterotrimeric G protein coupling to a 1-methyladenine receptor in the oocyte leads to an intracellular pH increase that can overcome the MI arrest even in the presence of active MAP kinase. Thus, the MAP kinase pathway is required for establishing MI arrest at lower pH (<pH 7.0). At higher pH (>pH 7.3), MI arrest does not occur, although the MAP kinase is still activated during meiosis. Before this study, MI arrest have not been reported, since starfish oocytes were usually treated with normal SW containing 1-MA for induction of GVBD, which caused an increase of pHi before GVBD. At lower pH of maturing oocytes in the ovary, the MAP kinase pathway may inhibit the APC-dependent degradation of cyclin B. After spawning, cyclin B degradation is induced by the pHi increase while the MAP kinase pathway is still active, which in turn, is involved in the block of DNA synthesis during meiosis.

Interface Molecules Connecting Mos-MAPK Pathways to Cell Cycle Regulators in Starfish Eggs

Kazunori Tachibana

Laboratory of Cell and Developmental Biology, Graduate School of Bioscience, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8501, Japan

While animal eggs await fertilization, their cell cycle needs to be arrested. The cytostatic factor (CSF) is the molecule responsible for this arrest. Mos protein is known as a key component of CSF activity in deuterosotmes and is required to maintain the metaphase II arrest in vertebrates and the G1 arrest in starfish, *Asterina pectinifera*. How have the Mos-MAP (mitogen activated protein) kinase signaling pathways been rewired different cell cycle regulators in different animals during the evolution? We thought that there might be the molecules that connect MAP kinase signals to cell cycle control, and named them "interface molecules". Evolution might have modified or changed the interface molecules and rewired the signaling pathways. To identify the interface molecules in starfish eggs, we approached to identify the molecules in two directions, downstream of MAP kinase and upstream of the cell cycle regulators.

We have determined that the next step just after MAP kinase to prevent initiation of DNA replication is activation of $p90^{Rsk}$ (ribosomal S6 kinase). Then, we have mapped the precise stage where starfish unfertilized eggs are arrested to identify the output of the interface molecules. Monitoring DNA replication proteins such as MCM, PCNA and Cdc45, we have found that the unfertilized starfish eggs are arrested at the stage before Cdc45 protein has not loaded into DNA replication origins after MCM proteins have loaded. These findings demonstrate that the putative interface molecules prevent DNA replication by inhibiting Cdc45 loading depend on $p90^{Rsk}$ activity.