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MAMO, a maternal BTB/POZ-Zn-finger protein enriched in germline progenitors is required for the production of functional eggs in *Drosophila*

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Abstract

A hallmark of germline cells throughout the animal kingdom is their ability to execute meiosis. However, despite its prime importance, little is known about how germline progenitors acquire this ability. In *Drosophila*, the primordial germ cells (PGCs) are characterized by the inheritance of germ plasm, which contains maternal factors that have sufficient ability to direct germline development. Here, we show that a novel maternal factor, MAMO, is autonomously required in PGCs to produce functional gametes. MAMO protein which contains both a BTB/POZ (Broad Complex, Tramtrack, Bric-a-brac/Pox virus and Zinc finger) domain and C_2H_2 zinc finger motifs is enriched in PGCs during embryogenesis. The PGCs with reduced maternal MAMO activity are able to undergo oogenesis, but fail to execute meiosis properly. In the resulting oocytes, meiosis-specific chromosomal configurations are impaired. We additionally show that the decondensation of fertilized sperm nuclei is also affected in the eggs. We propose that maternal MAMO activates downstream genes to promote specialized morphological changes of both female meiotic chromosomes and the sperm nucleus, which are critical in zygote formation.

Keywords: Maternal factor; BTB/POZ-Zn-finger protein; Meiosis; *Drosophila*

1. Introduction

In many animal groups, maternal factors required for germline development are localized in a histologically remarkable region in egg cytoplasm, or germ plasm, and are inherited in the PGCs (Beam and Kessel, 1974; Eddy, 1975). In *Drosophila*, germ plasm is referred to as polar plasm because it is localized in the posterior pole region of the early embryo and is inherited by pole cells, which are the PGCs in this animal. The pole cells pass through midgut epithelium into haemocoel and migrate within the embryos to reach the gonads, where they later undergo meiosis to generate functional gametes (Williamson and Lehmann, 1996; Santos and Lehmann, 2004). The polar plasm contains factors sufficient for germline development, as injecting polar plasm into the anterior pole induces ectopic pole cells with the ability to produce functional gametes (Illmensee and Mahowald, 1974; reviewed by Mahowald, 2001). These maternal factors direct germline development by regulating a variety of germline-specific events.

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Several maternal factors have been identified so far that are enriched in the polar plasm, and whose functions are required for early events of germline development (Mahowald, 2001). For example, mitochondrial large ribosomal RNA (mtlrRNA) and Germ cell-less (Gcl) protein are both involved in the formation of pole cell (Iida and Kobayashi, 1998; Robertson et al., 1999). Within the pole cells, maternal *Nanos* (*Nos*) is essential to repress the pathway leading to mitosis, apoptosis and somatic differentiation (Asaoka-Taguchi et al., 1999; Hayashi et al., 2004). The pole cells further require *wunen2* (*wun2*) and *Polar granule component* (*Pgc*) RNAs for their survival during their migration (Nakamura et al., 1996; Starz-Gaiano et al., 2001; Hanyu-Nakamura et al., 2004; Martinho et al., 2004) and *trapped in endoderm-1* (*tre1*) for their transepitherial movement through the midgut wall (Kunwar et al., 2003). However, neither genetic nor molecular screens have yet identified maternal factors that contribute to meiosis, a unique and essential property of the germline.

Meiosis is a specialized cell division that produces haploid gametes from diploid progenitors. This is accomplished through two chromosomal segregation events without an intervening DNA replication. To accommodate the specialized processes, meiotic chromosomes undergo specific morphological changes. In *Drosophila* oocytes, upon entry into meiosis, a synaptonemal complex (SC) is formed between homologous chromosomes to stabilize their pairing, and the meiotic recombination occurs. Following disassembly of the SC, the meiotic chromosomes condense to form specialized prophase I chromosome structures, "karyosomes", and these structures are maintained during later oogenesis (Ivanovska et al., 2005). After ovulation, the oocyte initiates meiotic division and produces both a single female pronucleus and three polar-body nuclei which later fuse to form a rosette structure (Foe et al., 1993). These dynamic changes by the meiotic chromosomes are critical for meiosis (Ivanovska et al., 2005; Cullen et al., 2005). Genetic screens have identified many genes involved in the regulation of the meiotic chromosomes. However, the mechanism by which the germline acquires the potential to execute meiosis remains elusive.

Here, we identified a novel gene, *mamo* (maternal gene required for meiosis), which encodes a maternal factor required autonomously in PGCs to produce functional gametes. *mamo* encodes a transcription factor that contains both a BTB/POZ domain and C_2H_2 zinc finger motifs, and the maternal MAMO protein is enriched in PGCs during embryogenesis. Reducing maternal *mamo* activity in PGCs prevents proper morphological changes of meiotic chromosomes in the resulting oocytes. In addition, decodensation of the fertilized sperm nucleus is also impaired in the eggs.

2. Results

2.1. Isolation of a maternal mutation, mamo

Maternal-effect sterile screens have identified many mutations that affect germ-plasm formation and early germline development (reviewed by Mahowald, 2001). However, these screens could only recover homozygous viable mutations and therefore missed many genes that play a critical role both in germline and somatic cells. Previous screens could not identify maternal genes encoding transcription factors which are involved in germline-specific gene expression and germline development. Considering the nature of transcription factors, it is possible that they may also be functional in somatic cells through their interaction with different cofactors, and their mutations may cause zygotic lethality. To overcome this problem, we generated homozygous germline clones by the FLP/FRT/DFS system in animals heterozygous for EMS-induced lethal mutations (Chou and Perrimon, 1992; Morris et al., 2003) (see Section 4), and the progenies derived from these germline clones were screened for phenotypes. To identify defects in germline-specific gene expression, we used enhancer-trap markers, *PZ198* and *BC69*, which express β-gal in pole cells (Kobayashi et al., 1996; Heller and Steinmann-Zwicky, 1998; Asaoka et al., 1998). From approximately 1000 lethal mutations on the X chromosome, we ultimately isolated one mutation,

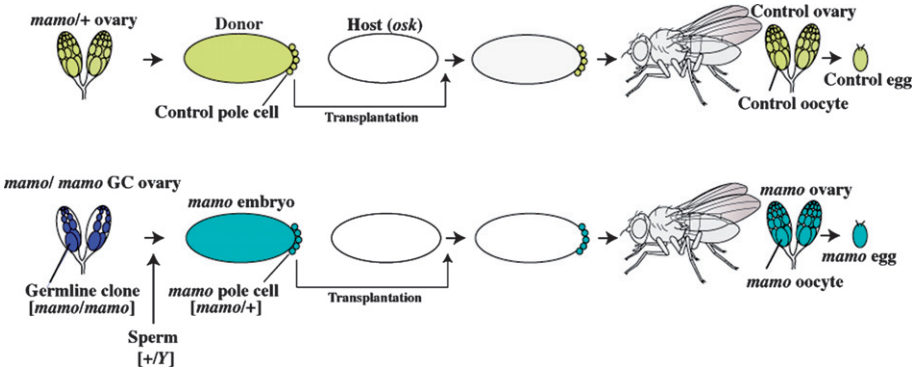

Fig. 1. Schematic representation of experimental procedures. To examine maternal phenotype of *mamo* mutation, we generated germline clones homozygous for $mamo^{SVA53}$ (blue). The eggs derived from the germline clones were fertilized by wild-type (+/Y) sperm to produce *mamo* embryos (light blue). The pole cells (*mamo* pole cells) formed in *mamo* embryos were transplanted into host embryos derived from osk^{301}-homozygous females. The transplanted embryos were allowed to develop into adults. We examined phenotypes in *mamo* oocytes and eggs.