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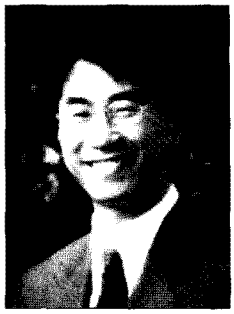

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Biological Functions of Dazl, a Male Infertility Gene Product, During Spermatogenesis

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Male germ cell development includes mitotic and meiotic cell division followed by dramatic morphological changes to produce spermatozoa. Genetic evidence indicated that DAZ family genes are critical for successful male germ cell development of diverse animals as well as human. In the present study, we investigated cellular functions of Dazl in the mouse male germ cells. We revealed specific interaction of Dazl with dynein light chain, a component of the dynein-dynactin motor complex. Subcellular distribution of Dazl was microtubule-dependent and the Dazl-bound mRNAs could be accumulated at perinuclear area. Based on the results, we propose that Dazl functions as an adaptor of a set of mRNAs to dyein motor complex. The Dazl-bound mRNA may be stored at specific sites available for future developmental processes. Our works confirmed the presence of an active transport system of mRNA in mouse male germ cells.

INTRODUCTION

A significant proportion of the azoospermic male infertility patients include micro-deletions at specific loci called AZF of the Y chromosome, suggesting that some of the genes in the loci are critical for spermatogenesis (Tiepolo and Zuffard, 1976). Among the genes at the AZF loci, DAZ has been considered as a male infertility gene (Reijo et al., 1995). Genetic evidence indicates that the DAZ family genes are critical for germ cell development of diverse animals as well as human. Disruption of the Dazl gene in mice has lead to loss of germ cells and complete absence of gamete production (Ruggiu et al., 1997). A careful examination of the Dazl-deficient mice revealed that the final point reached in male germ cell development was the leptotene-zygotene stage of meiotic prophase I (Saunders et al., 2003). The Drosophila boule mutant revealed the primary defect in the meiotic cell division of the male germ cell (Eberhart et al., 1996). In C. elegans, loss of Dazl function caused female sterility by blocking oogenesis at the pachytene stage of meiotic prophase I (Karashima et al., 2000).

There are three DAZ family genes in the human genome: Four copies of the DAZ gene are located at the AZFc locus of the Y chromosome and DAZL and BOULE are autosomal (Reijo et al., 1996;

Saxena et al., 2000; Kuroda-Kawaguchi et al., 2001). The Y chromosomal DAZ is found only in human and Old World primates while the autosomal DAZ family genes are present in all organisms tested (Xu et al., 2003). The genetic rescue experiments were carried out to determine functional redundancy among the DAZ family proteins. Male germ cell development in the Dazl-deficient mice was rescued partially by human DAZ or DAZL genes (Slee et al., 1999; Vogel et al., 2002). Similarly, introduction of Xenopus Dazl or human BOULE into the boule mutant fly made meiosis of the male germ completed (Houston et al., 1998; Xu et al., 2003). These results suggest that the DAZ family proteins take parts in related, but distinct roles during male germ cell development.

Regulation of gene expression at translational levels has been emphasized in germ cell development. In the present study, we report that Dazl interacts with dynein light chain, suggesting its function as an adaptor of specific mRNAs to the dynein motor complex. Our works confirmed the presence of an active transport system of mRNA in the mouse male germ cells.

RESULTS

In order to have a clue on biological functions of Dazl in male germ cells, we carried out yeast two-hybrid screenings with Dazl as bait and fished out dynein light chain 1 (Dlc1) predominantly. Interaction of Dazl and Dlc1 proteins was confirmed with biochemical methods. We examined association of Dazl with the dynein-dynactin complex. When testicular lysates were immunoprecipitated with the Dazl antibody, dynein intermediate chain (Dic) and p150Glued were co-immunoprecipitated, indicating that Dazl is in association with the dynein-dynactin complex in the mouse male germ cells. These results strongly suggest that Dazl is in contact with Dlc for its association to the dynein-dynactin complex.

Association of Dazl with dynein-dynactin complex allowed us to propose that Dazl travels through the microtubule network. Ectopic Dazl proteins in COS7 cells were located to peri-nuclear area in either diffused or aggregated forms and the endogenous Dlc co-localized with the Dazl protein. When nocodazole was added in the culture medium for disruption of the microtubule network, it resulted in dispersion of both ectopic Dazl and endogenous Dlc proteins throughout cytoplasm, suggesting that Dazl is linked to the microtubule network in cells. Finally, endogenous Dazl was co-precipitated with taxol-stabilized microtubule along with Dic, while Rbm, another male infertility protein, was not (Fig. 3C). These results collectively indicate that Dazl is in association to microtubule network through the dynein-dynactin complex.

Real time movement of the ectopic Dazl protein was measured in cultured cells. The CFP-Dazl protein moved straight or curvilinear tracks intermittently whereas the GFP-Dazl1-115 mutant protein was stationary. Polarity and speed of the movement of Dazl and Dazl1-115 were quantitated. Dazl moved to retrograde more frequently, while Dazl1-115 mutant protein was stationary without any directional preference. The median speed of the CFP-Dazl particles was about 0.3 (m/sec, which is

comparable to that of the dynein motor complex. These results are consistent with the hypothesis that Dazl travels through the microtubule network in association to the cytoplasmic dynein motor complex.

Dazl was reported to bind to a specific set of mRNAs. In order to examine whether Dazl functions as an mRNA transporter in cells, we determined subcellular distribution of the target mRNA using the MS2-GFP fusion protein system. In the presence of Dazl, the fluorescent signals were aggregated at perinuclear area in cells with MS2-Tpx1 and MS2-Cdc25C fusion mRNAs, but not in cells with MS2-Cdc25A fusion mRNA. The Dazl protein was also co-localized at the MS2-Tpx1 and MS2-Cdc25C aggregates. These results suggest that the Dazl-bound mRNAs, such as Tpx1 and Cdc25C, are linked to the cytoplasmic dynein motor complex and accumulated to specific sites within a cell.

One candidate storage place of the Dazl-bound mRNAs may be the nuage structure in the cytoplasm of the mouse spermatocytes. We asked if Dazl was localized at the chromatoid body precursor, the best characterized nuage structure in the mouse male germ cells. Our immunohistochemical analyses showed that a part of Tdrd1 overlapped with Dazl. At the same time, it is also clear that there are Tdrd1 signals which did not overlap with the Dazl signals, especially in spermatocytes near meiosis. These results opened a possibility that a part of Dazl-bound mRNA might be deposited at the chromatoid body precursors in the mouse spermatocytes.

DISCUSSION

In the present study, we observed specific interaction of Dazl with the dynein light chain in mouse male germ cells. Based on the results, we propose that Dazl functions as an adaptor of mRNAs on the cytoplasmic dynein motor complex. That is, Dazl recognizes specific mRNAs, links them to the dynein-dynactin complex and transports them to an RNA granule analogous to the stress granule in male germ cells. The Dazl-bound mRNAs at the RNA granule may be stable but translationally dormant. When the Dazl-bound mRNAs are released from the RNA granule, they may become translationally active. We do not rule out the possibility that some of the Dazl-bound mRNAs may be brought to ribosomes directly.

Presence of an active transport system of mRNA was somewhat unexpected in the mouse male germ cells where no polarized distribution of mRNA has been reported yet. Rather, its significance may reside in translational control of the transported mRNAs. The Dazl-bound mRNAs may be stored in protection of degradation until a proper developmental cue calls for their translation. In this way, the spermatocytes could undergo complex and highly elaborated developmental processes such as meiosis with precision.

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