Subcellular Localization of the Asp/ASPM Proteins

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Contributor: Alyona V. Razuvaeva , Lucia Graziadio , Valeria Palumbo , Gera A. Pavlova , Julia V. Popova , Alexey V. Pindyurin , Silvia Bonaccorsi , Maria Patrizia Somma , Maurizio Gatti

Investigations on different cell types showed that Asp (Drosophila abnormal spindle)/Aspm/ASPM (*Abnormal Spindle-like Microcephaly-associated*; or *MCPH5*) depletion disrupts one or more of the following mitotic processes: aster formation, spindle pole focusing, centrosome-spindle coupling, spindle orientation, metaphase-to-anaphase progression, chromosome segregation, and cytokinesis.

Drosophila Asp | mouse Aspm | human ASPM

1. Introduction

abnormal spindle (*asp*) was one of the first mitotic genes discovered in *Drosophila asp* mutants were shown to exhibit a strong mitotic phenotype in larval brain cells and morphologically abnormal spindles in male meiosis [1]. Molecular cloning of *asp* revealed that the gene encodes a large microtubule (MT)-binding protein that accumulates at the spindle poles [2]. The interest in *asp* was greatly heightened by the discovery that mutations in its human ortholog *ASPM* (*Abnormal Spindle-like Microcephaly-associated*) are the most common cause of autosomal recessive primary microcephaly (MCPH) [3][4][5]. MCPH is a rare and genetically heterogeneous disorder characterized by reduced head circumference of up to one-third of the normal volume at birth, resulting in mild-to-moderate intellectual disability. The principal cause of MCPH is a decreased production of neurons in the developing neocortex due to defects in progenitor cell proliferation and/or apoptosis (reviewed by [6][7][8]). To date (February 2023), 30 *MCPH* genes have been identified that are listed in the Online Mendelian Inheritance in Man (OMIM) database (https://omim.org), with *ASPM* designated as *MCPH5*. Most of these genes are involved in different aspects of mitotic division, including centriole biogenesis, centrosome-driven MT nucleation, kinetochore assembly and function, and spindle formation. However, a fraction of them are required for chromatin condensation and remodeling, DNA repair, and chromosome stability [7][8]. Remarkably, mutations in *ASPM* are responsible for more than 40% of MCPH cases [5].

Another reason for interest in the *ASPM* gene is its role in tumor development. *ASPM* expression is upregulated in several cancers, including prostate cancer, glioblastoma, and hepatocellular carcinoma, and increased *ASPM* expression is associated with tumor progression and poor clinical prognosis. It has also been shown that siRNA-mediated ASPM depletion strongly inhibits tumor cell proliferation ([9][10][11] and references therein). These and other studies suggested considering *ASPM* and the other *MCPH* genes as promising therapeutic targets in brain tumors (reviewed by [12][13]).

Although the Asp protein and its human (ASPM) and mouse (Aspm) orthologs have been studied for many years, a precise functional comparison between the three proteins has never been made.

2. Subcellular Localization of the Asp/ASPM Proteins

Early studies using antibodies against an Asp N terminal fragment of 512 amino acids showed that Asp localizes to the polar regions of the spindles and to the telophase central spindle in syncytial *Drosophila* embryos [2]. Subsequent studies expanded and refined these initial observations showing that Asp accumulates at the spindles poles of larval neuroblasts [14][15], epithelial cells [16], S2 tissue culture cells [17][18][19][20], and meiotic cells of both males and females [15][21][22]. Specifically, it has been reported that Asp accumulates at the transition region between the spindle and the centrosome, with Asp immunostaining partially overlapping the immunofluorescence signal elicited by γ-tubulin or Centrosomin (Cnn, the ortholog of the human centrosomal protein CDK5RAP2). However, a series of observations indicate that Asp localizes to the spindle poles independently of the centrosomes and that it is not an integral component of the centrosome. Namely, (i) Asp accumulates to the spindle poles of larval brain cells devoid of functional centrosomes, such as those of mutants in *asterless* (*asl*; encoding the ortholog of human CEP152), *cnn* or *dd4* (encoding a γ-tubulin ring component) [15][18][23], (ii) the γ-tubulin and Cnn signals in *asp* mutant metaphases are of similar intensity to those of controls [15], and, most importantly, (iii) in colchicine-treated embryos and S2 cells with fully depolymerized spindle MTs Asp does not localize to the centrosome [15][20].

In addition to the spindle poles, Asp accumulates at the central spindle. The central spindle, or intercellular bridge, is a prominent MT bundle that forms during late telophase. It consists of antiparallel MTs with the plus ends interdigitating at the center of the bundle, where they associate with many different proteins generating a discrete structure currently called the midbody. These proteins form a dense cluster (midbody ring) that impedes the access of anti-tubulin antibodies, resulting in a dark zone after tubulin immunostaining [24]. Asp associates with the sides of the MT bundle that face the telophase nuclei. This peculiar localization has been observed in different cell types, including larval neuroblasts, S2 tissue culture cells, and male meiotic cells [15][20][21]. Asp also accumulates at the extremities of the central spindle of embryonic telophases. In these syncytial divisions, where cytokinesis does not occur, the central spindle is not hourglass-shaped as in cells with a contractile ring; it is instead diamond-shaped with sharply focused extremities that are highly enriched in Asp [15]. The localization of Asp at the spindle poles and at the outer sides of the central spindle suggests that Asp binds and crosslinks the minus ends of the spindle MTs [15][18][19][21]. This suggestion was recently corroborated by in vitro studies showing that Asp accumulates at the MT minus ends [25].

Besides its accumulation at the spindle poles and central spindle extremities, antibody staining revealed a weak Asp signal along the spindle MTs of different cell types, including larval neuroblasts, spermatocytes, and S2 cells [15][19][21]. In addition, the prometaphase and metaphase spindles of live larval neuroblasts and S2 cells, both expressing Asp-GFP, displayed discrete fluorescent signals along the spindle MTs. Imaging of these Asp-GFP particles revealed that they stream towards the spindle poles and are eventually incorporated into the polar Asp

pool [17][18]. A poleward flow of Asp-GFP particles was also observed in the epithelial cell spindles of *Drosophila* pupal notum [16].

Aspm localization in neuroepithelial (NE) mouse cells is very similar to the Asp localization in *Drosophila* cells. Aspm localizes to the spindle poles throughout mitosis, accumulating in the immediate vicinity of the γ-tubulin signal of centrosomes. Aspm staining does not overlap the γ-tubulin immunoreactivity, and Aspm is absent from centrosomes during interphase [26]. In addition, Aspm localizes to the outer regions of the central spindle, but it is excluded from the midzone/midbody [27]. Thus, like its *Drosophila* ortholog, mouse Aspm appears to localize to spindle regions enriched in MT minus ends, consistent with the observation that Aspm preferentially associates with the MT minus ends in vitro [25].

Early studies on U2OS tissue culture cells suggested that human ASPM co-localizes with the centrosomes at the spindle poles and is enriched at the centrosomes in interphase nuclei [28]. Similar conclusions were reached in analyses performed in HeLa cells [29]. However, subsequent studies in U2OS cells showed that ASPM forms a ring around the centrosomes at the spindle poles, with little overlap with the γ-tubulin staining [30]. These studies did not provide evidence for ASPM localization at the interphase centrosomes. They also showed that after tubulin depolymerization with nocodazole, ASPM immunostaining at the spindle poles is lost, while γ-tubulin staining is unaffected [30]. However, in another investigation, antibody staining showed discrete ASPM signals located next to centrin (a centriolar marker) signals in interphase cells [31]. Thus, while it is clear that ASPM localization at the spindle poles is MT-dependent and centrosome-independent, the relationships between ASPM and the centrosome are not fully clarified.

ASPM localizes to the central spindle like its *Drosophila* and mouse counterparts, but there are some conflicting results about its precise localization to this structure. Using a commercial anti-human ASPM antibody and an antibody raised against amino acids 1-418 of rat ASPM, Paramasivam et al. [29] showed that both antibodies stain the midbody (dark zone) of HeLa cells. In a subsequent study, Higgins et al. [30] showed that an antibody directed to an ASPM N terminal peptide (aa 363–386) associates with the extremities of the central spindle but not with the midbody. In contrast, the same study showed that another antibody raised against a C terminal peptide (aa 3443–3458) of ASPM specifically decorates the midbody but fails to stain the lateral regions of the central spindle. This discrepancy was not addressed, and the authors focused on the relationships between ASPM and the centrosomes [30].

More recently, Jiang et al. [25] analyzed the properties and the mitotic behavior of a complete GFP-ASPM protein generated by the insertion of a GFP coding sequence just upstream of the first *ASPM* exon. In HeLa cells, GFP-ASPM accumulated to the spindle poles throughout mitosis; it also accumulated at central spindle extremities but was absent from the midbody. Importantly, Jiang et al. [25] also showed that ASPM binds the MT minus ends both in vitro and in living cells.

Collectively the extant results indicate that the orthologous Asp/ASPM proteins exhibit very similar, if not identical, localization patterns. They do not appear to be integral centrosome components but accumulate to the spindle

poles, where they are likely to bind and crosslink the minus ends of the MTs that detach from the centrosomes ([32]; reviewed by [33]). In addition, Asp and its orthologues accumulate at the extremities of the central spindle that are enriched in MT minus ends. It is unlikely that they are also part of the midbody, as this structure is thought to contain little to no MT minus ends [24][34][35]. The detection of ASPM at the midbody with some specific antibodies might reflect a cross-reaction with one of the many proteins that compose this structure [36].

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