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Temporal and spatial expression of *Drosophila* DLGS97 during neural development

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ABSTRACT

The products of the *Drosophila discs-large* (*dlg*) *gene* are members of the MAGUK family of proteins, a group of proteins involved in localization, transport and recycling of receptors and channels in cell junctions, including the synapse. In vertebrates, four genes with multiple splice variants homologous to *dlg* are described. *dlg* originates two main proteins, DLGA, similar to the vertebrate neuronal protein PSD95, and DLGS97, similar to the vertebrate neuronal and epithelial protein SAP97. DLGA is expressed in epithelia, neural tissue and muscle. DLGS97 is expressed in neural tissue and muscle but not in epithelia. The distinctive difference between them is the presence in DLGS97 of an L27 domain. The differential expression between these variants, makes the study of DLGS97 of key relevance to understand the *in vivo* role of synaptic MAGUKs in neurons. Here we present the temporal and spatial expression pattern of DLGS97 during embryonic and larval nervous system development, during eye development and in adult brain. Our results show that DLGS97 is expressed zygotically, in neurons in the embryo, larvae and adult, and is absent at all stages in glial cells. During eye development DLGS97 starts to be expressed in photoreceptor cells at early stages of differentiation and localizes basal to the basolteral junctions. In the brain, DLGS97 is expressed in the mushroom bodies and optic lobes at larval and adult stages; and in the antennal lobe in the adult stage. In addition we show that both, *dlgS97* and *dlgA* transcripts express during development multiple splice variants with differences in the use of exons in two sites.

1. Results and discussion

The MAGUK family is a conserved group of proteins, which play major roles in the assembly and maintenance of membrane domains such as septate junctions in epithelial cells and synapses in neurons (Pawson and Scott, 1997; Garner et al., 2000; Sheng and Sala, 2001; Tepass et al., 2001); in particular, the subfamily of Synaptic Associated Proteins (SAP), which has four members in vertebrates (SAP90/95, SAP102/NEDLG, SAP97/hDLG, SAP93/Chapsyn110) and is highly expressed in the vertebrate brain (Funke et al., 2005). *Drosophila* contains a single SAP homologue in its genome, namely the discs large (*dlg*) gene. *dlg* is required for the control of cell proliferation, the maintenance of neuroblasts polarity, the formation of septate junctions in epithelia and the correct development and function of the neuromuscular synapse. Mainly due to the epithelial cell defects in polarity and proliferation, *dlg* mutants die at the end of larval development precluding the analysis of DLG function in the adult nervous system. The multiple defects observed in *dlg* mutants were originally ascribed to the loss of a single gene product, DLGA. DLGA has three PDZ (PSD95-DLG-ZO1) domains, one SH3 (Src homologue 3) domain and one GUK (Guanylate kinase) domain (Woods and Bryant, 1991). However, additional proteins, products of the *dlg* gene are expressed in the central nervous system and muscles (Mendoza et al., 2003). These proteins are not expressed in epithelial cells and have an amino terminal region called S97N, which contains an L27 domain, not present in DLGA. L27 domains form homo or hetero-tetrameric complexes, providing a conserved platform for supramolecular assemblies (Feng et al., 2004). Some of functions of L27 domains in vertebrate culture neurons include vesicle transport (Setou et al., 2000), the regulation of neurotransmitter release and AMPA receptor trafficking (Nakagawa et al., 2004). Recently, L27 domains have been associated with activity induced synaptic modification in neurons (Schluter et al., 2006). We have recently shown, using mutants that selectively eliminate the expression of either DLGA or DLGS97, that the L27 containing DLG variant has specific functions in the larval neuromuscular synapse and in adult brain, which are not replaceable by DLGA. Although we have shown that the function of DLGS97 is necessary for the normal function of the larval neuromuscular synapse and of the adult brain, a detailed expression pattern of DLGS97 is still missing. Here we report a study of *Drosophila* DLGS97 expression in the central nervous system during development. In addition, we analyzed the expression during eye development given that in

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this tissue, the transition from an undifferentiated epithelium to a differentiated tissue that include neurons, can be easily observed. Finally, we studied *dlgA* and *dlgS97* transcripts by RT-PCR, in different tissue showing the alternative use of some of the exons present in both variants.

1.1. dlgS97 during embryonic development

We first studied the expression of *dlgS97* transcripts during embryonic development by *in situ* hybridization. From embryonic stage 10 a diffuse expression in the neurogenic region was detected (Fig. 1B). The same diffuse expression was observed at stage 11 (not shown). At stage 12, *dlgS97* expression became more defined and concentrated along the future ventral nerve cord region, including the dorsal germ band before its retraction (Fig. 1C, early stage 12, arrow). Beyond stage 13 *dlgS97* was clearly concentrated in the ventral nerve cord and later also in the brain (Fig. 1D and E). Although the expression of *dlg* is known to have a strong maternal component (Perrimon, 1988), a probe directed to the S97N coding region failed to detect *dlgS97* transcripts before embryonic stage 9 (Fig. 1A)

Next, we analyzed DLGS97 protein expression. For this, we used an antibody directed to its L27 domain (anti-DLGS97N antibody, (Mendoza et al., 2003; Mendoza-Topaz et al., 2008). DLGS97 protein started to be detected in the embryonic ventral cord at late embryonic stage 12, when the germ band is almost completely retracted (Fig. 1J, arrow). In stages 13 and 14, DLGS97 was observed in the commissures crossing the midline (Fig. 1K–L and P–Q). From stages 15 to 17 the label grew more intense as the ventral nerve cord retracts and became clear in the longitudinal fibers (Fig. 1M–O and R–T, arrow). At these stages, the expression was also detected in the brain (Fig. 1M, arrow), gonads (Fig. 1N, arrow), and the segmental and intersegmental nerve roots that leave the central nervous system to the periphery (Fig. 1S, arrow). In agreement with the *in situ* hybridization results, the protein was not detected before stage 11 (Fig. 1F–I).

As we did not detect *dlgS97* transcripts or DLGS97 protein during early stages of development, we sought to confirm this result by a more sensitive approach. For this, we conducted RT-PCR and Western blot experiments of staged embryos. As the zygotic transcription starts around embryonic stage five, we used RNA from embryos before stage five and compared it to RNA from late stage embryos (stages 15–17) where DLGS97 and DLGA are expressed. Using forward primers specific for either *dlgS97* or *dlgA* and reverse primers common to both variants in two different regions (SH3 and GUK coding regions), we were able to amplify from early embryos *dlgA* transcripts, but not *dlgS97* transcripts (Fig. 2A), while full length transcripts for both isoforms were detected in late embryos (stages 15–17; Fig. 2A). At late developmental stages, we also observed additional bands for both variants (*dlgA* and *dlgS97*). The exclusive zygotic expression of DLGS97 was confirmed by Western blot (Fig. 2B).

We then centered our study in late stage embryos (stage 15) using confocal microscopy to study in more detail the DLGS97 expression in the ventral nervous system. At this stage, DLGS97 was clearly seen in the intersegmental (ISN) and segmental nerve roots (SN), the anterior (ac) and posterior commissures (pc) and in the ventral cells of the ventral nervous system (Fig. 3A). In co-labeling experiments, we observed that all the ventral cord nerve cells labeled cortically with DLG_{S97N} antibody were co-labeled with anti-ELAV antibody, a established nuclear marker for all mature neurons (Fig. 3E). In contrast, labeling with anti-REPO antibody, a nuclear marker for glial cells, we did not detect co-labeling with anti-DLG_{S97N} (Fig. 3B, arrow).

DLG is expressed in neuroblasts where is necessary for the process of asymmetric division (Ohshiro et al., 2000; Peng et al., 2000). To determine the variant expressed in neuroblasts we used anti-Miranda antibody as a marker of mitotic neuroblasts. We did not detect DLGS97 expression in neuroblasts (Fig. 3C). Moreover, DLGS97 staining was only detected in a cell layer more dorsally located (inner) compared with the cells positive for anti-Miranda staining. The light signal in glia and neuroblasts observed using the anti-DLG_{S97N} was not different from background (data not shown).

In addition to the neurons of the central nervous system, DLGS97 was detected in the neurons of the peripheral nervous system and in the developing somatic muscles (Fig. 3D) (Mendoza et al., 2003).

These results reveal that the embryonic expression of DLGS97 is not maternally contributed, starting at embryonic stage 12 in

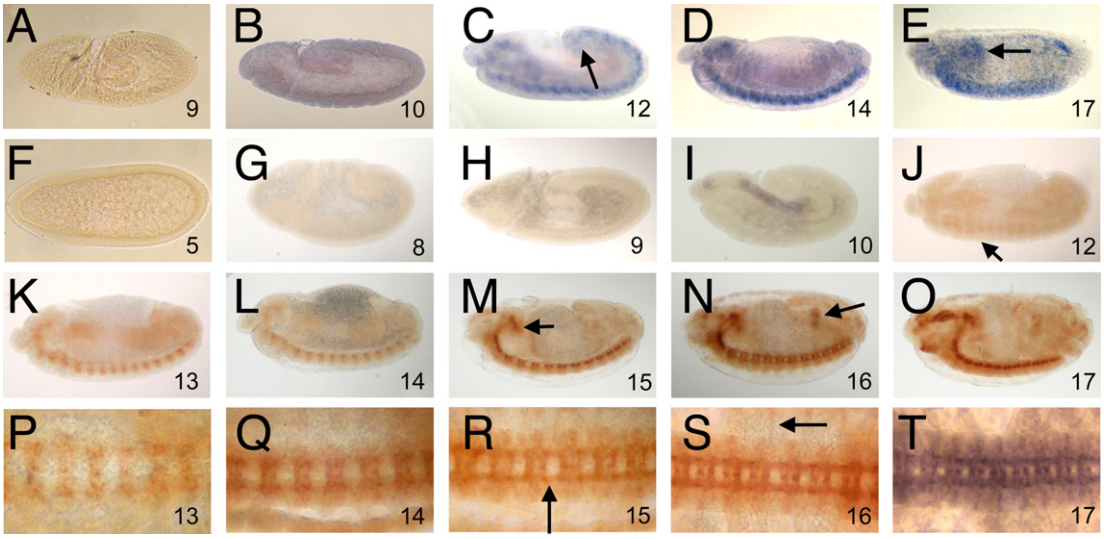

Fig. 1. DLGS97 during embryonic development. (A–E) *In situ* hybridization with a probe directed to the L27 region (see Section 2). (F–T) immunostaining with antiDLG_{S97N} antibody. The arrow in C points to the expression in the germ band before its retraction, and the arrow in E indicates the brain. (P–T) Higher magnification view of the ventral nerve cord. Arrowheads in J and M point to early DLGS97 protein expression in the ventral nerve cord and brain, respectively. Arrows in N, R and S indicate the gonads, the connectivity fibers in the anterior–posterior axis and the motor neurons that leave the CNS to the periphery, respectively. (A–O) are lateral and (P–T) are ventral views of whole embryos. The developmental stage is indicated in the bottom right corner in each panel. Anterior left (A–T) and dorsal up (A–O).