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Embryonic expression of *Drosophila* IMP in the developing CNS and PNS

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ABSTRACT

Drosophila IMP (dIMP) is related to the vertebrate RNA-binding proteins IMP1-3, ZBP1, Vg1RBP and CRD-BP, which are involved in RNA regulatory processes such as translational repression, localization and stabilization. The proteins are expressed in many fetal tissues, including the developing nervous system, and IMP up-regulation in solid tumors correlates with a high metastatic potential and poor prognosis. In this study, we used immunohistochemistry and live-imaging of an endogenous promoter-driven GFP-dIMP fusion protein to reveal the expression pattern of dIMP protein throughout embryogenesis. In the cellular blastoderm, immunoreactivity was seen in the entire cell-layer, where it was localized apically to the nucleus, and in the pole cells. Later, the GFP-dIMP fusion protein appeared in the developing central nervous system, both in the brain and in the ventral nerve cord. In the peripheral nervous system, immunoreactivity was detected in both neurons and accessory cells of chordotonal and external sensory organs.

1. Results and discussion

Drosophila melanogaster insulin-like growth factor II (IGF-II) mRNA-binding protein (dIMP) belongs to a family of RNA-binding proteins, comprising the human IMP1-3, chicken zipcode-binding protein 1 (ZBP1), murine c-*myc* coding region instability determinant binding protein (CRD-BP), and *Xenopus* Vg1 RNA-binding protein (Vg1RBP). During the last decade, different RNA targets such as *IGF-II, β-actin, Vg1, c-myc, Tau* and *Semaphorin* mRNAs, and the untranslated *H19* RNA, have been identified. IMPs and their orthologues are implicated in post-transcriptional events such as translational inhibition, RNA localization and RNA stabilization (Yisraeli, 2005). The mammalian proteins are expressed in an oncofetal manner, with high levels in many fetal tissues and low levels in most adult tissues. Moreover, they are frequently up-regulated in transformed cell-lines (Leeds et al., 1997) and solid tumors where they are associated with a high metastatic potential and poor prognosis (Dimitriadis et al., 2007; Kato et al., 2007; Kobel et al., 2007; Sitnikova et al., 2008; Tessier et al., 2004). In the mouse, fetal expression is high in neuronal tissue, and IMP is also found in heart, lung, liver, intestine, epidermis and reproductive organs as well as in the snout, tail and limb buds (Hansen et al., 2004; Mori et al., 2001; Nielsen et al., 1999). In cultured neurons, IMPs are found in discrete particles in both axons and dendrites, and participate in the transport of mRNA to the growth cone in response to stimulation (Eom et al., 2003; Leung et al., 2006; Zhang et al., 2001). Moreover, Vg1RBP is necessary for migration of neural crest cells in the *Xenopus* embryo (Yaniv et al., 2003), and IMP1 and 3 are critical for invadopodia formation in cultured mammalian cells (Vikesaa et al., 2006). Recently, isolation of IMP granules revealed that the ribonucleoprotein (RNP) particles contain about 1% of the entire transcriptome (Jonson et al., 2007).

Two dIMP proteins differing at the N-terminus are expressed from alternative promoters on the X chromosome (Fig. 1B). The two isoforms were recently reported to have different functions. SD-dIMP is functional in the oocyte, whereas RE-dIMP is active in motor neurons (Boylan et al., 2008). Both dIMP proteins contain four hnRNP K homology (KH) RNA-binding domains, but lack the two N-terminal RNA recognition motives (RRMs) present in verte-

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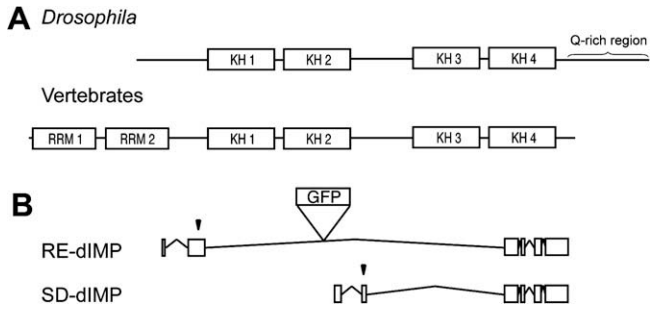

Fig. 1. Schematic view of dIMP transcripts and proteins. (A) The modular architecture of dIMP and its vertebrate homologues. dIMP contains four KH domains and exhibits a glutamine(Q)-rich C-terminus, but it lacks the N-terminal RRMs. (B) Schematic view of the two different *dIMP* transcripts reported by Boylan and colleagues (Boylan et al., 2008). Boxes designate exons and lines refer to introns. Arrowheads mark translational initiation codons. The figure is an outline of the complex transcript pattern reported in FlyBase (Wilson et al., 2008). The GFP-exon is only spliced into the transcripts encoding the RE polypeptide.

brate IMPs. Instead, the dIMP isoforms exhibit a glutamine-rich C-terminal tail that is absent in the vertebrate homologues (Fig. 1A).

The dIMP protein is expressed in both the oocyte and in the nurse cells (Munro et al., 2006), but its expression has so far not been thoroughly described in the embryo. Maternal *dIMP* transcripts are ubiquitous in the early embryo, but at embryonic stage 5, the transcript almost disappears except in the pole cells. Later, zygotic expression of the *dIMP* transcript is seen in the central nervous system (CNS) and epidermis (Nielsen et al., 2000). In the third instar larvae, dIMP protein is found in moving RNPs in motor axons, and somewhat paradoxically, both the removal of dIMP protein and its increased expression give rise to severe locomotion defects such as an inability to fly, to crawl up the sides of the vial, or to straighten themselves up when falling over (Boylan et al., 2008). Examination of the synapses in third instar larvae revealed that loss-of-function and gain-of-function mutants exhibited decreased and increased number of boutons, respectively (Boylan et al., 2008). In the adult fly, the expression of GFP-dIMP fusion protein in four different protein trap lines is seen in both somatic cyst cells and pre-meiotic germ cells of *Drosophila* testis (Fabrizio et al., 2008).

Two molecular studies of dIMP have been made previously, in which dIMP protein was shown to bind *gurken* and *oskar* transcripts (Geng and Macdonald, 2006; Munro et al., 2006). However, no abnormalities in *gurken* and *oskar* regulation were seen in oocytes lacking dIMP (Geng and Macdonald, 2006; Munro et al., 2006). Furthermore, dIMP has been identified in a gain-of-function screen, where third instar larvae overexpressing dIMP showed an abnormal branching pattern of the intersegmental nerve b (ISNb) (Kraut et al., 2001).

In this study, we describe the expression of dIMP protein throughout embryogenesis. In the cellular blastoderm, it was localized apically in the entire cell-layer, as well as in the pole cells. Later, it was found in the developing nervous system, both in the brain, the ventral nerve cord (VNC), and in the peripheral nervous system (PNS). In the latter, expression was detected in both neurons and accessory cells of sensory organs.

1.1. dIMP in the cellular blastoderm

To elucidate the expression and localization pattern of dIMP protein in the cellular blastoderm, we immunostained dIMP with a polyclonal anti-dIMP rabbit antibody (Fig. 2). In stage 5 embryos, immunoreactivity was observed in the entire blastoderm cell-layer. Staining was present throughout the cytoplasm, but it was stronger apically to the nucleus. To determine whether dIMP immunoreactivity could be seen in the nuclei, anti-dIMP and DAPI co-staining experiments were performed (Fig. 2 and Supplementary Fig. S1). No overlap was seen between the DAPI and the dIMP staining, showing that dIMP is cytoplasmic at steady-state. A co-immunostaining with the pole cell marker Vasa showed that dIMP immunoreactivity was also seen in the pole cells at the posterior end (Supplementary Fig. S2), implying that dIMP is expressed in the germline precursors similar to the murine homologue (Hammer et al., 2005).

1.2. GFP-dIMP expression

Whilst studying dIMP immunostainings, we also saw staining of the VNC, but because the VNC develops rapidly around stages 14–16 we decided to follow the development of live embryos to be able to clarify the precise timing of the VNC staining. Several gene trap projects have been performed in *Drosophila*, in which an exon encoding GFP is induced to jump into random positions in the genome. In one such line, called 126-1 (subsequently named GFP-dIMP flies), the GFP-exon is situated in the beginning of the *dIMP* gene (Fig. 1B), which corresponds to an intron present in the *RE-dIMP* transcript. Importantly, mutants without functional dIMP are semi-lethal (Boylan et al., 2008; Geng and Macdonald, 2006; Munro et al., 2006), whereas the GFP-dIMP flies are viable and fertile, suggesting that the fusion protein is functional. Moreover, the expression pattern of GFP-dIMP in 126-1 was identical to that observed when staining wild-type embryos with anti-dIMP antibody (Supplementary Fig. S3).

1.2.1. CNS

Embryos from GFP-dIMP flies were followed by confocal microscopy from the blastoderm stage and throughout the rest of embryogenesis. From the blastoderm stage and until stage 13, fluo-

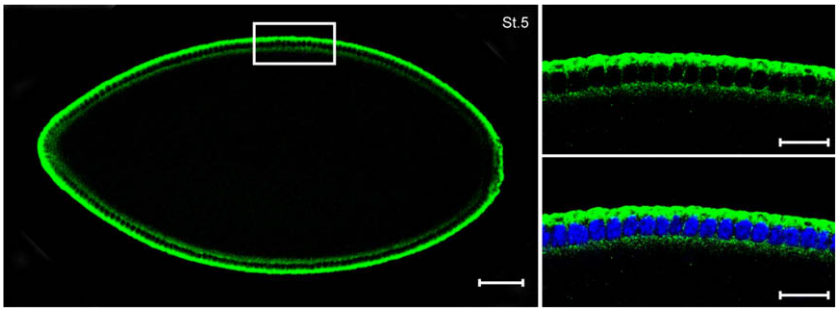

Fig. 2. dIMP expression in the blastoderm embryo. Lateral view of a stage 5 embryo stained with anti-dIMP antibody (anterior to the left, dorsal up). dIMP immunoreactivity is enhanced apically to the nucleus in the entire blastoderm cell-layer. At the right is shown an enlargement of the boxed area co-stained with DAPI. The scale bar is 50 μm in the overview and 20 μm in the enlargement.