immunoblot analysis showed that ALSM III was distributed in a wide variety of tissues, not only in embryos but also in adult squid. These observations indicate that ALSM is a secreted protease produced in the liver that later translocates to several tissues, including muscle and non-muscle tissues. In the present study, we observed ALSM III staining in the iris and in tissue connected to the cornea. In situ hybridization showed no expression of ALSM mRNA in these tissues. Therefore, expression in the iris and in tissue connected to the cornea may be translocated from the liver. We further examined whether ALSM III is present in adult squid eye tissues. However, no signals were detected in these tissues by immunoblot analysis (Fig. 8), or immunofluorescence inspection (data not shown). Thus, distribution of ALSM III in the eyes seems to be developmentally regulated. There are no reports that astacins are involved in eye development or physiological functions of the eye.

In this study, we first showed that expression of ALSM III was higher than that of ALSM I. This result is consistent with the biochemical evidence that in vitro MyHC hydrolysis activity of crude extracts is higher in ALSM III than in ALSM I (Tamori et al., 1999), and purification yields of ALSM III obtained from the same liver extract source as ALSM I are approximately 5-fold (unpublished data), showing that expression of ALSM isoforms is regulated at the transcription level. In summary, our current findings suggest that ALSMs are secreted proteases that are initially expressed at late stages of embryogenesis in the squid liver and then translocate to various muscle and non-muscle tissues. The distribution of ALSM isoforms is differentially regulated, and the isoforms appear to associate with different parts of the tissue. We provide evidence that at least one isoform (ALSM III) contributes to the developmental events of squid. Additional studies are needed to determine the physiologic functions of ALSM isoforms.

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