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Astacin gene family of metalloproteinases in planarians: Structural organization and tissue distribution

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ABSTRACT

Planarian flatworms possess extraordinary regenerative capability and body plasticity, which rely on a composite population of stem cells, the neoblasts. Despite impressive advances have been recently achieved in the knowledge of neoblast biology, few is still known about factors that are released by differentiated tissues and influence the neoblast fate. Extracellular matrix (ECM) is a fundamental component of the stem cell niche and its remodeling affects stem cell fate. Here we provide the characterization of the *astacin* gene family of metalloproteinases in planarians, good candidate enzymes for generating dynamicity in the ECM. Ten and eighteen astacin isoforms were identified in the planarian species *Schmidtea mediterranea* and *Dugesia japonica,* respectively. Besides the already characterized Smedolloid, in *Schmidtea mediterranea* are present eight astacins with a minimal structure (a signal peptide, an activation domain and a Zn-binding catalytic domain), that are colocalized in large cells organized in a peculiar, not yet morphologically characterized, two-ring-shaped structure located in the middle of the body. A single astacin, characterized by a ShK toxin domain in its C-terminal region, has been found to be produced in gastrodermal cells.

Section 1

Astacins are secreted and membrane-bound metzincin metallopeptidases widespread among different animal phyla and involved in various physiological processes, including digestion, extracellular matrix (ECM) remodeling, morphogenesis, hatching, tissue remodeling and differentiation (Gomis-Rüth et al., 2012 and references therein). All members of this family are characterized by two key elements: the 18-amino acid zinc-binding motif (HEXXHXXGFXHEXXRXDR), and the methionine-turn (Met-turn) sequence SXMHY (Gomis-Rüth, 2003; Bond and Beynon, 1995). The N-terminal hydrophobic signal peptide - that directs the proteins into the endoplasmic reticulum during biosynthesis - followed by an activation domain, is another remarkable common feature of astacins. While minimal astacins do not possess any C-terminal domain in addition to the protease domain, other members of the family contain one or more copies of different non-catalytic domains, promoting protein-protein and substrate interactions (Wermter et al. 2007; Hintze et al. 2006; Garrigue-Antar et al., 2001; Sieron et al., 2000). Astacins are temporally and spatially regulated through modulation of gene expression, compartmentalization, allostery or inhibition. However, their primary regulatory mechanism is the zymogenic latency, provided by the presence of the activation domain (Bond and Beynon, 1995; Nguyen et al., 1994). This segment physically blocks the access of substrates to the active site when the enzymatic activity is not required and it is removed during enzyme maturation (Khan and James, 1998).

Expression and function of astacins have been studied during embryogenesis of different animals, being BMP-1 and its *Drosophila* homologues, Tolloid and Tolloid-like, the best characterized members (Ge and Greenspan, 2006). BMP-1/Tolloid members perform multiple functions, including activation of the TGF-β signaling pathway and formation/remodeling of the ECM during development and metamorphosis (Reddi, 1996; Hopkins et al., 2007). BMP-1/Tolloid also plays crucial roles during regeneration. In *Hydra* two Tolloid-like proteinases, HMP-1 and HMP-2, are implicated in head regeneration and transdifferentiation of tentacle battery cells and in foot morphogenesis, respectively (Yan et al., 2000a,b). A BMP-1/Tolloid homolog is involved in morphogenetic movements leading to folding of the luminal epithelium and gut looping during visceral regeneration in the sea cucumber (Mashanov et al., 2012). A Tolloid-like gene (*Smedolloid-1*) has

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Table 1
S.mediterranea astacin sequences and Blastx analysis versus *Homo sapiens* non redundant protein sequences.

	Planmine ID	Genebank ID	Best human blastx hit
Smed-ast-1	dd_Smed_v6_85_0_1	HF952107	PREDICTED: astacin-like metalloendopeptidase isoform X2 [*Homo sapiens*] E-value: 1e-19; identity: 29%
Smed-ast-2	dd_Smed_v6_51_1_1	HF952108	PREDICTED: astacin-like metalloendopeptidase isoform X4 [*Homo sapiens*] E-value: 2e-16; identity: 30%
Smed-ast-3	dd_Smed_v6_345_0_1	HF952109	tolloid-like protein 1 isoform 2 precursor [*Homo sapiens*] E-value: 7e-19; identity: 28%
Smed-ast-4	dd_Smed_v6_76_0_1	HF952110	Select seq gb|AAH13871.1| TLL2 protein [*Homo sapiens*] E-value: 7e-19; identity: 32%
Smed-ast-5	dd_Smed_v6_497_0_1	HF952111	meprin A subunit beta isoform 1 precursor [*Homo sapiens*] E-value: 7e-14; identity: 33%
Smed-ast-6	dd_Smed_v6_51_1_2	HF952112	PREDICTED: astacin-like metalloendopeptidase isoform X4 [*Homo sapiens*] E-value: 1e-18; identity: 30%
Smed-ast-7	dd_Smed_v6_961_0_1	HF952113	PREDICTED: astacin-like metalloendopeptidase isoform X1 [*Homo sapiens*] E-value: 1e-18; identity: 28%
Smed-ast-8	dd_Smed_v6_254_0_1	HF952114	PREDICTED: meprin A subunit alpha isoform X1 [*Homo sapiens*] E-value: 2e-22; identity: 30%
Smed-ast-9	dd_Smed_v6_12688_0_1		PREDICTED: astacin-like metalloendopeptidase isoform X4 [*Homo sapiens*] E-value: 3e-20; identity: 32%
Smedolloid-1	dd_Smed_v6_5652_0_1		tolloid-like protein 1 isoform 1 precursor [*Homo sapiens*] E-value: 0.0; identity: 45%

Table 2
Percentages of identity between *S. mediterranea* astacin putative proteins. Font size increases with the increase in identity percentage.

	Smed-ast-1	Smed-ast-2	Smed-ast-3	Smed-ast-4	Smed-ast-5	Smed-ast-6	Smed-ast-7	Smed-ast-8	Smedolloid-1
Smed-ast-1	–	82%	60%	34%	50%	77%	38%	26%	27%
Smed-ast-2	82%	–	58%	33%	52%	75%	36%	28%	27%
Smed-ast-3	60%	58%	–	35%	49%	62%	36%	24%	25%
Smed-ast-4	34%	33%	35%	–	34%	39%	76%	31%	29%
Smed-ast-5	50%	52%	49%	34%	–	52%	33%	25%	27%
Smed-ast-6	77%	75%	62%	39%	52%	–	38%	27%	24%
Smed-ast-7	38%	36%	36%	76%	33%	38%	–	29%	30%
Smed-ast-8	26%	28%	24%	31%	25%	27%	29%	–	31%
Smedolloid-1	27%	27%	25%	29%	27%	24%	30%	31%	–

also been characterized during planarian regeneration and its functional ablation causes the loss of some differentiated tissues at the regenerative midline (Reddien et al., 2007).

Planarians are free-living Platyhelminthes (Collins, 2017). A population of adult stem cells called neoblasts (Aboobaker, 2011), which includes pluripotent stem cells (Wagner et al., 2011; van Wolfswinkel et al., 2014), accounts for the astonishing regenerative capabilities, high body plasticity and continuous cell turnover of these organisms. Following amputation, neoblasts activate a biphasic proliferative program and accumulate to form a regenerative blastema in which novel tissues and organs are quickly reformed. Following this early epimorphic regeneration, morphallatic rearrangements of the entire body are needed to restore the appropriate body proportion (Elliott and Sánchez Alvarado, 2013; Rossi et al., 2008). Despite sustained efforts to identify micro-environmental cues responsible for neoblast fate control, no conclusive evidence has been provided in demonstrating the existence of a “neoblast-niche” analogous to that described for adult stem cells in other organisms. Thus, the molecular mechanisms by which stem cells respond to differentiated tissue requests are still almost unknown. ECM remodeling undoubtedly serves a dynamic microenvironment for stem cell niche and previous work demonstrates that some matrix metalloproteinases (MMPs) play key roles in planarians, generating the microenvironment in which cells can migrate and express their proliferation/differentiation program (Isolani et al., 2013; Dingwall and King, 2016). With the aim to identify additional candidates for ECM remodeling that might be important for neoblast fate determination, here we provide the identification and characterization of a family of *astacin*-related genes in planarians.

Section 2

2.1. Structural organization of the astacin-like gene family in planarians

By performing a survey of *S. mediterranea* genome, published transcripts and available transcriptome databases, we managed to identify ten genes encoding putative astacins that we named *Smed-ast-1, Smed-ast-2, Smed-ast-3, Smed-ast-4, Smed-ast-5, Smed-ast-6, Smed-ast-7, Smed-ast-8 Smed-ast-9,* and *Smedolloid-1* (Reddien et al., 2007) (Tables 1 and 2). The analysis of genomic organization revealed that some astacin genes are amplified. Indeed, at least two copies for *Smed-ast-2* and *Smed-ast-5* and 3 copies for *Smed-ast-4* and *Smed-ast-6* were detected in the same genome contig. Moreover, the locus coding for Smed-ast-2, Smed-ast-3, and Smed-ast-6, as well as those coding for Smed-ast-4 and Smed-ast-9 are relatively close, being included in the same genomic contig (Supplementary Fig. S1 A).

Based on the genomic organization of introns and exons the planarian astacin genes can be subdivided in three groups. The first group is represented by *Smed-ast-1* and *Smed-ast-2*, each with four exons and three introns. The length and position of the introns and exons appear well conserved between the two genes. The second group is represented by *Smed-ast-3, Smed-ast-4 Smed-ast-6, Smed-ast-7* and *Smed-ast-9* with five exons and four introns, again preserved in length and position. Finally, the third group is represented by *Smed-ast-5* and *Smed-ast-8*, with six and seven exons, respectively. Both *Smed-ast-5* and *Smed-ast-8* are characterized by the presence of a long intron, of about 1 Kb, in position three and five, respectively (Supplementary Fig. S1 B).

Smed-ast-1, Smed-ast-2, Smed-ast-3, Smed-ast-4, Smed-ast-5, Smed-ast-6, Smed-ast-7 and *Smed-ast-9* encode astacins with a minimal structure, including a signal peptide, an activation domain and a Zn-binding