Seminars in Cell & Developmental Biology 60 (2016) 5–9

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Developmental roles of Rhomboid proteases

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ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form 12 July 2016
Accepted 12 July 2016
Available online 14 July 2016

Keywords:
Rhomboid
EGF receptor
Ligand processing
Spitz
Star
Drosophila
Endoplasmic reticulum

ABSTRACT

Rhomboid proteins have emerged as one of the most tantalizing and diverse families of proteases. Gene duplication events and structural alterations have sculpted the varied roles of this protein family, maintaining a conserved structural core throughout the bacterial, plant and animal kingdoms. Unresolved questions pop up at many junctions. This review will focus on a distinct class of Rhomboid proteins that plays an essential role in development. It will outline the diverse mechanisms by which these proteins are regulated, and the implications on the biological processes they control. While most of the review will deal with Rhomboids in *Drosophila*, a system that has been studied in the greatest detail, it will also explore parallels and differences in the function of Rhomboids in the flour beetle *T. casteneum* and the worm *C. elegans*.

Contents

1. Rhomboid1 intracellular localization and trafficking of EGFR ligands

Rhomboid proteases maintained a conserved structural core throughout the bacterial, plant and animal kingdoms [1,2]. Epidermal growth factor (EGF) receptor signaling plays a pivotal role in determination of cell fates at many junctions during *Drosophila* embryonic and post-embryonic development [3,4]. As a rule, the receptor and its downstream canonical Mitogen activated protein kinase (MAPK) pathway are ubiquitously expressed, "awaiting" the regulated activation by ligands. The tight spatial and temporal regulation of the pathway is dictated by the ligands. Three components are essential for ligand processing and secretion. The cardinal ligand, Spitz (Spi), is produced as an inactive transmembrane precursor [5], and retained in the endoplasmic reticulum (ER). It is trafficked by bulk flow to the Golgi, but returned to the ER by retrograde trafficking. In order to prevent retrograde trafficking and allow processing and secretion, a second protein, Star, is required. Star is a type II transmembrane protein that associates with Spi in the ER and blocks its retrograde trafficking [6,7]. Following trafficking from the Golgi to a Rab 4 and Rab14 enodosomal compartment [8], the Spi/Star complex encounters the Rhomboid1 protein, which cleaves the transmembrane domain of the Spi precursor [9]. This releases the Spi extracellular domain containing the

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http://dx.doi.org/10.1016/j.semcdb.2016.07.014
1084-9521/

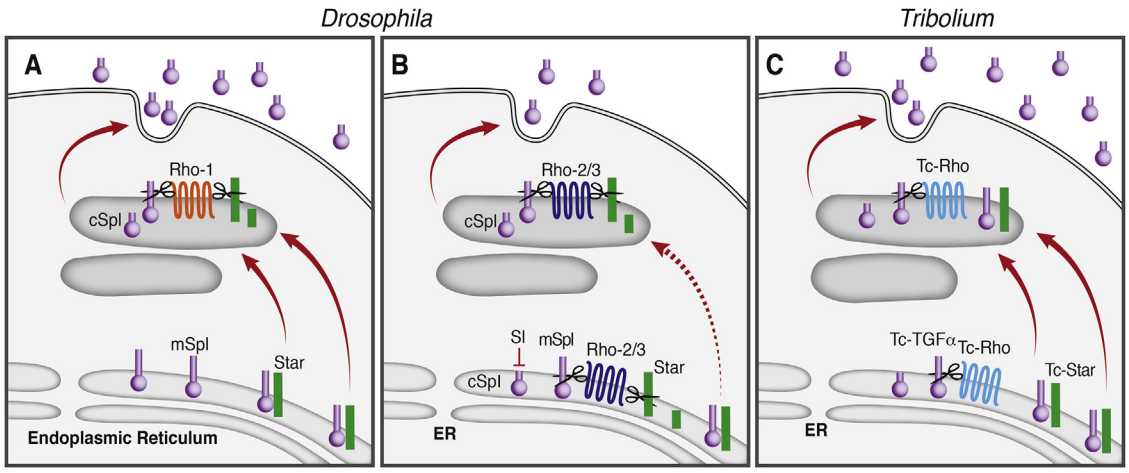

Fig. 1. Diversification of Rhomboid intracellular localization impinges on EGFR ligand processing.
A,B) In *Drosophila*, the existence of multiple Rhomboid proteins enables modulation of the processed ligand levels by differential compartmentalization. Rhomboid1 is located only at the secretory compartment and mediates high signaling levels (A). Rhomboids2/3 display a combined ER and secretory compartment localization and cleavage activity, leading to an attenuated signal, primarily due to cleavage in the ER of the ligand chaperone Star. (B). The cleaved ligand (cSpi) generated in the ER, is retained by Small wing (Sl) and is thus inactive. C) In the flour beetle *Tribolium*, the single Rhomboid is active in the combined ER and secretory compartment mode. Signaling levels remain high, however, since Tc-Star is refractive to Rhomboid cleavage and can efficiently traffic the Spi precursor to the secretory compartment.

EGF motif, allowing its subsequent secretion to the extracellular milieu (Fig. 1A).

Three elements, Spi, Star and Rhomboid, comprise a cassette that is sufficient for ligand processing. When expressed in *Drosophila* S2 cells or in mammalian cells, efficient trafficking and cleavage ensues [6,7]. This indicates that the signals that target each of these proteins to distinct cellular compartments are conserved. It also implies that once Rhomboid and Spi are located within the same compartment, efficient cleavage takes place, suggesting that no accessory proteins are required. The primary regulation of the processing cassette is thus executed at the level of intracellular compartmentalization and trafficking. It follows that diversification of function or regulation of Rhomboid proteins may emerge from alterations in their intracellular localization.

2. Rhomboid1 exhibits restricted substrate specificity

Proteins of the Rhomboid family exhibit a diverse array of substrate specificities. For example, members that are involved in quality control in the ER are required to recognize a broad range of substrates [10]. Rhomboid1, on the other hand, displays a very narrow substrate specificity. A powerful approach to gauge the roles and potential substrate specificity of Rhomboid1 is to define the mutant phenotype resulting from its absence. In view of the multiple roles of EGFR throughout development, there are many instances in which the phenotypes can be monitored. In all cases, the phenotype of Rhomboid1 loss-of-function is synonymous with loss of elements in the ligand cassette (Spi, other ligands and Star), or with mutants in EGFR [11,12]. This indicates that Rhomboid1 is dedicated to the EGFR pathway, and does not have substrates outside this cascade. Moreover, Rhomboid1 activity is essential and thus its absence results in a complete shutoff of the pathway.

Within the EGFR pathway, what are the Rhomboid1 substrates? There are four ligands that activate *Drosophila* EGFR [13]. Three of them (Spi, Gurken and Keren) are produced as type I transmembrane precursors that require Rhomboid cleavage for activation [5,14,15]. The fourth ligand, Vein, is produced as a secreted protein that does not require processing [16]. Surprisingly, another member of the ligand-processing cassette, Star, is also a substrate for Rhomboid1 [17]. This highlights the capacity of Rhomboid proteins to cleave both type I and type II transmembrane proteins. It is not clear however, if Star cleavage by Rhomboid1 has a significant regulatory outcome. The two proteins first "meet" in the late secretory compartment, after Star has already fulfilled its job of bringing Spi to Rhomboid1. Following Star cleavage and inactivation, the prevention of Star recycling may have a regulatory significance. As will be outlined below, the capacity of Rhomboids to cleave Star has substantial regulatory implications for other members of the Rhomboid family.

3. Regulation of *rhomboid1* expression

If spatial and temporal regulation of EGFR activation relies on the ligand-processing cassette, which of the three elements in this group is normally limiting? Expression of Spi and Star appears to be ubiquitous. Presumably, in the absence of Rhomboid the Spi/Star complex fails to reach the plasma membrane. Thus, the Spi precursor is protected from fortuitous cleavage by broad-specificity metalloproteases that reside on the plasma membrane. In contrast, expression of *rhomboid1* is extremely restricted and dynamic [18–20]. In fact, there is a strict correlation between the expression pattern of Rhomoid1 and the activation of the EGFR pathway as monitored by staining for phosphorylated MAPK [21,22]. Moreover, ectopic expression of *rhomboid1* in different tissues leads to broad EGFR activation, indicating that Rhomboid is the only limiting element in the ligand-processing cassette [20].

The entire plan for EGF activation during development is essentially "etched" into the enhancer of *rhomboid1*. Dissection of this enhancer has indeed identified discrete regulatory elements that are dedicated to the expression of Rhombodi1 in the different tissues [19,23]. Having the regulatory switch for the EGFR pathway rely on the expression of an enzyme is a "risky" prospect, since low levels of leaky expression of *rhomboid1* could give rise to fortuitous EGFR activation. Nevertheless, activation of EGFR is tight, suggesting that the switch of *rhomboid1* expression is firm. In addition, biochemical studies have implied that intra-membrane proteolysis is a slow inefficient process, and the turnover times for substrates are extremely long [24]. Thus, the number of processed ligand molecules that could be generated by a "stray" Rhomboid1 protein may not be high.

The normal expression of *rhomboid1* defines the cells that are actively processing the ligand. On the other hand, the pattern of phosphorylated MAPK in the tissues where EGFR is activated outlines the distribution profile of the active ligand itself. A comparison