Seminars in Cell & Developmental Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

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

Review

Hedgehog and its circuitous journey from producing to target cells

Isabel Guerrero[a,*], Thomas B. Kornberg[b]

[a] Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, E-28049 Madrid, Spain
[b] Cardiovascular Research Institute, University of California, San Francisco, CA 94158, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Hh gradient
Hh processing
Hh recycling
Cytonemes

ABSTRACT

The hedgehog (Hh) signaling protein has essential roles in the growth, development and regulation of many vertebrate and invertebrate organs. The processes that make Hh and prepare it for release from producing cells and that move it to target cells are both diverse and complex. This article reviews the essential features of these processes and highlights recent work that provides a novel framework to understand how these processes contribute to an integrated pathway.

Contents

1. Introduction

Hedgehog (Hh) was identified by genetic screens in *Drosophila melanogaster*, and much of our understanding of its roles and of the mechanisms involved in Hh signaling has come from studies in the fly. *hh* received its name from the phenotype of mutant *Drosophila* embryos, which have a lawn of disorganized hair-like cuticular protrusions reminiscent of a hedgehog's spines [1]. Subsequent findings show that Hh has many roles and is not dedicated only to embryo segmentation. Indeed, Hh is essential for most organs in the fly and for many metazoan tissues, and in many of these contexts it appears to function as a morphogen, spreading from the

* Corresponding author. Tel.: +34 91 196 4680; fax: +34 64 797 6897.
E-mail address: iguerrero@cbm.csic.es (I. Guerrero).

http://dx.doi.org/10.1016/j.semcdb.2014.06.016
1084-9521/

Please cite this article in press as: Guerrero I, Kornberg TB. Hedgehog and its circuitous journey from producing to target cells. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.06.016

cells that express it to trigger differential, concentration-dependent responses in target cells. The mechanism by which it spreads is both fascinating and important to understanding its function.

In mammals, the Hh protein family has three members: Sonic Hh (Shh) [2, reviewed in 3], Indian Hh (Ihh), and Desert Hh (Dhh). Shh has multiple roles and appears to function in several modes. In the ventral neural tube, it induces distinct neural fates in a concentration-dependent manner [reviewed in 3]. A temporal gradient of Shh gradient has been implicated in the specification of digit identities [4,5]. And in the developing nervous system, there is evidence for a role of graded Hh activity in the control of axon guidance such that the growth cone and axon respond to the gradient slope rather than to absolute concentration [6]. These findings suggested a model in which the Shh gradient initially patterns the ventral spinal cord, acting as a morphogen, and subsequently functions in axon guidance [7–11]. Although the signaling pathway that directs growth cone turning may differ from the Hh pathway in other contexts [12], Hh appears to function as a paracrine, non-autonomous signal in all contexts. Ihh and Dhh also appear to be paracrine signals. Ihh negatively regulates the differentiation of proliferating chondrocytes in the appendicular skeleton [13,14]. Dhh regulates the male germline [15–17].

A key feature of the Hh protein is its lipophilicity. It is modified with cholesterol and palmitate, and as a consequence has high affinity for membranes. Despite this, in many contexts Hh travels many cell diameters from the cells that produce it, for example up to 50 μm (30–40 cells) in the Drosophila wing imaginal disk. In this organ, Hh is expressed by posterior (P) compartment cells and distributes to form a concentration gradient that spans approximately 8–10 anterior (A) compartment cells from the A/P compartment border (Fig. 1). Recent findings now show how Hh moves to generate such concentration gradients [18,19]. Much prior work has identified and characterized the processes that produce, disperse and receive Hh, showing that these processes are complex and involve the contributions of many different proteins. Although fundamental aspects of these processes remain to be elucidated, our purpose in this review is to present what is known about them and to describe how these processes relate to and can be understood in the context of the mechanism that generates Hh gradients.

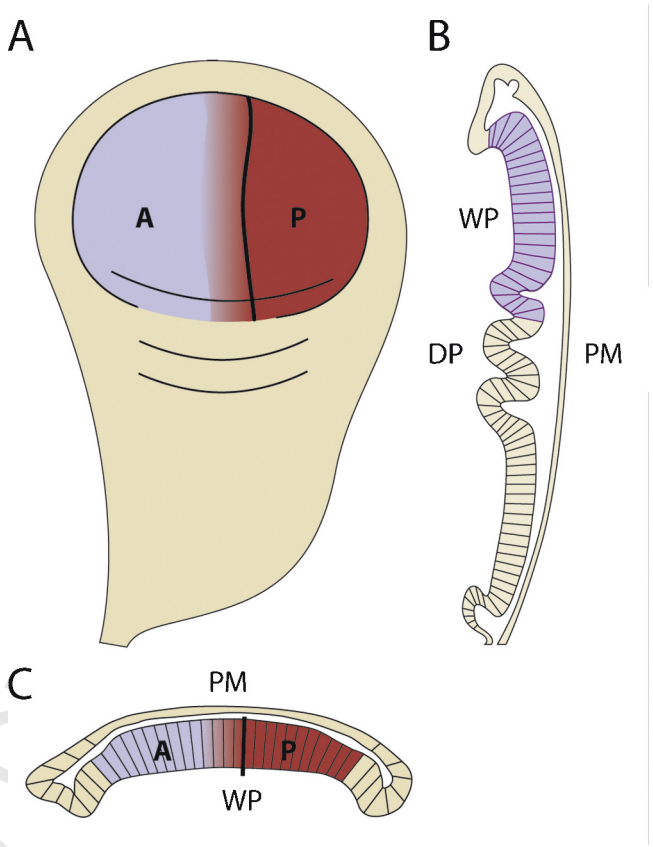

Fig. 1. Schematic representation of the epithelial layers of the *Drosophila* wing imaginal disk. (A) Frontal representation of the disk proper (DP) of the wing imaginal disk. The A compartment of the presumptive wing region (wing pouch) is indicated in blue and Hh expression in the P compartment in red. (B and C) Longitudinal section (B) and cross-section (C) of the wing disk showing the Peripodial Epithelium (PE) and DP epithelium. The wing pouch is also labeled as (WP) in (B and C). The Hh gradient at the A/P compartment border is graded red, and a thick line marks the A/P compartment border in (A, C).

2. Hh production

2.1. Hh processing

Post-translational processing of Hh removes N-terminal signal sequence residues (1–84 of Hh, and 1–23 of Shh) and attaches palmitate by a stable amide linkage to the N-terminal cysteine of the N-terminally truncated Drosophila Hh (c85) and vertebrate Shh (c24) proteins [20–22]. Palmitate addition is catalyzed in the endopasmic reticulum (ER) by membrane-bound O-acyltransferases [23]. In *Drosophila*, the acyltransferase encoded by *rasp* (also known as *sightless*, *skinny hedgehog*, and *central missing*) is required in cells that produce Hh (Fig. 2, right panel) [21,24–26]. Skn, the murine homolog of *rasp*, is essential for Shh activity and for generation of the Shh protein gradient [27], and the purified human Hhat, a Rasp homolog, palmitoylates Shh in cells and in vitro [20,28].

Hh also undergoes autoproteolytic cleavage in the ER, splitting into two parts to generate a modified N-terminal fragment (HhNp), which is linked to cholesterol, and an unmodified C-terminal fragment (HhC) (Fig. 2, right panel) [29]. Much evidence testifies to the importance of this post-translation processing and to the signaling functionality of the HhN domain. For example, mutations in human Shh that block cleavage and modification are associated with holoprosencephaly, a congenital malformation [27,30–32]. However, there is also evidence suggesting that Hh retains activity even if it has a defective protease active site (e.g., Hh-h329a) and has not undergone autocleavage and is not modified with cholesterol [33,34]. Recombinant uncleaved protein (HhU) activates signaling in cultured vertebrate cells, and HhU that is ectopically-expressed in fly eye imaginal discs partially rescues *hh* mutant phenotypes in the eye [34]. Nevertheless, it has not been established that HhU is present or is an active paracrine signal under normal conditions. Shh autoprocessing is inhibited by depletion of sterols [35]. Unprocessed Hh molecules are targeted for degradation by the ERAD (ER-associated degradation) cascade; this mechanism could affect the levels of Hh in the mutants with impaired processing (Fig. 2, right panel) [23].

The protease active site that catalyzes autoprocessing is located in the HhC domain. It has a catalytic histidine (h329), and its activity and structure are homologous the processing domains of inteins [36, reviewed in 37]. After cleavage, the C-terminal domain is rapidly degraded in the ER lumen by the ERAD pathway [23]. HhC may have additional functions. Studies of Hh signaling in the *Drosophila* eye suggest that the C-terminal domain targets Hh to axons and growth cones of photoreceptor neurons [38].

2.2. Roles of the Hh lipids

The roles of the palmitate and cholesterol modifications have been investigated by expressing altered forms of Hh proteins that either lack palmitate but have cholesterol (Hh-c85s,

Please cite this article in press as: Guerrero I, Kornberg TB. Hedgehog and its circuitous journey from producing to target cells. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.06.016