Hedgehog ligand biogenesis

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

18/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

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Reactome database release: 82

This document contains 2 pathways and 10 reactions (see Table of Contents)

https://reactome.org
Mammalian genomes encode three Hedgehog ligands, Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH). These secreted morphogens can remain associated with lipid rafts on the surface of the secreting cell and affect developmental processes in adjacent cells. Alternatively, they can be released by proteolysis or packaging into vesicles or lipoprotein particles and dispersed to act on distant cells. SHH activity is required for organization of the limb bud, notochord and neural plate, IHH regulates bone and cartilage development and is partially redundant with SHH, and DHH contributes to germ cell development in the testis and formation of the peripheral nerve sheath (reviewed in Pan et al, 2013).

Despite divergent biological roles, all Hh ligands are subject to proteolytic processing and lipid modification during transit to the surface of the secreting cell (reviewed in Gallet, 2011). Precursor Hh undergoes autoproteolytic cleavage mediated by the C-terminal region to yield an amino-terminal peptide Hh-Np (also referred to as Hh-N) (Chen et al, 2011). No other well defined role for the C-terminal region of Hh has been identified, and the secreted Hh-Np is responsible for all Hh signaling activity. Hh-Np is modified with cholesterol and palmitic acid during transit through the secretory system, and both modifications contribute to the activity of the ligand (Porter et al, 1996; Pepinsky et al, 1998; Chamoun et al, 2001).

At the cell surface, Hh-Np remains associated with the secreting cell membrane by virtue of its lipid modifications, which promote clustering of Hh-Np into lipid rafts (Callejo et al, 2006; Peters et al, 2004). Long range dispersal of Hh-Np depends on the untethering of the ligand from the membrane through a variety of mechanisms. These include release of monomers through the combined activity of the transmembrane protein Dispatched (DISP2) and the secreted protein SCUBE2, assembly into soluble multimers or apolipoprotein particles or release on the surface of exovesicles (Vyas et al, 2008; Tukachinsky et al, 2012; Chen 2004; Zeng et al, 2001; reviewed in Briscoe and Therond, 2013).

**Literature references**


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Glycosylation of Hh

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5362386

Type: omitted

Compartments: endoplasmic reticulum lumen

Proteomic studies show that the C-termini of SHH and IHH are N-glycosylated (Liu et al, 2005). A N278A mutant of SHH does not undergo cholesterol-mediated autoproteolysis suggesting that glycosylation is a prerequisite for this processing step (Huang et al, 2013).

Followed by: P4HB forms mixed disulphides with Hh precursors

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P4HB forms mixed disulphides with Hh precursors

**Location:** Hedgehog ligand biogenesis

**Stable identifier:** R-HSA-5358336

**Type:** binding

**Compartments:** endoplasmic reticulum lumen

Hh ligands undergo a autoproteolytic cleavage mediated by a conserved residue in the C-terminal region to yield an N-terminal fragment destined for further modification and secretion, and a C-terminal fragment that is subsequently degraded by ERAD (reviewed in Gallet, 2011). Recent work has shown that autoproteolytic cleavage of Hh ligands depends on prior formation of an intramolecular disulphide bond between the catalytic cysteine residue and another conserved cysteine residue in the C-terminal region of the precursor (Chen et al, 2011). Mutation of either of these cysteine residues abolishes cleavage, suggesting that the intramolecular disulphide bond is required to establish a catalytically active conformation of the precursor (Chen et al, 2011).

Prior to the autoproteolytic cleavage reaction, the protein disulphide isomerase P4HB is required to reduce the intramolecular disulphide, freeing the catalytic cysteine side chain for nucleophilic attack. Mutational analysis and co-immunoprecipitation studies support a model where the N-terminal CXXC motif of P4HB forms a mixed disulphide with the non-catalytic cysteine residue of the Hh precursor (Chen et al, 2011).

**Preceded by:** Glycosylation of Hh

**Followed by:** Autoproteolytic cleavage of Hh precursors

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Autoproteolytic cleavage of Hh precursors

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5358340

Type: transition

Compartments: endoplasmic reticulum lumen

Autoproteolytic processing of the Hh precursor is essential for the production of active secreted Hh ligand and mutants that disrupt this processing have been identified in the congenital nervous system disorder holoprosencephaly (Traiffort et al, 2004; Maity et al, 2005; Roessler et al, 2009; reviewed in Jiang et al, 2008). Cleavage of Hh occurs through two nucleophilic substitutions. The first step is mediated by the catalytic cysteine residue, which is found in a conserved G-C-F motif. The cysteine side chain attacks the carbonyl carbon of the main peptide chain between the glycine and cysteine residues, replacing the amino group in the peptide backbone with a thioester linkage (Lee et al, 1994; Porter et al, 1995; Porter et al, 1996a, b; Chen et al, 2011). The second step involves nucleophilic attack of the same carbonyl group by cholesterol. This step displaces the C-terminal fragment (Hh-C) of the Hh precursor and results in the formation of the N-terminal fragment (Hh-Np) modified at its C-terminus by an ester linkage with cholesterol (Porter et al, 1996a, b; Chen et al, 2011). Cholesterol modification appears to contribute to further processing and trafficking of the Hh ligand, as engineered forms of vertebrate and fly Hh that lack cholesterol are not efficiently palmitoylated (Pepinsky et al, 1998). Cholesterol also restricts the diffusion of the secreted ligand by interacting with the lipid bilayer of the secreting cell. Consistent with this, aberrant activation of Hh target genes is seen in the absence of cholesterol modification (Peters et al, 2004; Guerrero et al, 2007; Li et al, 2006; Huang et al, 2007).

Preceded by: P4HB forms mixed disulphides with Hh precursors

Followed by: HHAT palmitoylates Hh N-terminal fragment, C-terminal Hh fragments are bound by lectins

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In addition to being modified by cholesterol at its C-terminal end, the N-terminal fragment of Hh (Hh-Np) is also palmitoylated by the O-acyltransferase HHAT (Pepinsky et al, 1998; Charmoun et al 2001; Chen et al, 2004; Hardy and Resh, 2007). HHAT-mediated palmitoylation of Hh can be recapitulated in vitro and in vivo, and the cholesterol- and palmitoyl-modified N-terminal fragment represents the predominant secreted form of Hh in vivo (Buglino and Resh, 2008; Buglino and Resh, 2010; Taipale et al, 2000). Mutation or depletion of the HHAT enzyme and mutation of the palmitoyl acceptor cysteine in Hh itself abrogates palmitoylation of the ligand and reduces Hh signaling (Chen et al, 2004; Chamoun et al, 2001; Pepinsky et al, 1998; Callier et al, 2014).

**Preceded by:** Autoproteolytic cleavage of Hh precursors

**Followed by:** Hh-Np traffics to the plasma membrane

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Hh-Np traffics to the plasma membrane where it may cluster in lipid rafts. Cholesterol modification is thought to contribute to this transit and targeting, possibly by promoting interaction with the lipid raft components caveolin and flotillin2, although this has not been demonstrated in mammalian cells (reviewed in Gallet, 2011). Cholesterol and lipid modification also impact the effective signaling range of the secreted ligand: due to the hydrophobic nature of these modifications, Hh-Np remains closely associated with the plasma membrane of the secreting cell, where it is competent for short-range signaling to adjacent cells. Long-range signaling depends on a number of possible mechanisms to extract the ligand from the secreting cell, including oligomerization of ligand into micelle-like structures, cleavage of the C-terminal cholesterol moiety by metalloproteases, or interaction with additional factors that help promote release from the plasma membrane (reviewed in Gallet, 2011; Briscoe and Therond, 2013).

Preceded by: HHAT palmitoylates Hh N-terminal fragment

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Release of Hh-Np from the secreting cell

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5362798

Cholesterol and palmitoyl-modification of Hh-Np render the ligand highly hydrophobic and results in its close association with the plasma membrane of the producing cell after secretion. Hh-Np tethered in this way may cluster in sterol-rich lipid rafts where it is competent for short-range signaling. Cell surface Hh-Np also interacts with glypican components of the extracellular matrix and this interaction stabilizes the ligand and is required for its lateral spread. Together, clustering into lipid rafts and interaction with HSPGs may favour packaging of ligand into higher order forms required for ligand dispersal.

Long-range signaling requires release of Hh-Np from the secreting cell. Release is achieved through a number of possibly overlapping mechanisms. These include oligomerization into micelle-like structures, packaging into lipoprotein particles and interaction with cholesterol-binding adaptor proteins such as DISP and SCUBE2. In addition, Hh-Np can be released from the plasma membrane through proteolytic cleavage: NOTUM is a secreted enzyme that is thought to promote the release of Hh-Np by cleaving the GPI anchor of Hh-associated glypicans, while the transmembrane metalloprotease ADAM17 promotes long-range Hh signaling by removing the palmitoyl- and cholesterol-modified N- and C-termini of the membrane-associated ligand. How all these mechanisms are coordinated remains to be elucidated (reviewed in Briscoe and Therond, 2013; Gallet, 2011).

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C-terminal Hh fragments are bound by lectins

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5362437

Type: binding

Compartments: endoplasmic reticulum membrane

Promotion of the cholesterol-mediated autocleavage reaction is the only well documented role for the C-terminal region of intact Hh (reviewed in Briscoe and Therond, 2013), and recent in vitro studies suggest that the Hh-C fragment generated by autocleavage is subsequently targeted to the endoplasmic reticulum-associated degradation (ERAD) pathway (Chen et al, 2011; Huang et al, 2013). This pathway delivers N-glycosylated ER-resident substrates to a retrotranslocation channel, where they are ubiquitinated and translocated to the cytosol for proteasome-mediated degradation in an ATP-ase dependent fashion (reviewed in Vembar and Brodsky, 2008). Recognition and targetting of ER proteins for the ERAD pathway depends at least in part on modification and binding of the glycosyl groups. Consistent with this, depletion of the lectins OS9 and ERLEC1 abrogates degradation of the Hh-C fragments (Chen et al, 2011). OS9 and ERLEC1 may target Hh-C to the retrotranslocation channel by virtue of their interaction with SEL1, an ER membrane protein with established roles in the ERAD pathway (Christianson et al, 2008; Mueller et al, 2008; Hosokawa et al, 2008; Hosokawa et al, 2009).

Preceded by: Autoproteolytic cleavage of Hh precursors

Followed by: C-terminal Hh fragments are recruited to SEL1:SYVN1 at the ER membrane

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C-terminal Hh fragments are recruited to SEL1:SYVN1 at the ER membrane

**Location:** Hedgehog ligand biogenesis

**Stable identifier:** R-HSA-5362441

**Type:** binding

**Compartments:** endoplasmic reticulum membrane

After binding to OS9/ERLEC1, the Hh C-terminal fragments are recruited to the ER membrane through a lectin-SEL1 interaction. SEL1 is a component of a multiprotein retrotranslocation complex in the ER membrane that also includes the E3 ubiquitin ligase SYVN1 (also known as HRD1; present as a dimer), DERL2 and the hexameric ATPase VCP (Christianson et al, 2008; Hosokawa et al, 2008; Mueller et al, 2008; Chen et al, 2011; Huang et al, 2013; reviewed in Vembar and Brodsky, 2008). Depletion of SEL1, SYVN1, VCP or DERL2 results in the accumulation of the Hh-C in the ER lumen (Chen et al, 2011; Huang et al, 2013).

**Preceded by:** C-terminal Hh fragments are bound by lectins

**Followed by:** SYVN1 ubiquitinates Hh C-terminal fragments

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SYVN1 ubiquitinates Hh C-terminal fragments

**Location:** Hedgehog ligand biogenesis

**Stable identifier:** R-HSA-5362412

**Type:** transition

**Compartments:** endoplasmic reticulum membrane

SYVN1 ubiquitinates Hh-C as part of the retrotranslocon that targets these Hh fragments for degradation through the ERAD pathway. Both depletion of SYVN1 by siRNA and expression of a catalytically inactive form of the enzyme strongly inhibits Hh-C degradation. Consistent with this, a dominant negative version of SYVN1 abrogates the polyubiquitination of Hh-C as assessed by IP-Western from HEK293 cells (Chen et al, 2011).

**Preceded by:** C-terminal Hh fragments are recruited to SEL1:SYVN1 at the ER membrane

**Followed by:** VCP-catalyzed ATP hydrolysis promotes the translocation of Hh-C into the cytosol

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VCP-catalyzed ATP hydrolysis promotes the translocation of Hh-C into the cytosol

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5362459

Type: transition

Compartments: cytosol

The ATPase activity of VCP is required for the retrotranslocation of Hh-C across the ER membrane (Chen et al, 2011). Although in this pathway, the VCP hexamer is shown as part of the SEL1:SYVN1:DERL2 retrotranslocon, the details, order of events and even the full complement of protein players in this process are not known. In yeast, the VCP homologue Cdc48 is associated with two additional proteins Ufd1 and Npl4 -both of which are also conserved in mammals- and this complex interacts with several ER components including derlins and the yeast SYVN1 homologue, Hrd1 (reviewed in Vembar and Brodsky, 2009). Consistent with the yeast data, VCP interacts with DERL2 by co-immunoprecipitation in HEK293 cells (Huang et al, 2013).

Preceded by: SYVN1 ubiquitinates Hh C-terminal fragments

Followed by: Hh C-terminal fragments are degraded by the proteasome

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Hh C-terminal fragments are degraded by the proteasome

Location: Hedgehog ligand biogenesis

Stable identifier: R-HSA-5362448

Type: omitted

Compartments: cytosol

After retrotranslocation to the cytosol, Hh-C is degraded by the proteasome (Chen et al, 2011; Huang et al, 2013).

Preceded by: VCP-catalyzed ATP hydrolysis promotes the translocation of Hh-C into the cytosol

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