

SPOTLIGHT

Circulating Hedgehog: a fresh view of a classic morphogen

Elodie Prince, Julien Marcetteau and Pascal P. Théron*

ABSTRACT

Members of the Hedgehog family of morphogens mediate the intercellular communication necessary for the organisation and development of many animal tissues. They are modified by various lipid adducts, rendering them insoluble in hydrophilic environments and leading to the contentious question of how these molecules travel in the aqueous extracellular space. Seminal work carried out by Suzanne Eaton and her colleagues has shed light on how these morphogens can spread over long distances through their association with lipoprotein particles. In this Spotlight article, we discuss Suzanne's pioneering work and her contribution to our understanding of the transport and activity of morphogens, in particular Hedgehog. We also describe two other essential aspects of her work: the discovery and characterisation of endogenously present Hedgehog variants, as well as her proposition that, in addition to its role as a morphogen, Hedgehog acts as an endocrine hormone.

KEY WORDS: Endocrine hormone, Hedgehog/Wingless, Lipoproteins, Morphogens, Signalling, Endocannabinoids

Introduction

Secreted morphogens are classically thought to diffuse from their source, forming an extracellular gradient through which they communicate spatial information to surrounding tissues (Wolpert, 2016). The local morphogen concentration is then interpreted by receptive cells, triggering changes in cell fate and tissue patterning. Morphogens typically signal in a paracrine manner, acting over both short and long ranges (Ashe and Briscoe, 2006). This raises the question of how morphogens travel from their source to receiving cells, and how a functional morphogen gradient is established and maintained. Answering these questions has long presented both intellectual and technical challenges.

Hedgehog (Hh) family secreted proteins are classical morphogens that were originally identified in *Drosophila*. In *Drosophila*, Hh is responsible for patterning processes during embryonic segmentation and the development of the larval imaginal discs (Lee et al., 2016). Its close vertebrate orthologue, sonic hedgehog (Shh), is also essential for developmental patterning, for example during limb development and neural tube morphogenesis (Briscoe and Théron, 2013). Hh family proteins are modified by lipid adducts: a palmitic acid (saturated fatty acid) on their amino terminus and a cholesterol moiety covalently attached to their carboxy terminus (Pepinsky et al., 1998; Porter et al., 1996). These lipid modifications render Hh proteins hydrophobic, providing them with a high affinity for cell membranes. These characteristics would seem somewhat paradoxical for extracellular signalling molecules, and suggest the existence of

mechanisms to transport Hh proteins in the aqueous extracellular space.

As we highlight below, Suzanne Eaton (1959-2019) addressed this paradox during her career, approaching it using a variety of interdisciplinary approaches and yielding fundamental insights into our understanding of Hh release, spread and activity.

Lipoprotein particles as morphogen carriers

Suzanne's group initially proposed that Hh spreads in association with extracellular lipidic particles (Greco et al., 2001), which they later identified as lipoprotein particles (Panáková et al., 2005). Lipoprotein particles allow Hh to spread by providing a carrier to which Hh can bind, shielding its lipid hydrophobic moieties. This transport mechanism came as a surprise, as lipoproteins had primarily been studied for their roles in the transport of dietary and endogenous lipids between tissues.

In *Drosophila*, the major lipoprotein Lipophorin (Lpp; also known as Apolipophorin) is mainly produced in the fat body (Palm et al., 2012; Prasad et al., 1986), an energy storage and endocrine organ that controls nutrient response, before being released into the blood (haemolymph). By biochemically fractionating the wing imaginal disc, a classical model of Hh signalling, Suzanne and co-workers demonstrated that a portion of Hh could be isolated from the supernatant in association with lipoprotein particles (Panáková et al., 2005). Reducing the total level of circulating Lpp in larvae by specifically depleting Lpp expression in the fat body led to a decrease in the intercellular movement of Hh. This treatment did not cause a general suppression of Hh signalling; instead, only long-range Hh targets were reduced. This may have been the consequence of complementary mechanisms acting in parallel. Indeed, it has since been shown that Hh can also be secreted on extracellular vesicles (EVs), or transported along cellular extensions, called cytonemes, in order to carry out its patterning function (Bischoff et al., 2013; Gradilla et al., 2014; Kornberg, 2017; Matusek et al., 2014; Parchure et al., 2018). The association of Hh with these various carriers could fulfil distinct but complementary signalling roles, which together could make up the functional morphogen gradient. The molecular machinery responsible for selectively loading Hh onto its carriers is still poorly understood but is likely to be cell or tissue dependent and remains an important question in the field.

In order to test whether these findings could be generalised to other morphogens, Suzanne's group also looked at the impact of disrupting Lpp expression on another lipid-modified morphogen, Wingless (Wg) – a *Drosophila* Wnt family protein (Takada et al., 2006; Willert et al., 2003). They found that Wg was associated with lipoproteins, and that a reduction in Lpp was associated with a reduced range of Wg activity in the wing imaginal disc (Panáková et al., 2005). Since then, vertebrate Wnts have also been found to be associated with lipoprotein particles in different fluids, including the cerebrospinal fluid (Kaiser et al., 2019; Neumann et al., 2009).

In linking the fields of lipoprotein metabolism and morphogen signalling, this work provided a first glimpse at a new mechanism regulating the spread of morphogens. Moreover, as Lpp is not

Université Côte d'Azur, CNRS, INSERM, iBV, Parc Valrose, 06108 Nice Cedex 2, France.

*Author for correspondence (therond@unice.fr)

 P.P.T., 0000-0003-0434-2334

expressed in the wing imaginal disc, this study also highlighted for the first time that morphogen patterning activity, previously thought to be autonomously controlled within a tissue, could in fact be remotely regulated by another peripheral tissue source. It also provided evidence for a tight link between lipoprotein metabolism and animal development.

How Hedgehog and lipoproteins meet

As mentioned above, Lpp is not produced in the same tissue as Hh, naturally raising the question of how these molecules meet. Suzanne's group hypothesised that Lpp might regulate membrane-bound molecules involved in Hh spreading and signalling. Around that time, heparan sulphate proteoglycans (HSPGs) had been shown to bind lipidated Hh at the cell surface, and to be essential for both morphogen spreading and signalling (Yan and Lin, 2009), making them likely candidates. Suzanne's group demonstrated that the membrane-associated HSPGs Dally and Dally-like protein (Dlp) recruit circulating Lpp particles to the wing imaginal discs via their heparan sulphate moieties (Eugster et al., 2007). Importantly, the HSPGs remain associated with Lpp particles even upon their release from the membrane. The released form of Dally associates not only with Lpp but also with Hh in the same particles, and is found in endosomes with the Hh receptor Patched (Ptc). Suzanne's group found that released Dally increases the signalling activity of Hh on lipoprotein particles without affecting its spread or stability (Eugster et al., 2007). Soluble heparin is known to induce the dimerisation of Interference Hedgehog (Ihog), a Hh co-receptor, and increase Ihog-Hh binding *in vitro* (McLellan et al., 2006). Thus, the presence of Dally on lipoprotein-associated Hh could bridge heparin sulphate, Hh and Ihog, thereby promoting signalling.

Identification of Hh variants

Through detailed analyses of *Drosophila* tissues and mammalian cell lines by biochemical fractionation (Palm et al., 2013), Suzanne's group also uncovered additional, conserved forms of Hh/Shh: one sterol-modified, lipoprotein-associated variant and a second, lipoprotein-free form that is not sterol modified. Understanding how these different variants of Hh act together *in vivo* is a pressing question in the field.

In order to assess the functional relevance of these two secreted Hh/Shh variants, the Eaton group employed a signalling assay both in mammalian cells and in *Drosophila*. They found that the two released forms of Hh have complementary signalling activities, acting synergistically to fully activate the Hh pathway. Although the lipoprotein-associated Hh could induce the first step in signal transduction, it was not sufficient to activate downstream target genes. However, by adding low levels of the second, lipoprotein-free Hh in addition to the lipoprotein-associated Hh, the pathway could be fully activated (Palm et al., 2013). Although morphogen gradients had historically been considered to consist of a gradient of a single protein, these findings introduced the possibility that the Hh morphogen gradient is heterogeneous, integrating the activities of distinct Hh variants. These variants may differ in their ability to spread in tissues, or in their interaction with receptors and other regulators. The conclusions of these studies are based heavily on extensive *in vitro* and biochemical characterisations, and testing their observations in a tissue-specific, *in vivo* context has been restrained by technical limitations. Ongoing developments in high-resolution imaging and proteomics technologies will hopefully provide a means to gain a more fundamental understanding of these processes.

The role of lipoprotein-derived lipids in the Hh signalling pathway

A central process regulating the Hh signalling cascade is the inactivation of the transmembrane protein Smoothened (Smo), a G protein-coupled receptor, by the Hh receptor Ptc (Briscoe and Théron, 2013). In the absence of Hh, Ptc enzymatically inhibits Smo activity and induces its degradation (Taipale et al., 2002). By contrast, the binding of Hh to Ptc inactivates Ptc, resulting in the stabilisation and activation of Smo. In *Drosophila*, Smo activation prevents proteolysis of the transcriptional mediator of the Hh pathway, Cubitus interruptus [Ci, a member of the GLI protein family, simultaneously cloned by Suzanne and Kornberg and by Orenic and colleagues (Eaton and Kornberg, 1990; Orenic et al., 1990)], promoting the conversion of Ci from a transcriptional repressor to an activator.

Ptc is a member of the resistance-nodulation-division (RND) protein family and contains a sterol-sensing domain essential for Smo repression. Based on comparative structural analyses, it has been proposed that Ptc transports sterol or other small lipidic molecules (Chen et al., 2002; Frank-Kamenetsky et al., 2002; Kowatsch et al., 2019), and can act as a lipoprotein receptor in addition to being a Hh receptor (Callejo et al., 2008). Interestingly, several small lipidic molecules that could act as Smo agonists or antagonists were identified in chemical screens (reviewed by Peer et al., 2019). Although this suggested that lipid transport could be involved in the regulation of Smo by Ptc, the molecules mediating this process remained elusive.

Suzanne's group became interested in whether the sterol or lipid components of lipoprotein particles could act in the Ptc-mediated inhibition of Smo in the absence of Hh. Their experiments revealed that Ptc could sequester Lpp into an endosomal compartment (Eugster et al., 2007; Khaliullina et al., 2009) and that this process depends on the Ptc sterol-sensing domain. Moreover, when they mutated this domain, they observed an accumulation of lipids and Smo in Ptc-containing endosomes, suggesting that by trafficking through this compartment, Smo could be exposed to the lipids mobilised by Ptc (Khaliullina et al., 2009). The Eaton group also showed that reducing the levels of circulating Lpp in *Drosophila* larvae led to the accumulation of Smo and the stabilisation of both Smo and Ci, but was insufficient for Hh pathway target gene activation (Khaliullina et al., 2009), suggesting that Smo and Ci stabilisation can be regulated by Lpp-derived lipids but that their full-strength activation requires an additional level of regulation. This inhibitory function of lipoprotein-derived lipids could be blocked by the presence of Hh on lipoprotein particles (Palm et al., 2013). This led Suzanne's group to propose a model in which Ptc mediates Smo activity through lipoprotein-derived lipids, tuning the balance between the degradation and recycling of Smo (Khaliullina et al., 2009).

Lipoproteins therefore have two contrasting roles in Hh signalling: they help mobilise Hh ligands from the cell membrane, promoting their spread, but they also provide the lipids necessary for Ptc activity. In this model, the presence of Hh on lipoprotein particles acts as a switch, blocking the Ptc-mediated mobilisation of lipids from the lipoprotein particles, and inhibiting the Ptc-dependent repression of Smo.

Having established that lipoprotein-derived lipids could negatively regulate Hh signalling, Suzanne's group then worked to identify the inhibitory lipids using biochemical fractionation and mass spectrometry on human very-low-density lipoprotein particles (Khaliullina et al., 2015). In doing so, they identified lipoprotein-derived endocannabinoids as novel endogenous Smo inhibitors. As

endocannabinoids are present in the circulation, the link that Suzanne established suggested that systemic metabolism could directly influence development and tissue homeostasis. Endocannabinoids are unlikely to be the only endogenous Smo regulators, as recent structural and biochemical studies suggest that a sterol lipid, probably cholesterol, is one of the second positive messengers that communicate signals between Ptc and Smo (Kowatsch et al., 2019).

Hh can act as an endocrine hormone

Morphogens are typically thought to act in a paracrine manner, travelling a relatively short distance from their source cells to form a gradient that induces signalling in the receiving tissue. In contrast to the traditional model of morphogen signalling, Suzanne's group found Hh circulating in the haemolymph in association with Lpp (Palm et al., 2013; Panáková et al., 2005). By depleting Hh in a tissue-specific manner, they identified midgut enterocytes (ECs) as a major source of circulating Hh (Rodenfels et al., 2014). In this context, Hh is produced by ECs, secreted, and packaged with lipoproteins. Suzanne's group also showed that circulating Hh did not regulate tissue patterning but instead coordinated larval growth and pupariation timing by acting on two different tissues, the fat body and the prothoracic gland, respectively (Rodenfels et al., 2014). Specifically, Hh signalling in the fat body leads to the release of stored triacylglycerides (TAGs) and to a reduction in larval growth rate, whereas Hh signalling in the prothoracic gland inhibits production of the ecdysone hormone involved in pupariation timing, thereby increasing the duration of growth.

Previous studies have shown that growth and developmental timing are intrinsically linked to nutrient availability. The role of the gut in nutrient absorption hinted that midgut Hh could respond to the nutrient status of the animal, coupling it with growth and development. Interestingly, Suzanne's group showed that Hh production in the midgut, and consequently its secretion into the haemolymph, is physiologically increased upon protein and sugar starvation (Rodenfels et al., 2014). This elevated circulating Hh is essential for normal survival under starvation conditions, and thus provides a protective function against nutrient shortage by extending the larval growth period.

This pioneering work broke away from the established model of morphogen signalling, identifying not only a new function for Hh in the control of developmental timing but, more fundamentally, a new mode of action for Hh as an endocrine metabolic hormone able to facilitate inter-organ communication. This work also introduced Hh into a network that couples larval growth with developmental timing (Boulan et al., 2015). Importantly, the loading of Hh onto lipoprotein particles was also observed in mammalian tissues (Palm et al., 2013), suggesting that the endocrine function of Hh is conserved (Matz-Soja et al., 2014; Song et al., 2018). Suzanne was keen to expand on these findings, exploring a new role for circulating Shh and establishing a project analysing the role of Shh as a regulator of adult adrenal gland homeostasis (Swierczynska et al., 2013).

Conclusions

Together with her lab and collaborators, Suzanne catalysed the bridging of fields – from nutrition, lipidomics and metabolism, to biochemistry, cell biology and developmental biology – with the aim of understanding how morphogens, notably Hh, are transported and signal. Her group's studies, integrating lipoprotein particles into the Hh signalling pathway, formed the basis for a series of

discoveries revealing novel mechanisms of Hh function. First, they demonstrated that Lpp could regulate the spread and activity of Hh non-autonomously. Second, their work revealed that the Hh morphogen gradient consists of functionally distinct forms of Hh: one sterol modified and Lpp dependent, and another non-sterol modified and Lpp independent. Finally, contrary to the previously described models of morphogen activity, Suzanne and colleagues discovered that Lpp-associated Hh could act systemically as an endocrine factor, regulating organismal development. These innovative, thought-provoking studies added another level of complexity into the Hh field, pushing our thinking further and motivating the community to adopt novel, more integrative approaches.

Final remarks

'What a powerful thing it is to take two supposedly separate fields – cell biology and developmental biology – and wear both hats at the same time.'

The above phrase, taken from a 2013 interview with Suzanne (Sedwick, 2013), succinctly describes her approach to research. In this Spotlight, we have discussed representative work carried out at the interface of disparate fields, which yielded results that often provided answers to questions that had long remained enigmatic in the field, or renewed our understanding of classically established concepts. These answers were invariably accompanied by many more questions, but they laid the foundation for ongoing projects across many research groups.

Through the examples we have provided, we hope to convey the level of originality present in Suzanne's approaches to understanding the mechanisms of morphogen signalling. She was one of the first to embrace and link fields such as developmental biology, cell biology, organismal growth control and metabolism. Through her work on morphogen regulation, she successfully integrated different scales of biological organisation from the molecular, to the organismal.

We have had the privilege of being associated with Suzanne's career for a long time, and to work on similar questions regarding Hh regulation. Her sudden disappearance was a shock to us all. We will never forget our lively and passionate discussions during international meetings and PhD jury committees. Just knowing that Suzanne would be present at a conference provided an incentive to attend and we would encourage students and colleagues to seek her input on their own projects. Despite our often differing views on the ways in which Hh is transported, and how the different vehicles cooperate, our discussions invariably remained respectful and, above all, inspiring. We shared in her desire to confront dogmas and established concepts in the field. Suzanne's passion for scientific discussion transcended scientific rivalry; she amplified our own excitement, opened our eyes to new ideas and, always positively, encouraged us to explore new avenues. We deeply appreciated her endless, contagious curiosity and drive to solve scientific puzzles, without inflating her own ego, simply to share the joy of contributing her reflections and inspire research. Discussions with Suzanne were not limited to scientific exchanges but embraced subjects ranging from art and literature to more personal exchanges on personal and family lives, subjects on which she was also a source of inspiration. This combination of rare qualities made her an exceptional friend and colleague whose spirit will stay with us forever.

This article is part of a collection that commemorates the work of Suzanne Eaton. See also Dahmann and Classen (2020), Mlodzik (2020), and Palm and Rodenfels (2020) in this issue.

Acknowledgements

We thank Laurent Ruel and Tamas Matussek for discussions and advice.

Competing interests

The authors declare no competing or financial interests.

Funding

This work is supported by the Agence Nationale de la Recherche (ANR-18-CE13-0003) and by LABEX SIGNALIFE (ANR-11-LABX-0028-01).

References

- Ashe, H. L. and Briscoe, J. (2006). The interpretation of morphogen gradients. *Development* **133**, 385. doi:10.1242/dev.02238
- Bischoff, M., Gradilla, A.-C., Seijo, I., Andrés, G., Rodríguez-Navas, C., González-Méndez, L. and Guerrero, I. (2013). Cytosomes are required for the establishment of a normal Hedgehog morphogen gradient in *Drosophila* epithelia. *Nat. Cell Biol.* **15**, 1269-1281. doi:10.1038/ncb2856
- Boulan, L., Milán, M. and Léopold, P. (2015). The systemic control of growth. *Cold Spring Harb. Perspect. Biol.* **7**, a019117. doi:10.1101/cshperspect.a019117
- Briscoe, J. and Théron, P. P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat. Rev. Mol. Cell Biol.* **14**, 416-429. doi:10.1038/nrm3598
- Callejo, A., Culi, J. and Guerrero, I. (2008). Patched, the receptor of Hedgehog, is a lipoprotein receptor. *Proc. Natl. Acad. Sci. USA* **105**, 912. doi:10.1073/pnas.0705603105
- Chen, J. K., Taipale, J., Young, K. E., Maiti, T. and Beachy, P. A. (2002). Small molecule modulation of smoothened activity. *Proc. Natl. Acad. Sci. USA* **99**, 14071. doi:10.1073/pnas.182542899
- Dahmann, C. and Classen, A.-K. (2020). Charting the unknown currents of cellular flows and forces. *Development* **147**, dev186403. doi:10.1242/dev.186403
- Eaton, S. and Kornberg, T. B. (1990). Repression of *ci-D* in posterior compartments of *Drosophila* by engrailed. *Genes Dev.* **4**, 1068-1077. doi:10.1101/gad.4.6.1068
- Eugster, C., Panáková, D., Mahmoud, A. and Eaton, S. (2007). Lipoprotein-heparan sulfate interactions in the Hh Pathway. *Dev. Cell* **13**, 57-71. doi:10.1016/j.devcel.2007.04.019
- Frank-Kamenetsky, M., Zhang, X. M., Bottega, S., Guicherit, O., Wichterle, H., Dudek, H., Bumcrot, D., Wang, F. Y., Jones, S., Shulok, J. et al. (2002). Small-molecule modulators of Hedgehog signaling: identification and characterization of smoothened agonists and antagonists. *J. Biol.* **1**, 10. doi:10.1186/1475-4924-1-10
- Gradilla, A.-C., González, E., Seijo, I., Andrés, G., Bischoff, M., González-Mendez, L., Sánchez, V., Callejo, A., Ibáñez, C., Guerra, M. et al. (2014). Exosomes as Hedgehog carriers in cytoskeleton-mediated transport and secretion. *Nat. Commun.* **5**, 5649. doi:10.1038/ncomms6649
- Greco, V., Hannus, M. and Eaton, S. (2001). Argosomes: a potential vehicle for the spread of morphogens through Epithelia. *Cell* **106**, 633-645. doi:10.1016/S0092-8674(01)00484-6
- Kaiser, K., Gyllborg, D., Procházka, J., Salašová, A., Kompaníková, P., Molina, F. L., Laguna-Goya, R., Radaszkiewicz, T., Harnoš, J., Procházková, M. et al. (2019). WNT5A is transported via lipoprotein particles in the cerebrospinal fluid to regulate hindbrain morphogenesis. *Nat. Commun.* **10**, 1498. doi:10.1038/s41467-019-09298-4
- Khaliullina, H., Panáková, D., Eugster, C., Riedel, F., Carvalho, M. and Eaton, S. (2009). Patched regulates Smoothened trafficking using lipoprotein-derived lipids. *Development* **136**, 4111-4121. doi:10.1242/dev.041392
- Khaliullina, H., Bilgin, M., Sampaio, J. L., Shevchenko, A. and Eaton, S. (2015). Endocannabinoids are conserved inhibitors of the Hedgehog pathway. *Proc. Natl. Acad. Sci. USA* **112**, 3415-3420. doi:10.1073/pnas.1416463112
- Kornberg, T. B. (2017). Distributing signaling proteins in space and time: the province of cytosomes. *Curr. Opin. Genet. Dev.* **45**, 22-27. doi:10.1016/j.gde.2017.02.010
- Kowatsch, C., Woolley, R. E., Kinnebrew, M., Rohatgi, R. and Siebold, C. (2019). Structures of vertebrate patched and smoothened reveal intimate links between cholesterol and Hedgehog signalling. *Curr. Opin. Struct. Biol.* **57**, 204-214. doi:10.1016/j.sbi.2019.05.015
- Lee, R. T. H., Zhao, Z. and Ingham, P. W. (2016). Hedgehog signalling. *Development* **143**, 367-372. doi:10.1242/dev.120154
- Matussek, T., Wendler, F., Polés, S., Pizette, S., D'Angelo, G., Fürthauer, M. and Théron, P. P. (2014). The ESCRT machinery regulates the secretion and long-range activity of Hedgehog. *Nature* **516**, 99-103. doi:10.1038/nature13847
- Matz-Soja, M., Aleithe, S., Marbach, E., Böttger, J., Arnold, K., Schmidt-Heck, W., Kratzsch, J. and Gebhardt, R. (2014). Hepatic Hedgehog signaling contributes to the regulation of IGF1 and IGFBP1 serum levels. *Cell Commun. Signal.* **12**, 11. doi:10.1186/1478-811X-12-11
- McLellan, J. S., Yao, S., Zheng, X., Geisbrecht, B. V., Ghirlando, R., Beachy, P. A. and Leahy, D. J. (2006). Structure of a heparin-dependent complex of Hedgehog and Ihog. *Proc. Natl. Acad. Sci. USA* **103**, 17208. doi:10.1073/pnas.0606738103
- Mlodzik, M. (2020). Planar cell polarity: moving from single cells to tissue-scale biology. *Development* **147**, dev186346. doi:10.1242/dev.186346
- Neumann, S., Coudreuse, D. Y. M., Van Der Westhuyzen, D. R., Eckhardt, E. R. M., Korswagen, H. C., Schmitz, G. and Sprong, H. (2009). Mammalian Wnt3a is released on lipoprotein particles. *Traffic* **10**, 334-343. doi:10.1111/j.1600-0854.2008.00872.x
- Orenic, T. V., Slusarski, D. C., Kroll, K. L. and Holmgren, R. A. (1990). Cloning and characterization of the segment polarity gene *cubitus interruptus* Dominant of *Drosophila*. *Genes Dev.* **4**, 1053-1067. doi:10.1101/gad.4.6.1053
- Palm, W., Sampaio, J. L., Brankatschk, M., Carvalho, M., Mahmoud, A., Shevchenko, A. and Eaton, S. (2012). Lipoproteins in *Drosophila melanogaster*—assembly, function, and influence on tissue lipid composition. *PLoS Genet.* **8**, e1002828. doi:10.1371/journal.pgen.1002828
- Palm, W., Swierczynska, M. M., Kumari, V., Ehrhart-Bornstein, M., Bornstein, S. R. and Eaton, S. (2013). Secretion and signaling activities of lipoprotein-associated hedgehog and non-sterol-modified hedgehog in flies and mammals. *PLoS Biol.* **11**, e1001505. doi:10.1371/journal.pbio.1001505
- Palm, W. and Rodenfels, J. (2020). Understanding the role of lipids and lipoproteins in development. *Development* **147**, dev186411. doi:10.1242/dev.186411
- Panáková, D., Sprong, H., Marois, E., Thiele, C. and Eaton, S. (2005). Lipoprotein particles are required for Hedgehog and wingless signalling. *Nature* **435**, 58-65. doi:10.1038/nature03504
- Parchure, A., Vyas, N. and Mayor, S. (2018). Wnt and Hedgehog: secretion of lipid-modified morphogens. *Trends Cell Biol.* **28**, 157-170. doi:10.1016/j.tcb.2017.10.003
- Peer, E., Tesanovic, S. and Aberger, F. (2019). Next-generation Hedgehog/GLI pathway inhibitors for cancer therapy. *Cancers* **11**, 538. doi:10.3390/cancers11040538
- Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K. et al. (1998). Identification of a palmitic acid-modified form of human Sonic hedgehog. *J. Biol. Chem.* **273**, 14037-14045. doi:10.1074/jbc.273.22.14037
- Porter, J. A., Young, K. E. and Beachy, P. A. (1996). Cholesterol modification of Hedgehog signaling proteins in animal development. *Science* **274**, 255. doi:10.1126/science.274.5285.255
- Prasad, S. V., Fernando-Warnakulasuriya, G. J., Sumida, M., Law, J. H. and Wells, M. A. (1986). Lipoprotein biosynthesis in the larvae of the tobacco hornworm, *Manduca sexta*. *J. Biol. Chem.* **261**, 17174-17176.
- Rodenfels, J., Lavrynenko, O., Ayciriex, S., Sampaio, J. L., Carvalho, M., Shevchenko, A. and Eaton, S. (2014). Production of systemically circulating Hedgehog by the intestine couples nutrition to growth and development. *Genes Dev.* **28**, 2636-2651. doi:10.1101/gad.249763.114
- Sedwick, C. (2013). Suzanne Eaton: the beautiful logic of development. *J. Cell Biol.* **202**, 184-185. doi:10.1083/jcb.2022pi
- Song, M., Han, L., Chen, F., Wang, D., Wang, F., Zhang, L., Wang, Z., Zhong, M., Tang, M., Zhang, W. et al. (2018). Adipocyte-derived exosomes carrying sonic hedgehog mediate M1 macrophage polarization-induced insulin resistance via Pth and PI3K pathways. *Cell. Physiol. Biochem.* **48**, 1416-1432. doi:10.1159/000492252
- Swierczynska, M. M., Lamounier-Zepter, V., Bornstein, S. R. and Eaton, S. (2013). Lipoproteins and Hedgehog signalling—possible implications for the adrenal gland function. *Eur. J. Clin. Invest.* **43**, 1178-1183. doi:10.1111/eci.12145
- Taipale, J., Cooper, M. K., Maiti, T. and Beachy, P. A. (2002). Patched acts catalytically to suppress the activity of smoothened. *Nature* **418**, 892-896. doi:10.1038/nature00989
- Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T. and Takada, S. (2006). Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev. Cell* **11**, 791-801. doi:10.1016/j.devcel.2006.10.003
- Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R. and Nusse, R. (2003). Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448-452. doi:10.1038/nature01611
- Wolpert, L. (2016). Chapter Thirty-Five - positional information and pattern formation. In *Current Topics in Developmental Biology* (ed. P. M. Wassarman), pp. 597-608. Academic Press.
- Yan, D. and Lin, X. (2009). Shaping morphogen gradients by proteoglycans. *Cold Spring Harb. Perspect. Biol.* **1**, a002493. doi:10.1101/cshperspect.a002493