Preface

hedgehog, n.

1. An insectivorous quadruped of the genus *Erinaceus*, armed above with innumerable spines, and able to roll itself up into a ball with these bristling in every direction; an urchin.

2. *Genetics* and *Embryol.* A gene originally identified in the fruit fly *Drosophila melanogaster* which encodes a protein involved in the determination of segmental polarity and cell-to-cell signalling during embryonic development and metamorphosis; the protein encoded by this gene.

Oxford English Dictionary

The development of a new animal starts with fertilization of the egg, the fusion of the male and female gametes – the spermatocyte and the oocyte. Immediately following the fusion of these two cells, a change in electric potential of the egg cell membrane changes essential properties of the cell membrane, making entry of another spermatocyte impossible and importantly, fires the starting gun for the developmental program.

What follows is the cleavage of the zygote, giving rise to a spherical group of cells that later on becomes hollow and subject to a range of movements that shape the developing organism. As the developing organism grows more complex, gradients of different signaling molecules guide the shaping of the features that we associate with a normally developed individual. These signaling molecules are often called morphogens, and the Hedgehog proteins are prime examples of these. It is here that the presented thesis contributes.

The history of the family of Hedgehog (Hh) proteins starts in 1980, after Christiane Nüsslein-Volhard following sifting through thousands of fruit fly embryos, published the identification of a group of genes responsible for polarity within the segments across the embryo's body axis. One of the mutant fruit flies showed a denticle pattern reminiscent of a hedgehog's back. Undoubtedly strapped for gene names, the researcher considered *hedgehog* was a good name for the gene involved. Although a Nobel Prize was awarded for the work that gave birth to what is now a highly competitive and productive research field, it is probably fair to speculate that at the time, Nüsslein-Volhard did not realize the implications of the identification of a gene named after the spiny mammal.

The biology of the Hh proteins is remarkably complex and compared to other biological systems, unusual. An example of such a remarkable feature is the receptor pair that relays the Hh signal. Unlike other pathways, in which one receptor transduces a signal into the cell to the downstream pathway components, the Hh pathway does this the hard way and uses an "on" receptor (Smoothened) and an "off" receptor (Patched) to manage its activation status. In the absence of Hh ligand, Patched employs vitamin D_3 (as shown in Chapter 2) and probably also similar molecules to inhibit Smoothened, inactivating the pathway. In the presence of the ligand, Patched is inactivated and Smoothened is free to activate the pathway, ultimately leading to transcription of target genes. The ligand itself is also very interesting, being for instance the only sterolated protein in the animal kingdom, and to achieve this sterolation, it cleaves itself from a precursor form.

As a consequence of these idiosyncrasies, the Hh proteins are subject to an intense research effort, and the first part of this thesis deals with the elucidation of some unusual signaling mechanisms that were expected to exist but had remained obscure (Chapter 2 and 3), but also the discovery of an entirely new way of transducing the Hh signal (Chapters 4 through 6).

Knowing how the Hh signaling pathway works is also important to uncover novel roles and functions for Hh proteins. For instance, the work presented in the second part of this thesis (Chapters 7 through 9) , focusing on novel roles of Hh proteins in (patho)physiology is critically dependent on mechanistic knowledge of the pathway, specifically the strong negative feedback mechanism that controls the pathway.

The introduction serves to provide a comprehensive overview to the uninitiated reader interested in the material presented within this doctoral thesis. However, with the research field advancing as fast as it does, the original publication contained what can now (4 years later) be considered factual errors. The original manuscript has therefore been altered to be truthful, but not fully exhaustive. The aim of the experimental work that follows the introduction is to enlighten the reader's knowledge on more specific aspects of Hedgehog protein biology and to hopefully entice him/her to take an interest in this astonishing signaling pathway.