Chapter 1

General Introduction

General Introduction

The origin of multicellularity: a major evolutionary transition

The mesmerizing diversity of life on Earth showcases the wonders of evolution over billions of years. Underpinning the complexity of life forms are major evolutionary transitions, landmark events which have drastically shaped the trajectory of life and paved the way for the rich biodiversity we observe today (Smith and Szathmary 1997). Major evolutionary transitions include for instance the origin of eukaryotes from the merging of an archaeal host and a bacterial endosymbiont (McInerney et al. 2015; Zaremba-Niedzwiedzka et al. 2017; Donoghue et al. 2023), the advent of reproduction by sexual means (REFS) and the emergence of multicellularity (Ruiz-Trillo and Nedelcu 2015). Multicellularity has arisen several times independently in various eukaryotic lineages resulting in a diverse set of complex multicellular organisms, including brown algae, red algae, green algae and land plants, fungi, and animals (Ruiz-Trillo and Nedelcu 2015). The characteristics of the ancestral unicellular eukaryote and the mechanisms driving the emergence of multicellularity vary between lineages and remain subjects of ongoing research (Ruiz-Trillo and Nedelcu 2015).

The origin of animals through multicellularity has seen various hypotheses, each centred around the nature of the unicellular ancestor. This has been recently reviewed by Brunet and King (Brunet and King 2022) and is here summarized. Prior to the establishment of molecular phylogenies, proposed ancestral lineages spanned a range from amoebozoans (Haeckel 1876) to choanoflagellates (Metchnikoff 1886) and ciliates (Saville-Kent 1882). This lack of consensus throughout the 19th and 20th centuries, was amplified by both technical and conceptual limitations. A notable point of contention was the debate over animal monophyly. Some researchers questioned the relatedness of sponges to other animals, postulating the possibility of distinct ancestors for sponges (choanoflagellates) and the remainder of animals (ciliates) (Saville-Kent 1882). Contemporary molecular phylogenies unequivocally support the monophyly of animals and choanoflagellates as their sister group, together forming the clade Choanozoa, within the broader Holozoan clade (Wainright et al. 1993; Lang et al. 2002; Ruiz-Trillo et al. 2008). Choanozoa is corroborated by morphological and biochemical evidence: the collar complex surrounding the flagellum, a defining feature of choanoflagellates, is not only found in sponge choanocytes but across various animals and is composed of cytoskeletal filaments that are homologous among choanoflagellates, sponges, and other animals (Nerrevang and Wingstrand 1970; Lyons 1973; Rieger 1976; Brunet and King 2017; Colgren and Nichols 2020). While the choanoflagellate-like ancestor hypotheses is now the most widely accepted, the specific mechanisms behind the evolution of animals from such an ancestor remain to be clarified. Theories have revolved around the two hypotheses of aggregative and clonal multicellularity, with the latter currently gaining wider acceptance (Brunet and King 2017). However, a recent theory posits that the mutual ancestor of animals and choanoflagellates presented a complex life-cycle, including transitions between amoeboid and flagellate phenotypes, similar to the cell types present in modern sponges (Arendt et al. 2015; Brunet and King 2017; Brunet et al. 2021; Brunet and King 2022). These alternative phenotypes were temporally segregated into different cells in the ancestor, however, following a process of clonal multicellularity these different phenotypes became spatially rather than temporally segregated. This combined with division of labour and innovation lead to the evolution of animals (Brunet and King 2017). The notion that living choanoflagellates present multiple phenotypes including sessile, swimming, and colonial forms, plus the fact that other closely related holozoans such as ichthyosporeans and filastereans also assume diverse cellular forms (Suga and Ruiz-Trillo 2013; Hehenberger et al. 2017; Parra-Acero et al. 2018; Brunet et al. 2019; Parra-Acero et al. 2020; Tikhonenkov, Hehenberger, et al. 2020; Tikhonenkov, Mikhailov, et al. 2020), support this line of investigation, that is currently topic of active research, driven by the emergence of holozoans as model organisms (Booth and King 2022).

Expansion of signal transduction systems in animals

Regardless of the precise mechanisms behind the origin of multicellularity in animals, this major transition has had profound implications. Obligate multicellular organisms such as animals must interact with the environment as a whole entity rather than as individual cells. Equally vital is the internal communication and coordination amongst cells within the organism. Consequently, cells must undergo subspecialisations for different tasks, whilst contemporarily maintaining the ability to collaborate with each other (Ruiz-Trillo et al. 2007). Ultimately this paved the way for the vast diversity of animal forms, ranging from relatively simple to extremely complex organisms with intricate systems for self-coordination and interaction with the non-self, such as the nervous and immune systems (Bich et al. 2019; Jékely 2021; Jékely et al. 2021).

From a genetic perspective, we expect the emergence of novel genes to accompany the evolution of animals in response to these new challenges. Indeed, research into genes originating at the stem of metazoa point towards an increase in new genes for nucleic acid binding molecules, transcription factors and molecules involved in cell signalling (Paps and Holland 2018). Cell signalling plays a pivotal role in facilitating biological processes requiring communication amongst cells. Typically, it involves chemical messages or ligands—either endogenous or exogenous—that engage cellular receptors. This activation triggers a sequence of intracellular events, the signal transduction, involving second messengers and various effectors (REFS). Ultimately, this allows cells to detect and react to extracellular cues either deriving from other cells, like hormones, neurotransmitters, and neuropeptides, or from external stimuli such as light (REFS). There are many different types of receptors that generally fall within the categories of ligand-gated ion channels, enzyme linked receptors, G-protein-coupled receptors (GPCRs) and even intracellular receptors (Foreman et al. 2010). GPCRs, in particular, play a key role in numerous signalling pathways in animals, from neural communication, light reception and other sensory systems and immunity. Given the importance of cell signalling for animals, it is not surprising that one of the categories of gene families that was found to have significant emergence of new genes in the stem of metazoa is signalling molecules (Paps and Holland 2018). Additionally, even when there has not been a *de novo* origin of novel genes, there can be expansions within existing gene families effectively introducing novel genes that are often associated with new functions. This seems to have been the case for GPCR receptors. GPCR signalling is ancient, being present throughout eukaryotes, however, a huge expansion of this gene family occurs in animals. This is not seen neither in close relatives of animals nor in other multicellular organisms (e.g., plants have a comparatively limited set of GPCRs) (de Mendoza et al. 2014). This dramatic increase of GPCRs in animals is likely linked to their heightened need for rapid responsiveness to their environment.

Given the centrality of these receptors in orchestrating myriad biological processes, they have long been a primary subject of research, with a particular focus in deciphering their evolution to gain insights into the fundamental biological processes that they govern (Fredriksson et al. 2003; Foster et al. 2019). Understanding the evolution of these molecules, sheds light on animal evolution, especially during its early stages when critical adaptations were likely to have occurred following the transition to the novel multicellular lifestyle. Similarly, unravelling the evolutionary histories of other molecules involved in GPCR signalling, such as the second messengers and effectors, is also important in understanding the evolution of cell signalling in animals.

General Aims of the Thesis

During my PhD, I was interested in investigating the evolution of signalling systems in animals. For this, I focused my attention on two different biological processes that rely on signal transduction systems. The first is vision, a widespread phenomenon in animals (Land and Nilsson 2012); the second is the vertebrate-specific chemokine signalling, best known for its role in immunity but also involved in other physiological and developmental processes (REFS). Each presented unique challenges but were both primarily addressed with phylogenetic methods and in some cases with additional bioinformatic approaches such as single cell sequencing analyses. In this short General Introduction, I will delineate the basic background and aims for both systems studied. In the next chapter, General Methods, I will introduce the basics of the methodologies used. Further details about both the background and the methodologies are then provided in the respective chapters.

The origin and evolution of vision in animals

Vision is an example of sensory system that functions through GPCR signalling. It is a quintessential feature of animals, deeply influencing their ecology and behaviour (REFS). At its core, vision consists in a photo-sensitive molecule coupled to a signal transduction machinery within a highly specialised photoreceptor cell. The photo-sensitive molecule is an opsin, a GPCR of class A, bound to a derivative of vitamin A, the retinal (REFS). When the retinal is hit by light it changes conformation (from 11-cis to all-trans), inducing a structural change of the opsin which in turn triggers the G alpha protein it is coupled with activating a signal transduction pathway called phototransduction. There are two major types of phototransduction, rhabdomeric and ciliary, depending on the type of opsins that initiate them, but both culminate in the modulation of ion channels initiating electrical signalling of the photoreceptor cell (REFS).

Photoreceptor cells (PRCs) are classified based on the type of opsins and phototransduction pathway employed (REFS). A general peculiarity of PRCs is the enlargement and folding of the membrane surface to increase the area with the photopigment and therefore enhance light sensitivity. This characteristic membrane folding is present within the cilia of ciliary PRCs of vertebrates, while in rhabdomeric PRCs of insects such as *Drosophila melanogaster* this folding is in the apical surface of the cell forming the rhabdomere (Arendt 2003). Traditionally these morphological differences were the primary basis for PRC classification, and it was believed that rhabdomeric PRCs were characteristic of the protostome (e.g. insects) lineage of Bilateria, while ciliary PRCs belonged to visual systems of the deuterostome clade, that includes vertebrates (Eakin 1979). However, it is now known that ciliary PRCs are present within protostomes (Arendt et al. 2004; Passamaneck et al. 2011; von Döhren and Bartolomaeus 2018) and rhabdomeric PRCs within deuterostomes (Hattar et al. 2002; Ullrich-Lüter et al. 2011) and that a molecular definition of the PRC type is most appropriate, especially when trying to characterise the PRCs of non-bilaterian animals. Regardless of differences in morphology and phototransduction pathways employed, all photoreceptor cells are generally considered homologous cell types (REFS), meaning there may be a core set of regulatory genes constant amongst all animal PRCs.

Beyond the phototransduction machinery and photoreceptor cells, vision encompasses another layer of molecular complexity. After the retinal is isomerized from it cis to its trans state by light, it must return to its cis state in order to be receptive to new light stimuli. This recycling occurs through a series of enzymatic reactions occurring as part of the retinol metabolism (REFS). As the opsin alone cannot carry out the visual function, this pathway that allows constant replenishment of the cis-retinal is just as essential part of the molecular assembly of vision.

Vision is present even in some early-branching animals, such as cnidarians and ctenophores (REFS), suggesting that it must have originated early in animal evolution. Some molecular components underpinning it, such as core signal transduction elements, likely trace back more anciently, while others, such as the regulatory genes involved in photoreceptor cell identity, may be animal innovations. Unravelling the evolutionary history of all these molecular players, identifying key innovations and major family expansions, will not only elucidate the emergence of vision but also enrich our understanding of animal evolution more broadly.

Even when there is this myriad of molecular components involved in the basic setup of vision, the phototransduction machinery, the regulome that specifies the photoreceptor cell identity and the enzymes of the retinol metabolism pathway, efforts in understanding the evolution of vision have focused on the evolution of opsins. This collective effort has allowed to discover x,y,z. While being a crucial starting point, ultimately the other molecular components are just as important for the full picture.

Thus, one of my PhD goals was to fill in some of these gaps by investigating the evolution of the complex molecular assembly of vision. For this, I identified two main aims:

**Aim 1: Evolution of the molecular components of photoreceptor cells**

The first aim is to understand the evolution of the molecular setup of photoreceptor cells, including both the phototransduction machinery and the regulatory toolkit that define the cell type. The objectives of aim 1 are addressed in Chapter 3.

**Aim 2: Evolution of the retinol metabolism**

The second major aim is to investigate the evolution of the retinol metabolism that includes enzymes involved in the recovery of the cis-retinal. And to identify whether specific components may have followed specific evolutionary paths in animals. Aim 2 is addressed in Chapter 4.

Evolution and diversity of chemokine signalling systems.

**Background**

The immune system is another example of organism-wide system that requires cell coordination to identify and target external invaders. An example of this is the chemokine system.

- Canonical CK signalling

- Non-canonical CK

**Aims**

- Second, the evolution of chemokine signalling: here I wanted to understand what are the evolutionary relationships between molecular components that compose the system; when they originated; and describe their evolutionary histories. Since canonical chemokine signalling has only been described in vertebrates, the focus was in searching for ancestral molecules in animals and specifically, in sister groups of vertebrates. This work was conducted in collaboration with my coworkers Matthew Goulty etc and is currently a pre-print.

***Uncover the relationships among canonical and non-canonical components***

***Evolution of all canonical and non-canonical ligands***

***Evolution of all canonical and non-canonical receptors***

All of these aims are addressed in Chapter 5.

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