Chapter 1

General Introduction

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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 (REFS). 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 clonal and aggregative multicellularity (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 exact details of the mechanisms of the origin of multicellularity in animals, it is clear that there are major implications. Obligate multicellular organisms such as animals need the ability to interact with the environment as a whole organism rather than as a single cell, there is a need for the cells of the organism to communicate and coordinate amongst each other, therefore cells have to undergo subspecialisations to address different tasks whilst still being able to collaborate with each other. (Trillo et al 2007 ?? or others.) .

From a genetic/genomic perspective, we expect genetic differences that accompanies the evolution of animals whilst they responded to these new challenges.

Analysis of novel genes arising at the stem of metazoa indicate an expansion in new genes for nucleic acid binding, transcription factors and signalling molecules (Paps and Holland 2018).

The cells developed a way of communication known as cell signalling. Cell signalling is a complex process that involves many different components, including the chemical message (ligand), receptors, second-messengers, effectors, etc. Receptors play a crucial role in signal transduction, allowing cells to sense and respond to various extracellular signals such as hormones, neurotransmitters, and other signaling molecules. which include a very long range of molecules, like hormones, neurotransmitters, and other signalling molecules.

There are many different types of receptors, including ligand-gated ion channels, enzyme linked receptors, g-protein-coupled receptors (GPCRS) and even intracellular receptors.

GPCRs in particular play a key role in signalling pathways in animals, from neural communication, sensory systems and immunity.

And even when some genes are not “novel”, there can be phenomena of expansions, that are often linked to new function/situation. For example, GPCR signalling is generally ancient, while other components of the system do not increase with the advent of animals, the receptors themselves (GPCRs) expand a lot with animals. This is not seen neither in close relatives of animals not in other multicellular organisms (e.g., plants have a limited set) (Mendoza et al 2014 (GBE)). This increase of GPCRs is connected to the fact that for animals it becomes even more essential to be reactive to their environment.

Consequently they have been heated topic of investigation for any years, with a particular focus also in trying to understand the evolution of these receptors as a starting point to understand the evolution of the biological processes that they underpin.

(Interest in understanding evolution of GPCRs has been going on for a while, e.g. Fredriksson 2003.)

- These systems involve all aspects of animal biology. For example, a large number of these are related to perceiving the environment and elaborating responses. These are the senses. For example vision.

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

Power of Evolutionary Studies

Final paragraph could have some concluding remarks about the power of evolutionary studies to understand how signalling systems work in animals and introducing that in the next chapter (methods). I will explain about the methodological approaches used to answer these questions in this thesis.

General Aims of the Thesis

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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; the second is the vertebrate-specific chemokine signalling, best known for its role in immunity but also involved in other physiological and developmental processes. Each presented unique challenges but were both addressed with phylogenetic approaches and to some degree other bioinformatic approaches such as single cell sequencing analyses.

In this general introduction, I will delineate the basic background for my projects, although further details can be found in the respective chapters.

- In this thesis, I used phylogenetic and bioinformatic approaches to understand the molecular evolution of two main systems in animals.

- First, the evolution of vision: here I wanted to understand when all the molecular and cellular components that are the minimum basic setup of vision originated. As multiple non-bilaterian organisms have vision, vision must have originated at some point in early evolution of animals, either prior to the split of extant phyla, or at one of the early splits of extant phyla. However, some of the components are involved in other cellular tasks and likely originated more anciently, so my investigation extended to all Eukraya.

- 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.

**Introduction about vision**

(different levels of complexity in vision: molecular, cellulare, cellular integration, eye organ – Nilsson 2009)

- Phototransduction

- Photoreceptor cells

- Retinol metabolism that synthesises the 11-cis-retinal

**Introduction about chemokine signalling**

- Canonical CK signalling

- Non-canonical CK

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