Chapter 1

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 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 by multicellularity, has had various hypotheses (reviewed by Brunet and King 2020), all centred around the nature of the unicellular ancestor. Prior to the establishment of molecular phylogenies, proposed ancestral lineages spanned a range from amoebozoans (REF) to choanoflagellates (REF) and ciliates (REF). This lack of consensus throughout the 20th century, was amplified by both technical and conceptual limitations. A notable point of contention was the debate over animal monophyly, with some questioning the relatedness of sponges to other animals. With some postulating the possibility of distinct ancestors for sponges (choanoflagellates) and the remainder of animals (ciliates). Contemporary molecular phylogenies unequivocally support the monophyly of animals and choanoflagellates as their sister group. This is further corroborated by morphological and biochemical evidence: the collar complex surrounding the flagellum, a defining feature of choanoflagellate cells, is not only found in sponge choanocytes but across various animals and has been established to be composed of cytoskeletal filaments that are homologous among choanoflagellates, sponges, and other animals. While the choanoflagellate-like ancestor hypotheses is now widely the most accepted one, the specific mechanisms behind the evolution of animals from such an ancestor remain to be clarified. One theory posits that the mutual ancestor of animals and choanoflagellates presented a complex life-cycle, including transitions between amoeboid and flagellate phenotypes. 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, support this line of investigation, that is currently topic of active research, driven by the emergence of holozoans as model organisms.

We discuss two hypotheses for the origin of animal cell types: division of labor from ancient plurifunctional cells and conversion of temporally alternating phenotypes into spatially juxtaposed cell types.

- From Brunet and King. 2017

Regardless of the details of the mechanisms of origin of multicellularity in animals, it is clear that there are major implications.

- Major implications/consequences of multicellularity:

1-the ability to interact with the environment as a whole organism rather than as a single cell

2-subspecialisation of different cells for different tasks

3- need for cells to communicate and coordinate amongst each other

created new challenges, including the need for cooperation and communication between cells, and the division of labor among different cell types. – Trillo et al 2007.

Expansion of signal transduction systems in animals

More details of point 3 above.

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

Our analyses show some striking similarities between the genomes of the first animal and other protist ancestors, copious in genes involved in gene regulation and metabolic activities. However, we also find a large number of gene novelties in the stem lineage of the Metazoa compared to other opisthokont ancestors, with the animal Novel Core HG having functions related to gene regulation, signalling, cell adhesion and cell cycle. ..

There are two alternative scenarios that could explain these patterns depending on the length of the branch leading to the metazoan LCA. The first assumes that the birth rate of new genes was constant over time, thus the branch leading to the first metazoan was longer than other opisthokont internodes. ..

The second possibility involves many new genes emerging during a short ‘popcorn’ stage, caused either by a higher gene birth rate (perhaps produced by environmental factors elevating mutation rates, or due to whole-genome duplications), and/or a lower gene death rate (due to high integration of new genes into regulatory networks).

- Paps and Holland.

And even when some genes are not “novel”, e.g. GPCRs are ancient, there can be phenomena of expansions, that are often linked to new function/situation.

- while signal transduction occurs also in unicellular eukaryotes (check) in animals it becomes even more essential – examples to defend this claim, e.g. stats of number of gpcrs.

E.g. Mendoza et al 2014 (GBE): components of GPCR signalling are generally ancient, while other components of the system do not increase with the advent of animals, the recepotrs themselves (GPCRs) expand a lot with animals. This is not necessarily seen in other multicellular organisms (e.g. plants have a limited set).

Regardless the exact details of the passage to multicellularity in animals (different hypotheses), one thing s clear: when the multicellular organisms evolved from their unicellular ancestors, they started to become larger and more complex, and a problem arose – there was now a need to maintain homeostasis and to nown the internal state of the whole organism and monitor the environmental changesThe 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. However, we will be focusing on the first steps of this process, which include a ligand (or a chemical message) and a receptor which works as a received or decoder of that message. 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. – LUIS

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.

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.

Aims of the Thesis

- 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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