Chapter 1

General Introduction

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The origin of multicellularity: a major evolutionary transition

The astonishing diversity of life on Earth showcases the profound impact 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) 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 and this requires complex mechanisms for internal communication and coordination amongst cells. 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 (Foreman et al. 2010). 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 (Elphick et al. 2018; Moroz et al. 2021; Oteiza and Baldwin 2021). 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 fundamental for the response to external light stimuli (Land and Nilsson 2012). The second is chemokine signalling, best known for its role in immunity but also involved in other physiological and developmental processes that require internal organismal communication (Murphy 2023). 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 a sensory system that functions through GPCR signalling. It is a quintessential feature of animals, deeply influencing their ecology and behaviour (Nilsson 2009). At its core, vision consists of 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 (Terakita 2005). 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 (Hardie and Juusola 2015; Lamb 2020).

Photoreceptor cells (PRCs) are classified based on the type of opsins and phototransduction pathway employed (Arendt 2003). 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). Historically, these morphological differences dictated PRC classification. It was believed that rhabdomeric PRCs were characteristic of the protostome (e.g. insects) lineage of Bilateria, while ciliary PRCs were specific to deuterostomes, including 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). Therefore, molecular definitions offer a more accurate classification, especially for non-bilaterian animals. While complex visual structures, such as eyes, are believed to have evolved independently on multiple occasions (Land and Nilsson 2012; Picciani et al. 2018), their fundamental units—photoreceptor cells—stem from a limited number of subtypes that may share a common ancestral cell type. This suggests that, despite variations in phototransduction machinery, there may exist a core set of regulatory genes defining this broad cell type (Arendt 2008; Arendt et al. 2016), consistent across all animal photoreceptor cells.

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 (Palczewski and Kiser 2020). 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.

Photoreceptor cells are present even in some early-branching animals, such as cnidarians (Nordström et al. 2003; Kozmik et al. 2008; Picciani et al. 2018) and potentially ctenophores (Horridge 1964; Jékely et al. 2015; Tamm 2016), suggesting that vision 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, can not only elucidate the emergence of vision but also enrich our understanding of animal evolution more broadly.

Numerous studies have delved into the evolution of opsins, illuminating the vast diversity of these molecules across animals, including non-bilaterians (Feuda et al. 2012; Feuda et al. 2014; D’Aniello et al. 2015; Roberts et al. 2022; De Vivo et al. 2023; McCulloch et al. 2023). Such research has led to significant discoveries, including the identification of phylogenetically related placopsins in placozoans, a non-bilaterian phylum lacking neurons (Feuda et al. 2012). Nevertheless, comprehensive investigations into the evolution of all molecular components involved in vision remain sparse.

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: Reconstructing the 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:** **Reconstructing the 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, discerning whether specific components may have undergone distinct evolutionary events in animals. Aim 2 is addressed in Chapter 4.

Evolution and molecular diversity of chemokine signalling systems.

The immune system exemplifies an organism-wide system necessitating cellular coordination to detect and counteract external invaders. Present across the animal kingdom, immune systems function through an intricate range of subsystems (Yuan et al. 2014). In vertebrates, the chemokine signalling system plays a fundamental role in both innate and adaptive immunity (Murphy 2023). Best known for the chemoattraction of leukocytes during host defence (Wong and Fish 2003; Blanchet et al. 2012); chemokine signalling is also implicated in homeostasis, development (Zlotnik and Yoshie 2000; Tran and Miller 2003; López-Cotarelo et al. 2017), and neuronal communication (Tran and Miller 2003; de Haas et al. 2007; Rostène et al. 2007). Failure of the system can lead to various diseases (Tran and Miller 2003; Blanchet et al. 2012), including cancer (Nagarsheth et al. 2017).

The chemokine system comprises two primary components: the chemokine ligands, small cytokines possessing chemotactic attributes, and the chemokine receptors, Class A GPCRs. Canonical chemokine ligands possess in their N-terminal portion characteristic cysteine patterns that can be used to classify them into subgroups. Canonical chemokine receptors are in turn classified based on the type of ligands they respond to, although there tends to be a high degree of promiscuity in the system (Zlotnik and Yoshie 2000; Nomiyama et al. 2011). Additionally, several other molecules have been implicated in the system. For example, ligands bearing varying degrees of sequence similarity to canonical chemokines have been found to activate some canonical receptors and/or have chemotactic properties (Zhang et al. 2018). Conversely, some so-called atypical chemokine receptors can bind canonical ligands, but do not trigger the signal transduction pathway necessary for chemokine function (Bonecchi and Graham 2016; Chen et al. 2018). Therefore, these additional players can be considered as “non-canonical” chemokine components. Yet, their relationship with the canonical components and amongst each other is unclear, hampering our understanding of the origin and evolution of the system. Applying evolutionary approaches can aid in clarifying the relatedness of these molecules and ultimately help clarify the evolution of the whole system. Thus, the second goal of my PhD was to explore the evolution of the chemokine system including both its canonical and non-canonical components. For this, three main aims were identified:

**Aim 1: Uncovering the relationships among canonical and non-canonical components.**

The first aim was to investigate the evolutionary relationships among all chemokine ligands and amongst all receptors, including all known canonical and non-canonical molecules. This served as a first step for subsequent phylogenetic analyses.

**Aim 2: Reconstructing the evolution of all canonical and non-canonical ligands.**

The following aim was to perform phylogenetic analyses for each ligand family identified to discern their evolution in animals.

**Aim 3: Reconstructing the evolution of all canonical and non-canonical receptors.**

Similarly, the last aim was to understand the evolutionary history of receptor groups.

The work addressing these aims is detailed in Chapter 5 and was carried out in collaboration with other members of the Feuda Group.

References

Arendt D. 2003. Evolution of eyes and photoreceptor cell types. *Int J Dev Biol* 47:563–571.

Arendt D. 2008. The evolution of cell types in animals: emerging principles from molecular studies. *Nat Rev Genet* [Internet] 9:868–882. Available from: https://www.nature.com/articles/nrg2416

Arendt D, Benito-Gutierrez E, Brunet T, Marlow H. 2015. Gastric pouches and the mucociliary sole: setting the stage for nervous system evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 370:20150286. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2015.0286

Arendt D, Musser JM, Baker CVH, Bergman A, Cepko C, Erwin DH, Pavlicev M, Schlosser G, Widder S, Laubichler MD, et al. 2016. The origin and evolution of cell types. *Nat Rev Genet* 17:744–757.

Arendt D, Tessmar-Raible K, Snyman H, Dorresteijn AW, Wittbrodt J. 2004. Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* 306:869–871.

Bich L, Pradeu T, Moreau J-F. 2019. Understanding Multicellularity: The Functional Organization of the Intercellular Space. *Frontiers in Physiology* [Internet] 10. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2019.01170

Blanchet X, Langer M, Weber C, Koenen R, von Hundelshausen P. 2012. Touch of Chemokines. *Frontiers in Immunology* [Internet] 3:175. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2012.00175

Bonecchi R, Graham GJ. 2016. Atypical Chemokine Receptors and Their Roles in the Resolution of the Inflammatory Response. *Frontiers in Immunology* [Internet] 7:224. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2016.00224

Booth DS, King N. 2022. Chapter Three - The history of Salpingoeca rosetta as a model for reconstructing animal origins. In: Goldstein B, Srivastava M, editors. Current Topics in Developmental Biology. Vol. 147. Emerging Model Systems in Developmental Biology. Academic Press. p. 73–91. Available from: https://www.sciencedirect.com/science/article/pii/S0070215322000011

Brunet T, Albert M, Roman W, Coyle MC, Spitzer DC, King N. 2021. A flagellate-to-amoeboid switch in the closest living relatives of animals.Wittkopp PJ, Ruiz-Trillo I, López-García P, editors. *eLife* [Internet] 10:e61037. Available from: https://doi.org/10.7554/eLife.61037

Brunet T, King N. 2017. The Origin of Animal Multicellularity and Cell Differentiation. *Developmental Cell* [Internet] 43:124–140. Available from: https://www.cell.com/developmental-cell/abstract/S1534-5807(17)30769-4

Brunet T, King N. 2022. The Single-Celled Ancestors of Animals: A History of Hypotheses. In: The Evolution of Multicellularity. CRC Press.

Brunet T, Larson BT, Linden TA, Vermeij MJA, McDonald K, King N. 2019. Light-regulated collective contractility in a multicellular choanoflagellate. *Science* [Internet] 366:326–334. Available from: https://www.science.org/doi/full/10.1126/science.aay2346

Chen K, Bao Z, Tang P, Gong W, Yoshimura T, Wang JM. 2018. Chemokines in homeostasis and diseases. *Cell Mol Immunol* [Internet] 15:324–334. Available from: https://www.nature.com/articles/cmi2017134

Colgren J, Nichols SA. 2020. The significance of sponges for comparative studies of developmental evolution. *WIREs Developmental Biology* [Internet] 9:e359. Available from: https://wires.onlinelibrary.wiley.com/doi/abs/10.1002/wdev.359

D’Aniello S, Delroisse J, Valero-Gracia A, Lowe EK, Byrne M, Cannon JT, Halanych KM, Elphick MR, Mallefet J, Kaul-Strehlow S, et al. 2015. Opsin evolution in the Ambulacraria. *Marine Genomics* [Internet] 24:177–183. Available from: https://www.sciencedirect.com/science/article/pii/S1874778715300349

De Vivo G, Crocetta F, Ferretti M, Feuda R, D’Aniello S. 2023. Duplication and Losses of Opsin Genes in Lophotrochozoan Evolution. *Molecular Biology and Evolution* [Internet] 40:msad066. Available from: https://doi.org/10.1093/molbev/msad066

von Döhren J, Bartolomaeus T. 2018. Unexpected ultrastructure of an eye in Spiralia: the larval ocelli of Procephalothrix oestrymnicus (Nemertea). *Zoomorphology* [Internet] 137:241–248. Available from: https://doi.org/10.1007/s00435-017-0394-3

Donoghue PCJ, Kay C, Spang A, Szöllősi G, Nenarokova A, Moody ERR, Pisani D, Williams TA. 2023. Defining eukaryotes to dissect eukaryogenesis. *Current Biology* [Internet] 33:R919–R929. Available from: https://www.sciencedirect.com/science/article/pii/S0960982223009879

Eakin RM. 1979. Evolutionary Significance of Photoreceptors: In Retrospect. *Am Zool* [Internet] 19:647–653. Available from: https://academic.oup.com/icb/article-lookup/doi/10.1093/icb/19.2.647

Elphick MR, Mirabeau O, Larhammar D. 2018. Evolution of neuropeptide signalling systems. *Journal of Experimental Biology* [Internet] 221:jeb151092. Available from: https://doi.org/10.1242/jeb.151092

Feuda R, Hamilton SC, McInerney JO, Pisani D. 2012. Metazoan opsin evolution reveals a simple route to animal vision. *PNAS* [Internet] 109:18868–18872. Available from: https://www.pnas.org/content/109/46/18868

Feuda R, Rota-Stabelli O, Oakley TH, Pisani D. 2014. The Comb Jelly Opsins and the Origins of Animal Phototransduction. *Genome Biology and Evolution* [Internet] 6:1964–1971. Available from: https://doi.org/10.1093/gbe/evu154

Foreman JC, Johansen T, Gibb AJ. 2010. Textbook of Receptor Pharmacology. CRC Press

Foster SR, Hauser AS, Vedel L, Strachan RT, Huang X-P, Gavin AC, Shah SD, Nayak AP, Haugaard-Kedström LM, Penn RB, et al. 2019. Discovery of Human Signaling Systems: Pairing Peptides to G Protein-Coupled Receptors. *Cell* [Internet] 179:895-908.e21. Available from: https://www.sciencedirect.com/science/article/pii/S0092867419311262

Fredriksson R, Lagerström MC, Lundin L-G, Schiöth HB. 2003. The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. *Mol Pharmacol* [Internet] 63:1256–1272. Available from: https://molpharm.aspetjournals.org/content/63/6/1256

de Haas AH, van Weering HRJ, de Jong EK, Boddeke HWGM, Biber KPH. 2007. Neuronal Chemokines: Versatile Messengers In Central Nervous System Cell Interaction. *Mol Neurobiol* [Internet] 36:137–151. Available from: https://doi.org/10.1007/s12035-007-0036-8

Haeckel E. 1876. The history of creation, or, The development of the earth and its inhabitants by the action of natural causes : doctrine of evolution in general, and of that of Darwin, Goethe, and Lamarck in particular / from the German of Ernst Haeckel ; the translation revised by E. Ray Lankester. London: Henry S. King Available from: https://www.biodiversitylibrary.org/item/99234

Hardie RC, Juusola M. 2015. Phototransduction in Drosophila. *Current Opinion in Neurobiology* [Internet] 34:37–45. Available from: https://www.sciencedirect.com/science/article/pii/S0959438815000173

Hattar S, Liao HW, Takao M, Berson DM, Yau KW. 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295:1065–1070.

Hehenberger E, Tikhonenkov DV, Kolisko M, del Campo J, Esaulov AS, Mylnikov AP, Keeling PJ. 2017. Novel Predators Reshape Holozoan Phylogeny and Reveal the Presence of a Two-Component Signaling System in the Ancestor of Animals. *Current Biology* [Internet] 27:2043-2050.e6. Available from: https://www.sciencedirect.com/science/article/pii/S0960982217307078

Horridge GA. 1964. Presumed photoreceptive cilia in a ctenophore. *Quarterly Journal of microscopic science* [Internet]. Available from: https://openresearch-repository.anu.edu.au/handle/1885/167542

Jékely G. 2021. The chemical brain hypothesis for the origin of nervous systems. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 376:20190761. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2019.0761

Jékely G, Godfrey-Smith P, Keijzer F. 2021. Reafference and the origin of the self in early nervous system evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 376:20190764. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2019.0764

Jékely G, Paps J, Nielsen C. 2015. The phylogenetic position of ctenophores and the origin(s) of nervous systems. *EvoDevo* [Internet] 6:1. Available from: https://doi.org/10.1186/2041-9139-6-1

Kozmik Z, Ruzickova J, Jonasova K, Matsumoto Y, Vopalensky P, Kozmikova I, Strnad H, Kawamura S, Piatigorsky J, Paces V, et al. 2008. Assembly of the cnidarian camera-type eye from vertebrate-like components. *PNAS* [Internet] 105:8989–8993. Available from: https://www.pnas.org/content/105/26/8989

Lamb TD. 2020. Evolution of the genes mediating phototransduction in rod and cone photoreceptors. *Progress in Retinal and Eye Research* [Internet] 76:100823. Available from: https://www.sciencedirect.com/science/article/pii/S1350946219301107

Land MF, Nilsson D-E. 2012. Animal Eyes. Second Edition, Second Edition. Oxford, New York: Oxford University Press

Lang BF, O’Kelly C, Nerad T, Gray MW, Burger G. 2002. The Closest Unicellular Relatives of Animals. *Current Biology* [Internet] 12:1773–1778. Available from: https://www.sciencedirect.com/science/article/pii/S0960982202011879

López-Cotarelo P, Gómez-Moreira C, Criado-García O, Sánchez L, Rodríguez-Fernández JL. 2017. Beyond Chemoattraction: Multifunctionality of Chemokine Receptors in Leukocytes. *Trends in Immunology* [Internet] 38:927–941. Available from: https://www.sciencedirect.com/science/article/pii/S1471490617301655

Lyons KM. 1973. Collar cells in planula and adult tentacle ectoderm of the solitary coral Balanophyllia regia (anthozoa eupsammiidae). *Z.Zellforsch* [Internet] 145:57–74. Available from: https://doi.org/10.1007/BF00307189

McCulloch KJ, Babonis LS, Liu A, Daly CM, Martindale MQ, Koenig KM. 2023. Nematostella vectensis exemplifies the exceptional expansion and diversity of opsins in the eyeless Hexacorallia. *EvoDevo* [Internet] 14:14. Available from: https://doi.org/10.1186/s13227-023-00218-8

McInerney J, Pisani D, O’Connell MJ. 2015. The ring of life hypothesis for eukaryote origins is supported by multiple kinds of data. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 370:20140323. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2014.0323

de Mendoza A, Sebé-Pedrós A, Ruiz-Trillo I. 2014. The Evolution of the GPCR Signaling System in Eukaryotes: Modularity, Conservation, and the Transition to Metazoan Multicellularity. *Genome Biology and Evolution* [Internet] 6:606–619. Available from: https://doi.org/10.1093/gbe/evu038

Metchnikoff É. 1886. Embryologische Studien an Medusen : Ein Beitrag zur Genealogie der Primitiv-organe. Wien: A. Hölder Available from: https://www.biodiversitylibrary.org/item/27274

Moroz LL, Romanova DY, Kohn AB. 2021. Neural versus alternative integrative systems: molecular insights into origins of neurotransmitters. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 376:20190762. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2019.0762

Murphy PM. 2023. 15 - Chemokines and Chemokine Receptors. In: Rich RR, Fleisher TA, Schroeder HW, Weyand CM, Corry DB, Puck JM, editors. Clinical Immunology (Sixth Edition). New Delhi: Elsevier. p. 215–227. Available from: https://www.sciencedirect.com/science/article/pii/B9780702081651000150

Nagarsheth N, Wicha MS, Zou W. 2017. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nature Reviews Immunology* [Internet] 17:559–572. Available from: https://doi.org/10.1038/nri.2017.49

Nerrevang A, Wingstrand KG. 1970. On the Occurrence and Structure of Choanocyte-like Cells in Some Echinoderms. *Acta Zoologica* [Internet] 51:249–270. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1463-6395.1970.tb00436.x

Nilsson D-E. 2009. The evolution of eyes and visually guided behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences* [Internet] 364:2833–2847. Available from: https://royalsocietypublishing.org/doi/10.1098/rstb.2009.0083

Nomiyama H, Osada N, Yoshie O. 2011. A family tree of vertebrate chemokine receptors for a unified nomenclature. *Developmental & Comparative Immunology* [Internet] 35:705–715. Available from: https://www.sciencedirect.com/science/article/pii/S0145305X11000206

Nordström K, Wallén null, Seymour J, Nilsson D. 2003. A simple visual system without neurons in jellyfish larvae. *Proceedings of the Royal Society of London. Series B: Biological Sciences* [Internet] 270:2349–2354. Available from: https://royalsocietypublishing.org/doi/10.1098/rspb.2003.2504

Oteiza P, Baldwin MW. 2021. Evolution of sensory systems. *Current Opinion in Neurobiology* [Internet] 71:52–59. Available from: https://www.sciencedirect.com/science/article/pii/S0959438821000969

Palczewski K, Kiser PD. 2020. Shedding new light on the generation of the visual chromophore. *Proc Natl Acad Sci U S A* [Internet] 117:19629–19638. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7443880/

Paps J, Holland PWH. 2018. Reconstruction of the ancestral metazoan genome reveals an increase in genomic novelty. *Nat Commun* [Internet] 9:1730. Available from: https://www.nature.com/articles/s41467-018-04136-5

Parra-Acero H, Harcet M, Sánchez-Pons N, Casacuberta E, Brown NH, Dudin O, Ruiz-Trillo I. 2020. Integrin-Mediated Adhesion in the Unicellular Holozoan Capsaspora owczarzaki. *Current Biology* [Internet] 30:4270-4275.e4. Available from: https://www.cell.com/current-biology/abstract/S0960-9822(20)31169-6

Parra-Acero H, Ros-Rocher N, Perez-Posada A, Kożyczkowska A, Sánchez-Pons N, Nakata A, Suga H, Najle SR, Ruiz-Trillo I. 2018. Transfection of Capsaspora owczarzaki, a close unicellular relative of animals. *Development* [Internet] 145:dev162107. Available from: https://doi.org/10.1242/dev.162107

Passamaneck YJ, Furchheim N, Hejnol A, Martindale MQ, Lüter C. 2011. Ciliary photoreceptors in the cerebral eyes of a protostome larva. *EvoDevo* [Internet] 2:6. Available from: https://doi.org/10.1186/2041-9139-2-6

Picciani N, Kerlin JR, Sierra N, Swafford AJM, Ramirez MD, Roberts NG, Cannon JT, Daly M, Oakley TH. 2018. Prolific Origination of Eyes in Cnidaria with Co-option of Non-visual Opsins. *Current Biology* [Internet] 28:2413-2419.e4. Available from: https://www.sciencedirect.com/science/article/pii/S0960982218306912

Rieger RM. 1976. Monociliated epidermal cells in Gastrotricha: Significance for concepts of early metazoan evolution. *Journal of Zoological Systematics and Evolutionary Research* [Internet] 14:198–226. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0469.1976.tb00937.x

Roberts NS, Hagen JFD, Johnston RJ. 2022. The diversity of invertebrate visual opsins spanning Protostomia, Deuterostomia, and Cnidaria. *Developmental Biology* [Internet] 492:187–199. Available from: https://www.sciencedirect.com/science/article/pii/S0012160622002007

Rostène W, Kitabgi P, Parsadaniantz SM. 2007. Chemokines: a new class of neuromodulator? *Nat Rev Neurosci* [Internet] 8:895–903. Available from: https://www.nature.com/articles/nrn2255

Ruiz-Trillo I, Burger G, Holland PWH, King N, Lang BF, Roger AJ, Gray MW. 2007. The origins of multicellularity: a multi-taxon genome initiative. *Trends in Genetics* [Internet] 23:113–118. Available from: https://www.cell.com/trends/genetics/abstract/S0168-9525(07)00023-6

Ruiz-Trillo I, Nedelcu AM. 2015. Evolutionary Transitions to Multicellular Life: Principles and Mechanisms edited by Iñaki Ruiz-Trillo and Aurora M. Nedelcu. *Advances in Marine Genomics 2. Springer* [Internet] 91:370–371. Available from: https://www.journals.uchicago.edu/doi/abs/10.1086/688137

Ruiz-Trillo I, Roger AJ, Burger G, Gray MW, Lang BF. 2008. A Phylogenomic Investigation into the Origin of Metazoa. *Molecular Biology and Evolution* [Internet] 25:664–672. Available from: https://doi.org/10.1093/molbev/msn006

Saville-Kent W. 1882. A Manual of the Infusoria: Including a Description of All Known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and Foreign, and an Account of the Organization and the Affinities of the Sponges. D. Bogue

Smith T late PJM, Szathmary E. 1997. The Major Transitions in Evolution. Oxford, New York: Oxford University Press

Suga H, Ruiz-Trillo I. 2013. Development of ichthyosporeans sheds light on the origin of metazoan multicellularity. *Developmental Biology* [Internet] 377:284–292. Available from: https://www.sciencedirect.com/science/article/pii/S0012160613000146

Tamm SL. 2016. Novel Structures Associated with Presumed Photoreceptors in the Aboral Sense Organ of Ctenophores. *Biol Bull* 231:97–102.

Terakita A. 2005. The opsins. *Genome Biology* [Internet] 6:213. Available from: https://doi.org/10.1186/gb-2005-6-3-213

Tikhonenkov DV, Hehenberger E, Esaulov AS, Belyakova OI, Mazei YA, Mylnikov AP, Keeling PJ. 2020. Insights into the origin of metazoan multicellularity from predatory unicellular relatives of animals. *BMC Biology* [Internet] 18:39. Available from: https://doi.org/10.1186/s12915-020-0762-1

Tikhonenkov DV, Mikhailov KV, Hehenberger E, Karpov SA, Prokina KI, Esaulov AS, Belyakova OI, Mazei YA, Mylnikov AP, Aleoshin VV, et al. 2020. New Lineage of Microbial Predators Adds Complexity to Reconstructing the Evolutionary Origin of Animals. *Current Biology* [Internet] 30:4500-4509.e5. Available from: https://www.cell.com/current-biology/abstract/S0960-9822(20)31251-3

Tran PB, Miller RJ. 2003. Chemokine receptors: signposts to brain development and disease. *Nature Reviews Neuroscience* [Internet] 4:444–455. Available from: https://doi.org/10.1038/nrn1116

Ullrich-Lüter EM, Dupont S, Arboleda E, Hausen H, Arnone MI. 2011. Unique system of photoreceptors in sea urchin tube feet. *Proc Natl Acad Sci U S A* 108:8367–8372.

Wainright PO, Hinkle G, Sogin ML, Stickel SK. 1993. Monophyletic Origins of the Metazoa: an Evolutionary Link with Fungi. *Science* [Internet] 260:340–342. Available from: https://www.science.org/doi/10.1126/science.8469985

Wong MM, Fish EN. 2003. Chemokines: attractive mediators of the immune response. *Seminars in Immunology* [Internet] 15:5–14. Available from: https://www.sciencedirect.com/science/article/pii/S1044532302001239

Yuan S, Tao X, Huang S, Chen S, Xu A. 2014. Comparative Immune Systems in Animals. *Annual Review of Animal Biosciences* [Internet] 2:235–258. Available from: https://doi.org/10.1146/annurev-animal-031412-103634

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, et al. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* [Internet] 541:353–358. Available from: https://www.nature.com/articles/nature21031

Zhang K, Shi S, Han W. 2018. Research progress in cytokines with chemokine-like function. *Cellular & Molecular Immunology* [Internet] 15:660–662. Available from: https://doi.org/10.1038/cmi.2017.121

Zlotnik A, Yoshie O. 2000. Chemokines: A New Classification System and Their Role in Immunity. *Immunity* [Internet] 12:121–127. Available from: https://doi.org/10.1016/S1074-7613(00)80165-X