**Eumelanin and Pheomelanin Misc.**

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I have already written about the evolution of melanosomes and melanocytes, but I would like to try to recount some of the controversial issues that I have encountered with respect to the biology of melanin. In terms of pigment there are two types of melanin. Eumelanin is the black or brown pigment and pheomelanin produces the yellow to red colors. Figure 1 was used in a previous article, and I use it here to show the animal lineages that were making eumelanin and pheomelanin as described by D’Alba and Shawkey, 2019. D’Alba and Shawkey, 2019 note that insects use eumelanin and pheomelanin as pigments, but do not make melanin in melanocytes. Both basic groups of bilateral animals (protostomes and deuterostomes) can make eumelanin and pheomelanin, but the use of pheomelanin as a pigment produced in melanocytes and packaged in melanosomes is only found in tetrapod vertebrates (amphibians, reptiles, birds and mammals). One interesting aspect of melanin pigmentation that I have encountered while researching this topic is that the biology of melanin is not well understood. This is surprising in light of decades of research on human melanoma (skin cancer involving melanocytes) that one would think would have led to a better scientific understanding of melanin than is apparent in all the reviews on the topic that I have encountered. It remains the general consensus of the various review articles that I have used in previous articles that the structure of eumelanin polymers within melanosomes is not known. Neither is the structure of pheomelanin in pheomelanosomes known. The chemical reactions have been elucidated for vertebrates and the enzymatic functions of tyrosinase (TYR), and tyrosinase related proteins 1 and 2 (TYRP1 and TYRP2) are known, but it is not known how these enzymes integrate into the physical structure of the protein scaffold onto which eumelanin is polymerized and arranged within eumelanosomes. Pheomelanosomes lack an extensive protein scaffold structure and pheomelanin is stacked in the pheomelanosome in some unknown structure.

D'Alba and Shawkey 2019 was a review about the evolution of melanosomes (Melanosomes: Biogenesis, Properties, and Evolution of an Ancient Organelle) but some details were not known, glossed over, or left out. In bacteria melanin is “stacked” into granules and associated with the bacterial membrane (Simon and Peles, 2010). Animals like squid (protostomes), fish and tetrapod vertebrates (deuterostomes) package melanin into membrane bound organelles called melanosomes. Squid do not have melanocytes (derived from the neural crest of early embryos), but do have melanosome containing melanophores. Squid melanosomes are derived from the golgi or endoplasmic reticulum, and these vesicles contain multiple granules of melanin (Palumbo, 2003). Vertebrate melanocyte melanosomes are derived from the endosomal membrane (Raposo and Marks, 2007) and enclose a eumelanin shell with a pheomelanin core (Simon and Peles, 2010). Squid and vertebrates also use different paralogs of tyrosinase to make melanin (Aguilera et al., 2013). Insects produce melanin in different cell types in lysosomal related organelles using different enzymes. I haven’t found any papers claiming that protostome melanosomes have a similar structure as vertebrate melanosomes, and protostomes never seem to have evolved PMEL17 which is an integral part of the vertebrate melanosomal protein scaffold onto which eumelanin is polymerized. Lopes et al., 2007 note that melanosomes of both vertebrate retinal pigment epithelial (RPE) cells and melanocytes have a PMEL17 protein scaffold that forms the eumelanin shell of the melanosome. This indicates that the protostome invertebrate and the deuterostome vertebrate melanosomes may have evolved independently even though vertebrate melanocytes are also referred to as melanophores. All the reviews that I have read acknowledge that for vertebrate melanosomes how the eumelanin polymers are stacked in the eumelanin shell and how pheomelanin is arranged in the core are not known.

Slominski et al., 2004 cites over 700 references in their review of the topic. The biosynthesis of neuromelanin, pheomelanin and eumelanin are depicted in their Figure 1. Singh et al., 2013 update what is known about melanin biosynthesis in their Figure 1 by the addition of pyo-melanin, DHN-melanin of fungi, and HPQ-melanin of bacteria. What should be noted is that pheomelanin production is spontaneous and will occur during eumelanin biosynthesis if cysteine and glutathione are present under acidic pH conditions. Pheomelanin synthesis is inhibited under neutral to basic pH conditions. This is relevant because D’Alba and Shawkey do not identify all the taxa that make pheomelanin in their figure 1 (reproduced in my Figure 1). McNamara et al., 2021 agree with D’Alba and Shawkey’s distribution of pheomelanin as a pigment, but they note that pheomelanin has been detected in the retinal pigment epithelial (RPE) cells of squid, jawless and jawed fish. It is not known if the squid RPE melanosomes have the same structure as the vertebrate RPE melanosomes. Squid and cuttlefish along with jawless and jawed fish are not known to use pheomelanin as a pigment, but they do synthesize pheomelanin in the melanosomes of their RPE cells (Rogers et al., 2019). It is unknown why pheomelanin is synthesized in the melanosomes of RPE cells, and my take is that it is just a by-product of eumelanin synthesis in the presence of cysteine and glutathione (an antioxidant). Pheomelanin has likely existed since aerobic organisms were making eumelanin. As noted previously pheomelanin would be expected to be synthesized when eumelanin was synthesized if glutathione and cysteine were present under acidic conditions.

It is not only controversial as to what lifeforms make pheomelanin, but Galvan et al., 2012 note that a good reason for the evolution of making pheomelanin, before animals had eyes to see the pigment qualities of pheomelanin, has not yet reached any consensus. In terms of the photo protective aspects of melanin, pheomelanin may make the cell more sensitive to UV light, and may contribute to deleterious ROS (reactive oxygen species) production. Pheomelanin and high levels of cysteine are also associated with compromising the metal binding ability of eumelanin. There seems to be good reasons for selecting against the production of pheomelanin. Galvan et al., 2012 note that production of pheomelanin uses limited resources such as glutathione (used in reducing oxidative stress) and that pheomelanic morphs of some bird species are more sensitive to stress than are the eumelanic morphs. Galvan et al., 2012 also note that it has been suggested that initial production of pheomelanin may have been accidental (a by-product of eumelanin synthesis). In the case of neuromelanin, both eumelanin and pheomelanin, seems to be synthesized spontaneously from catecholamines as the neurons age (Haining and Achat-Mendes, 2017). The neuromelanin that is called pheomelanin is made when glutathione and cysteine are present during the biosynthesis of the neuromelanin that is called eumelanin. It may be that pheomelanin production has always been a by-product of the production of eumelanin, at least, within aerobic organisms that produced glutathione to deal with oxidative stress. Galvan et al., 2012 propose that pheomelanin began to be made in a controlled fashion in order to maintain cysteine levels in the melanocyte. My take is that the main factor working against this hypothesis is that lysosomes already perform that function in a reversable way. Cystine (cysteine dimer) is transported in and out of the lysosome, but it is difficult to recycle the cysteine from pheomelanin. Once eyes evolved during the Cambrian explosion pheomelanin may have started to be used as a pigment for camouflage and display in spite of the negative attributes that would have selected against pheomelanin production. For some unknown reason fish do not use pheomelanin as a pigment, and the use of pheomelanin as a pigment seems to have started, in the vertebrate lineage, when terrestrial tetrapods evolved. Pheomelanin also evolved to be used as a pigment in insects, but insects use different cell types to make melanin, do make melanin in lysosome related organelles (a type of melanosome), but different enzyme paralogs are used to make melanin.

It may be that melanosomes evolved in early photosensitive cells before eyes evolved, though protostomes and deuterostomes may have evolved melanosomes independently. Making melanin produces toxic intermediates and by-products, so multicellular organisms have evolved membrane enclosed organelles in which melanin is made. The photosensitive cells of protostomes and deuterostomes may have evolved melanosomes independently because of factors such as the different basic structure of melanosomes in protostomes and deuterostomes. Protostomes such as squid evolve retinal pigment epithelial (RPE) cells as did deuterostome vertebrates. I can find no evidence that squid use PMEL17 related protein in the construction of their melanosomes. PMEL17 is the major protein involved in making the protein scaffold associated with the inner melanosomal membrane of vertebrates, and the protein scaffold is where melanin polymerization occurs to form the eumelanin shell of vertebrate melanosomes. I can find no references that have found PMEL17 in either Ciona nor amphioxus. Chrystal et al., 2021 traced the evolutionary history of PMEL17 and their analysis indicates that GPNMB (glycoprotein nonmetastatic melanoma protein B) and PMEL17 are paralogs that may have been created in the R1 whole genome duplication that characterizes extant jawless fish. Chrystal et al., 2021 could not find orthologs of these genes in amphioxus and Ciona genomes. Presumably, either GPNMB or PMEL17 existed in Ciona and amphioxus before the R1 whole genome duplication, but the ortholog has not been identified among the genomic sequence currently available for those two chordates. Something like PMEL17 had to exist in order to have been duplicated in the R1 whole genome duplication event that had to have occurred in or before the common ancestor of all vertebrates existed. It may be that there might exist a PMEL17-like protein in the chordate RPE-like melanosomes, but that analysis has not been done at this time. Lopes et al., 2007 note that vertebrate RPE cells and melanocytes have melanosomes with the same basic structure (including the PMEL17 protein scaffold), and that RPE melanosomes appear to be derived from endosomal vesicles as are melanocyte melanosomes. Burgoyne et al., 2025 also claim that RPE melanosomes are derived from endosomal vesicles and have the same basic structure as melanocyte melanosomes, but they confuse the issue by citing Seiji et al, 1963 when that old review was dealing with melanocytes and had melanosomes derived from golgi vesicles in their Figure 1. Later in the paper Burgoyne et al., 2025 does as was done in Lopes et al., 2007, and only cite melanocyte research for the identification of the vesicles that they are dealing with as endosomal vesicles. RPE melansomes are claimed to be derived from endosomal vesicles because they are claimed to look like the vesicles identified in the melanocyte research papers. Though these two papers did not do the same analysis that identified melanosomes as being derived from endosomes of melanocytes as Raposo et al., 2001, what they did do is likely good enough to claim that vertebrate RPE melanosomes are also derived from endosomal vesicles.

Vopalensky et al., 2012 determined that the basal chordate amphioxus had pigment cells associated with the amphioxus eye spot that had RPE-like qualities. They did not determine that the RPE-like pigmented cells had melanosomes with the same structure as vertebrate melanosomes, but they determined that pigmentation was restricted by the same inhibitors that prevented pigmentation of vertebrate RPE cells, and genes expressed in the cells were similar to vertebrate RPE cells. Vopalensky et al., 2012 concluded that the frontal eye pigment cells were homologous to vertebrate RPE cells. Fatieieva et al., 2025 note that the tunicate Ciona has RPE-like pigmented cells in their pigmented sensory vesicle. Their analysis of the Ciona RPE-like pigmented cells and vertebrate RPE cells and melanocytes indicated that RPE-like pigmented cells could have evolved among the chordate ancestors of vertebrates (tunicates are the closest chordate relatives of vertebrates). Fatieieva et al., 2025 propose that RPE cells and melanocytes evolved from ancestral chordate photosensory cells. They think that melanocytes are homologous to RPE cells, and that the evolution of melanocytes and their differentiation from RPE cells drove the evolution of the neural crest. The neural crest not only produce melanocytes, but it produces the neurons, glial cells, bone, muscle, and connective tissue that transformed the invertebrate filter feeding ancestors of vertebrates into mobile predators (noted in Smith, 2020). The similarities between the vertebrate melanosomes of RPE cells and melanocytes would indicate that RPE cells evolved first and that melanocytes are homologous to RPE cells. Melanocytes evolved from RPE cells that are derived from the neural ectoderm, and the neural crest evolved to produce further differentiated cells such as melanocytes.

My guess is that the RPE-like melanosomes of amphioxus and Ciona will be found to also contain pheomelanin. It is likely a by-product of eumelanin synthesis that remains in the core of the eumelanin shell of the melanosome. This notion seems to be supported by the example of Neuromelanin. Slominski et al., 2004 note that neuromelanin synthesis begins in neurons with the decarboxylation of L-DOPA and ends up with the catecholamines (dopamine, norepinephrine, and epinephrine) that can be used to make neuromelanin. Haining and Achat-Mendes, 2017 claim that neuromelanin spontaneously self assembles as the neurons age, but they describe neuromelanin as being carefully packaged in double membrane granules within the neurons. These granules are later described as being the same size as human eye and hair melanosomes. They further go on to say that neuromelanin has a pheomelanin core and eumelanin surface. Haining and Achat-Mendes, 2017 initially give no reference to support this claim but later cite Bush et al., 2006 after repeating the claim, but they do not know what structure the pheomelanin has in order to make that core nor do they know the structure of the eumelanin making up the eumelanin shell of the neuromelanin granules. Vertebrate eumelanosomes are supposed to have a eumelanin shell with pheomelanin core inside the shell, and as noted it is not known what final structure pheomelanin and eumelanin polymers have within the melanosome. Despite the similarities neuromelanin granules are not melanosomes, but for some reason the eumelanin and pheomelanin are organized in a similar way in both melanin granule types. Neuromelanin is sequestered in a double membrane vesicle that sounds more like the mitochondrial double membrane while melanosomes are single membrane lysosome-like vesicles. The current reviews that I have read are mainly interested in melanin as a pigment, and basically only mention that neuromelanin exists. Neuromelanin is composed of aminochromes and noradrenalinochromes (Slomionski et al., 2004) but are still classified as pheomelanin and eumelanin in references such as Bush et al., 2006. The production of pheomelanin and eumelanin in neurons appears to be spontaneous and the melanins accumulate as the neurons age and are sequestered in the double membrane vesicle, probably, to protect the neurons from biochemical properties of neuromelanin (Haining and Achat-Mendes, 2017). Neuromelanin seems to support the notion that pheomelanin production is a by-product of eumelanin production in RPE cells, and that it was likely a by-product of eumelanin synthesis within the melanosomes of the melanocytes that evolved in the early vertebrates. Eumelanosomes existed long before pheomelanosomes evolved as a pigment in tetrapod vertebrate melanocytes.

It may be that melansomes evolved in the RPE-like cells of early chordates. The embryonic neural crest and melanocytes derived from the neural crest are limited to vertebrates (review York et al., 2020).

“Both neural crest cells and placodes are found only in vertebrate animals and they are responsible for constructing many of the traits that uniquely define the vertebrate clade (Figure 1), including the cartilage and bone of the head and jaw skeleton, neurons and glia of the peripheral sensory nervous system, colorful patterns of pigmentation, and much more.” (quote from York et al., 2020).

Smith, 2020 puts up references indicating that the tunicate Ciona may have a rudimentary neural crest and calls pigment cells produced, by the Ciona neural crest-like cells, melanocytes. Smith, 2020 cite Abitua et al., 2012 to support calling the Ciona pigmented photosensitive cells melanocytes, but Abitua et al., 2012 may be misidentifying the cell type by claiming that they are cephalic melanocytes. The cells that are called cephalic melanocytes are the RPE-like pigmented cells of the Ciona light detecting ocellus. They call them melanocytes because MITF (Microphthalmia-associated transcription factor) is expressed. MITF regulates pigmentation in both vertebrate melanocytes and RPE cells. Calling the Ciona photosensitive pigmented cells melanocytes seems to have been a minority view, as Vopalensky et al., 2012 claimed that their chordate pigmented photo receptor cells were not melanocytes and their data indicated that they were RPE-like cells in amphioxus a more basal chordate. Vopalensky et al., 2012 do consider the RPE-like photoreceptor cells to be homologous with melanocytes, and York et al.,2020 limit the neural crest to vertebrates, excluding Ciona. The issue may be that the vertebrate RPE cells are derived from the neural ectoderm and not the neural crest and RPE cells are not considered to be melanocytes in vertebrates. Todorov et al., 2024 presents additional evidence that neural crest-like cells exist in Ciona, and referred to the neural crest-like cell type as producing sensory pigment cells and cite references that claimed to have determined that these sensory pigment cells were homologous to melanophores (melanocytes) of zebra fish. The Ciona RPE-like cells are similar to the RPE-like cells described in amphioxus (Fatieieva et al., 2025 and Vopalensky et al., 2012) and amphioxus definitely does not have a neural crest. In Ciona if a neural crest does exist it seems to be limited to a few cells at the neural plate border, and could mean that the evolution of the neural crest occurred in the closest relatives (tunicates) to vertebrates and not jawless fish. It might also be that these cells are derived not from an early neural crest, but what is called the neural ectoderm in vertebrates, and that the Ciona photo sensitive cells evolved into RPE pigmented cells. RPE cells would have existed first, and then a true neural crest evolved producing the melanocytes of vertebrates. The Ciona photo sensitive pigmented cells would still be homologous to fish melanocytes, but would have an RPE intermediate phase of pigmented cells derived from the neural ectoderm. As noted it has already been proposed that RPE cells are homologous to melanocytes. One issue is that the vertebrate embryo is composed of thousands of cells at the stage that the neural crest first exists in the embryo. Todorov et al., 2024 note that what they are calling a possible neural crest in Ciona exists in an embryo of just a few hundred cells and that the Ciona “neural crest” may be limited to just a couple of cells of that smaller (in cell number) embryo.

Ciona has MC4R-like genes, but these receptors do not bind melanocortin ligands as do the MCa and MCb melanocortin receptors of jawless fish (Ji et al., 2024). This means that MC1R-like functions had not evolved in Ciona and was not involved in regulating pigmentation of the sensory pigment cells that are supposed to be homologous to fish melanophores. Melanocytes derived from the neural crest are known to exist in jawless fish along with melanocortin receptors. RPE-like cells exist in basal chordates like amphioxus and tunicates like Ciona that are more closely related to vertebrates than amphioxus. These RPE-like cells are not derived from neural crest cells in amphioxus. If the RPE-like cells of Ciona are melanocytes derived from neural crest cells, Ciona would be missing RPE-like cells that are not derived from neural crest cells in other chordates, but these Ciona “melanocytes” have RPE-like function in Ciona.

My take is that since Ciona have a mobile tadpole stage in their early development that their embryos do not have a true neural crest, but they have embryonic cells doing double duty as neural ectoderm and neural crest cells as some type of developmental intermediate. These cells may not be one or the other, but some type of transitional phase in the development of true neural crest cells. The common ancestor of extant jawless fish had evolved the basic vertebrate eye. The neural crest likely evolved in ancestral vertebrates that were evolving eyes and the neural crest derived tissues such as the head, muscle, bone and melanocytes. Neural ectoderm derived RPE cells would be needed for evolving the eye, and the neural crest cell types would be needed to evolve the head and mobile body of these ancestral vertebrates. These neural crest derived cell types would have to be produced without losing the original function of the neural ectoderm derived cells. Melanocytes would be among the homologous cell types produced by the neural crest, and RPE cells would continue to be produced by the neural ectoderm. Fatieieva et al., 2025 propose that RPE cells and melanocytes evolved from the ancestral RPE-like photosensory cells of Ciona. They think that melanocytes are homologous to Ciona RPE-like cells, and that the evolution of melanocytes and their differentiation from RPE cells drove the evolution of the neural crest. The evolution of the neural crest would have facilitated the differentiation of melanocytes from RPE cells (both cell types could be produced one from the neural ectoderm and the other from the neural crest). The development of the neural crest may have been required to allow the differentiation of many of the homologous cell types derived from the neural crest in order to allow the neural ectoderm derived cell types to retain their functions. My take is that Ciona may not represent an intermediate transitional state, but a dead end where some neural ectoderm cells transformed to be more neural crest-like, and this worked for Ciona that only temporarily needed a head like structure, and had not evolved eyes. The ancestors of vertebrates may have developed neural crest cells that may have evolved from cells related to neural ectoderm cells so that the neural ectoderm derived cells could retain their original functions. The ancestors of vertebrates were able to evolve the neural crest in a larger (more cells when the neural crest emerges) embryo, possibly, after the R1 whole genome duplication, at the time both neural ectoderm and neural crest derived cells would be differentiating.

In Figure 1 I have the R1 and R2 whole genome duplication events labeled in the phylogeny (reviewed in Yu et al., 2024). Ray and Medeiros, 2023 propose that the neural crest may have evolved before the R1 whole genome duplication event. They think that several genes associated with neural crest cells existed before the R1 genome duplication event. This is consistent with the findings of Todorov et al., 2024. Jawless and jawed vertebrates share the R1 genome duplication event. York et al., 2020 note that most neural crest derived cell types evolved before the evolution of jawed vertebrates and the R2 whole genome duplication event that all jawed vertebrates share, but a few cell types such as the myelin sheath surrounding neurons and sympathetic chain ganglia are not found in jawless fish, so the neural crest was continuing to evolve within jawless fish at the time the R2 whole genome duplication occurred in the ancestor of all extant jawed vertebrates. Melanocytes are found in jawless fish, and would have been among the original neural crest derived cell types.

The Ciona data indicate that a few neural crest-like cells may exist in Ciona embryos. These cells express neural crest specific genes and may produce neural crest derived cell types, and this is consistent with the neural crest developing before the R1 whole genome duplication event. The issue may be that Ciona is coopting what may be neural ectoderm cells to become neural crest-like cells, and what is left of the neural ectoderm no longer produces cell types such as pigmented RPE-like photoreceptor cells. Instead Ciona produces some neural crest derived cell types, but some of the presumed neural crest cells continue to produce RPE-like cells instead of melanocytes. Todorov et al., 2024 have their neural crest-like cells producing the RPE-like cells. These Ciona neural crest-like cells would have to revert to being more neural ectoderm-like in order to produce the cell types needed to make structures such as eyes, while continuing to be able to produce the neural crest derived cell types. It may be that early in the process of converting neural ectoderm cells to have neural crest functions that the R1 whole genome duplication produced embryos with more cells at the developmental stage where the neural crest was developing in the embryo, and allowed the neural ectoderm and neural crest to develop separately. This division of cell types might be more plausible in an allotetraploid between a species developing neural crest derived cell types (such as Ciona) and another species that was not as far along in that process, but Yu et al., 2024 claims their data is consistent with R1 being an autotetraploid event (one species genome duplicated).

Melanocortin 1 receptor (MC1R) function may have evolved in jawless fish with the evolution of MCa (melanocortin receptor a) after the R1 whole genome duplication event (Ji et al., 2024). The R1 whole genome duplication may have produced MCa and MCb, so the ancestor whose genome was duplicated may have had a functional melanocortin receptor that could bind melanocortin ligands. The function of the original melanocortin receptor is not known. The embryonic neural crest and neural crest derived melanocytes evolved by the time the common ancestor of extant jawless vertebrates evolved. It may be that the MC1R function of the regulation of the production of eumelanin or pheomelanin containing melanosomes (found in tetrapod vertebrates) evolved long after MC1R functioned in the proliferation of melanocytes and the development of eumelanosomes in the melanocytes of jawless fish, but what MCa does for melanocytes in jawless fish is not known. MC1R was evolving when the neural crest evolved and started producing melanocytes that only made eumelanosomes. Melanocytes evolved in jawless fish before the R2 whole genome duplication event that occurred in the ancestor of all jawed vertebrates. The current scenario is that MC1R would have existed at the R2 whole genome duplication event and would have continued it’s melanocyte associated activity. MC1R would have been co-opted into regulating eumelanosome and pheomelanosome production in the ancestor of terrestrial tetrapods, and this MC1R function would not have been needed during the evolution and radiation of jawed fish. If jawless fish eumelanosomes do have a pheomelanin core, like the eumelanosomes of tetrapod vertebrates, pheomelanin was being made long before MC1R was needed to limit the production of pheomelanosomes. Currently MC1R is activated by agnonist melanocortin ligand binding and eumelanosome production is initiated within the melanocyte. If the ASIP (agouti signaling protein) antagonist binds to MC1R, agonist binding is prevented and eumelanin production is blocked allowing pheomelanosomes to be produced. Loss of function mutations of MC1R results in the production of pheomelanosomes instead of eumelanosomes.

There seems to be a consensus that tetrapod eumelanosomes contain both pheomelanin and eumelanin. Among tetrapods eumelanin is embedded in the protein scaffold that is associated with the inner melanosomal membrane. Pheomelanin is believed to accumulate inside this eumelanin membrane bound shell that is characteristic of eumelanosomes. If Rogers et al., 2019 are correct in claiming that pheomelanin is produced in the RPE cells of jawless and jawed fish, pheomelanin was being produced when the function of MC1R in the development and proliferation of melanocytes was evolving. This could mean that MC1R may have evolved to limit the production of pheomelanin by-product as one of it’s initial functions, and would have already evolved that function before the adoption of pheomelanin as a pigment by tetrapod vertebrates. Pheomelanin is synthesized in the presence of tyrosinase, tyrosine, glutathione, and cysteine when the internal pH of the melanosome is acidic. The initial function of MC1R may have been to facilitate the proliferation of melanocytes and the production of eumelanosomes within those melanocytes, and restricting the production of pheomelanin as a by-product of eumelanin biosynthesis. It would have done this by limiting the cysteine levels in the melanosome and regulating the pH to be less acidic. The original function of MC1R may have been more similar to what it does in the tanning process (Review: Upadhyay et al., 2024).

In order to use pheomelanin as a pigment a regulatory system would have had to evolve to block MC1R’s negative regulation of pheomelanin synthesis, disrupt the protein scaffold needed to produce the eumelanic shell of the melanosome, decrease the pH of the melanosome so that pheomelanin synthesis was favored over eumelanin synthesis, and allow pheomelanin accumulation in the melanosome without having pheomelanin enclosed in a eumelanin shell. MC1R antagonists like ASIP that may have evolved to regulate melanocyte proliferation and the production of eumelanosomes would have been co-opted to interfere with eumelanin production and positively regulate pheomelanin production, in part, by interfering with the activation of MC1R.

**Conclusion:**

There remain some unknowns or controversial topics about melanin biology and the evolution of melanin as a pigment in multicellular animals.

1. The final structure of pheomelanin and eumelanin polymers and their association with proteins within the mature melanosome is not known. There is a consensus that vertebrate melanosomes have a eumelanin shell that surrounds a pheomelanin core, but how far back down the vertebrate evolutionary lineage that this is the case for chordate melanosomes is not known. Vertebrate retinal pigment epithelial (RPE) cells and melanocytes have melanosomes that have the same basic structure. Squid (protostomes) RPE cells have melanosomes that contain both pheomelanin and eumelanin as do the RPE cells of jawless and jawed vertebrates (deuterostomes), but protostomes use different enzymes and cell types to produce melanin, and their melanosomes may not be derived from endosomes, so intracellular melanosome organelles may have evolved independently in these two bilateral animal lineages. Both protostome and deuterostome lineages have evolved RPE cells in their evolution of eyes, and both have evolved melanophores (melanocytes) and their associated melanosomes, but they seem to have come independently to these ends.
2. There is no consensus on why pheomelanin production was selected for before it could be used as a pigment (before the evolution of eyes). Pheomelanin does not have the radiation protective aspects of eumelanin, produces reactive oxygen species under UV irradiation, and it interferes with the metal binding properties of eumelanin. Pheomelanin is spontaneously produced when eumelanin is being synthesized when glutathione (an antioxidant) and cysteine are present. My take is that initially, pheomelanin may have been a by-product of eumelanin synthesis in aerobic organisms that produced glutathione as an antioxidant.
3. The full extent as to the existence of pheomelanin among lifeforms is likely not known at this time. For multicellular animals pheomelanin is used as a pigment in insects (protostomes) and in tetrapod vertebrates (deuterostomes) but the use of pheomelanin as a pigment seems to have evolved independently in the two lineages since different cell types make the melanin and different paralogs of the enzymes are used to make melanin. Pheomelanin has been found to exist in the melanosomes of RPE cells of squid, jawless, jawed fish and tetrapod vertebrates, but it has not been identified in the RPE-like cell melanosomes of basal chordates such as amphioxus and the closest chordate relatives (tunicates) to vertebrates such as Ciona in their RPE-like cells. This may be due to the fact that no one has looked for pheomelanin in the RPE-like melanosomes in the chordate close relatives of vertebrates.
4. Melanosomes may have an ancient origin in the vertebrate lineage, but I could not determine if chordates like amphioxus and Ciona have melanosomes in their RPE-like cells with the same structure as vertebrate RPE and melanocyte melanosomes. PMEL17 was not found in the amphioxus and Ciona genomes, but neither were the other paralogs for this family of structural proteins. It may be that the genes related to PMEL17 have not been sequenced in the current genome builds of Ciona and amphioxus. Melanosomes evolved in pigmented photosensitive cells and the RPE cells of squid and vertebrates have melanosomes that contain both eumelanin and pheomelanin, but, as noted above, RPE cells and melanosomes may have evolved independently in the protostome and deuterostome lineages. The two lineages may have evolved different lysosome-like organelles to contain melanin that have similar characteristics due to their purpose of housing melanin biosynthesis separated from other cellular processes, and both lineages evolved pigmented photosensitive cells that evolved lysosome-like melanin containing organelles. Due to pheomelanin’s production of reactive oxygen species under UV irradiation the pheomelanin by-product may have been sequestered inside of a shell of eumelanin for both protostomes and deuterostomes. So far vertebrate RPE cells and melanocytes are known to have melanosomes with the same basic structure (eumelanin embedded into a protein scaffold, associated with the melanosomal membrane, composed of mostly PMEL17 producing a eumelanin shell with a pheomelanin core), but it is not known if the chordate ancestor of vertebrates also had RPE cell-like melanosomes with this structure.
5. There seems to be some controversy as to when melanocytes evolved. Some researchers are claiming that the Ciona pigmented photosensitive cells are melanocytes. The claim is that these pigmented photosensitive cells are derived from a couple neural crest-like cells in the Ciona embryo, but these pigmented photosensitive cells are also claimed to be RPE-like cells and would be homologous to vertebrate RPE cells. The vertebrate ancestor that evolved eyes and became a mobile predator would need RPE cells along with other neural ectoderm derived cells needed to form the central nervous system, and evolve a neural crest to generate the cell types that not only included melanocytes, but also the cell types required to become a mobile predator. RPE cells and melanocytes are likely homologous and vertebrate RPE cells and melanocyte melanosomes seem to have the same structure and are both derived from endosomal vesicles. The Ciona photo sensitive cells are believed to be homologous with vertebrate RPE cells, but some researchers call the Ciona pigmented photosensitive cells melanocytes. This may be incorrect because the pigmented cells have RPE cell-like properties and do not seem to act as melanocytes.

The majority view seems to be that the neural crest evolved in jawless vertebrates, possibly, after the R1 genome duplication event. Jawless fish have RPE cells and melanocytes. Jawless fish also have functional melanocortin receptors (MCa and MCb) while Ciona have MC4R-like genes, but these MC4R-like receptors do not bind melanocortin ligands. MCa is thought to have MC1R like function. My take is that the Ciona pigmented photosensitive cells are not melanocytes and that the vertebrate neural crest likely evolved in the vertebrate ancestor that evolved eyes and a head with melanocytes evolving with the rest of the mobile body plan. It may be that the R1 whole genome duplication event may have produced an embryo with enough cells to allow the differentiation of neural ectoderm cells into neural crest cells while retaining enough neural ectoderm cells to continue to produce the neural ectoderm derived cell types. For Ciona, the embryo consists of only a few hundred cells at the time that the presumed neural crest-like cells develop, but for vertebrates, the embryo is larger and is composed of more than 3 times the number of cells when the vertebrate neural crest develops. My take is that Ciona may be some type of dead end where the tadpole phase of development required some neural crest-like cells, but these cells were only temporarily needed and some cells derived from these “neural crest” derived cells kept their neural ectoderm derived cell functions (such as the RPE-like function instead of melanocyte function). The ancestor of vertebrates would have had to retain neural ectoderm cells that produce RPE cells and evolve a neural crest that would produce melanocytes and other cells needed to make a mobile predator.

1. Since pheomelanin seems to be produced in melanosomes when eumelanin is being synthesized it may be that the original function for MC1R was to control the proliferation of melanocytes and production of eumelanosomes. There was no need to produce pheomelanosomes in jawless and jawed fish. MC1R may have evolved to limit the production of unwanted pheomelanin by being involved in limiting cysteine concentration in the melanosome and increasing the pH of the melanosome so that it was less acidic and less prone to spontaneously produce pheomelanin. When pheomelanin began to be used as a pigment in the ancestors of tetrapod vertebrates MC1R would have been co-opted into it’s present role of regulating the production of either eumelanosomes or pheomelanosomes. My take is that the original roll of MC1R was likely closer to what it does in the tanning process of stimulating melanocyte proliferation and eumelanosome production.



Figure 1. This is a reduced representation of D’Alba and Shawkey, 2019 Figure 1 with some of the information from McNamara et al., 2021 Figure 2. D’Alba and Shawkey, 2019 did not have mussel or nematodes on their figure, but those are the taxa that I could find dated by Carlisle et al., 2024. The divergence estimates are in Ma (million years in the past). I rounded to whole numbers because the precision of the estimates do not seem to warrant any decimal places. I took their mean estimates from their model #1 (Carlisle et al., 2024 supplementary data). They are quite a bit different than the estimates of McNamara et al., 2021 in their Figure 2. I only have Chordata representing deuterostomes, and Carlisle et al., 2024 estimates that deuterostomes evolved around 565 Ma. R1 and R2 indicate the two whole genome duplication events in the vertebrate lineage. The black squares indicate that eumelanin is produced in those lineages. The red squares indicate that pheomelanin is produced in those lineages. The long terminal branches are not to scale and were truncated to fit into a reasonably sized phylogeny. The node branches are set to scale relative to each other.

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