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Have We Achieved a Unified Model of Photoreceptor Cell Fate Specification in Vertebrates?

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Abstract

How does a retinal progenitor choose to differentiate as a rod or a cone and, if it becomes a cone, which one of their different subtypes? The mechanisms of photoreceptor cell fate specification and differentiation have been extensively investigated in a variety of animal model systems, including human and non-human primates, rodents (mice and rats), chickens, frogs (*Xenopus*) and fish. It appears timely to discuss whether it is possible to synthesize the resulting information into a unified model applicable to all vertebrates. In this review we focus on several widely used experimental animal model systems to highlight differences in photoreceptor properties among species, the diversity of developmental strategies and solutions that vertebrates use to create retinas with photoreceptors that are adapted to the visual needs of their species, and the limitations of the methods currently available for the investigation of photoreceptor cell fate specification. Based on these considerations, we conclude that we are not yet ready to construct a unified model of photoreceptor cell fate specification in the developing vertebrate retina.

I) INTRODUCTION

Normal sight depends upon the coordinated activity of specialized retinal cells that are embryonically derived from a simple, apparently homogeneous neuroepithelium. Elucidation of the mechanisms underlying the developmental transition from a sheet of proliferating neuroepithelial cells to the complex, highly specialized and multilaminar array of distinct types of retinal neurons, ranging from sensory receptors (photoreceptors) to projection neurons (retinal ganglion cells), has intrinsic scientific interest as well as clinical relevance. For example, the transplantation of embryonic retinal tissue, neural progenitors or stem cells offers possible therapeutic strategies for diseases such as age-related macular degeneration and retinitis pigmentosa, in which photoreceptor degeneration leads to visual loss, and eventually to blindness. However, experimental approaches using cell transplantation have so far achieved limited success, and overcoming these limitations will require better understanding of the molecular mechanisms that regulate the differentiation of retinal cells in general and of photoreceptor cells in particular.

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Considerable progress has been made in the investigation of extracellular signaling molecules and intracellular regulatory mechanisms controlling retinal cell fate specification and differentiation in vertebrates, and a number of excellent reviews have been published recently [1,14,21,38,65,75,84,102,119,124,128,129,157,163,167,190,206]. Despite this progress there are still many unanswered questions regarding photoreceptor specification, including 1) Is the retinal neuroepithelium a homogeneous collection of multipotent neuroepithelial cells throughout retinal neurogenesis, or does it contain committed retinal progenitors with more restricted cell fate - *i.e.* cone progenitors and rod progenitors? 2) At what stage in retinal development do neuroepithelial derivatives become "committed" to the photoreceptor fate? 3) Is photoreceptor subtype determination a distinct (and direct) choice among different fates or, instead, do subtypes derive from a 'generic' immature photoreceptor precursor that indiscriminately transcribes low levels of photoreceptor-specific genes (*i.e.* genes specific for rods and for all subtypes of cones)? 4) Is the time of terminal mitosis/cell birth a determinant of specific photoreceptor cell fates, (*e.g.* cone vs. rod), or even of cone subtypes? 5) Does the competence of retinal progenitors / photoreceptor progenitors to produce cones versus rods change as development progresses? 6) Are there specific lineage relationships among different cone photoreceptor cell types? 7) Is the determination of cone subtype identity limited to control of the expression of a specific opsin gene, or is it a much more complex phenomenon? 8) Related to this, is the use of opsin markers sufficient to identify photoreceptor subtypes? 9) Do postmitotic cells retain the plasticity to commit / switch to a photoreceptor *vs*. non-photoreceptor fate, and/or between the rod and the cone fate? 10) Are postmitotic cone precursors committed to a specific cone subtype, or are they plastic? 11) What extrinsic and intrinsic signals determine photoreceptor cell fate, the choice to be a rod or a cone, and/or the choice between cone subtypes? 12) How is photoreceptor cell fate specification regulated in species in which photoreceptors are added to the differentiated retina during normal growth and/or during retinal regeneration?

In this review, we will limit our focus to vertebrate photoreceptors and consider cell fate specification and differentiation with special attention to what we know about how the different types of photoreceptors are generated, the methods used to identify photoreceptor subtypes, and their life history. Limitations of space and knowledge prevent us from addressing individually each one of the questions posed above. While it is clear that these questions are applicable to all vertebrates, mechanisms of photoreceptor cell fate specification and differentiation have only been investigated in a limited number of animal model systems, including human and non-human primates, rodents (mice and rats), chickens, frogs (*Xenopus*) and teleost fish (several species).

To build a truly unified model of photoreceptor cell fate specification in the developing vertebrate retina requires a comparative analysis that takes into account very substantial differences in photoreceptor subtypes, development and specializations that are found in vertebrates. In this review we have attempted to highlight fundamental questions that remain unanswered, or have only been partially answered, or even have different and sometimes contradictory answers in different animal model systems. We also point out that the many very powerful methods currently available for the study of photoreceptor cell fate specification are not free of limitations, which in some cases are quite significant, and we suggest that new experimental approaches will be needed before we can construct a unified model.

II) VERTEBRATE VISUAL PIGMENTS AND THE EVOLUTIONARY ORIGINS OF RODS AND CONES

The duplex theory of vision is based on the idea that rod photoreceptors mediate scotopic vision and cone photoreceptors are responsible for photopic vision. In brief, the characteristics of rod-mediated visual function include response to low light intensity, black and white vision, low

acuity and high sensitivity, with slow kinetics (rate of pigment regeneration and dark adaptation). In contrast, cone-mediated vision functions at high light intensity, allows color discrimination, has high acuity and low sensitivity, and rapid kinetics.[1] Ebrey and Koutalos [55] suggest four complementary criteria for classifying vertebrate photoreceptors as rods or cones: i) their visual pigment, ii) the components of their phototransduction cascade, iii) their morphology, and iv) their electrophysiological properties. Many studies of photoreceptor cell fate determination rely largely or exclusively on the first criterion, so we will first review what is known about the vertebrate visual pigments. It will quickly become clear to the reader that reliance on opsin expression as a proxy for photoreceptor identity (and lineage) is inadequate because of notable exceptions to the standard 'rule' that rhodopsin is found in rods and all the remaining opsins are in cones. The various components of the visual transduction cascade appear to be distinct in rods and cones (and correspond to separate but related genes), and the biochemical properties of rod and cone isoforms account for some of the differences in physiological properties [55]. Expression of rod or cone components of the transduction cascade may provide a more robust measure of photoreceptor identity, but to date only a few developmental studies have examined this question [42,146].

Vertebrate visual pigments are grouped by molecular phylogeny into five evolutionarily distinct opsin gene families (Fig. 2): RH1, RH2, SWS1, SWS2 and LWS/MWS [203].[2] The RH1 (rhodopsin) gene is generally associated with rods and the other four opsin genes are usually expressed in cones. Substitutions in specific amino acid residues in the opsin apoprotein spectrally tune the light sensitivity of visual pigments so that each class has maximal absorption (λ_{max}) within a specific range of wavelengths: i) RH1 $\lambda_{max} \sim$ 500 nm (but blue-shifted in marine species, as described below), ii) RH2, rhodopsin-like pigments expressed in cones with λ_{max} 470-535 nm, iii) SWS1 (short-wavelength-sensitive-1) with λ_{max} 355-440 nm, iv) SWS2 with λ_{max} 410-490 nm, and v) LWS/MWS (long and middle-wavelength-sensitive) with λ_{max} 490-575 nm [25,55,203].[3]

The number and nature of the visual pigments expressed in retinal photoreceptors varies significantly among different species

Shallow water teleost fishes and diurnal birds and reptiles generally express all five of the spectral classes of opsins in different photoreceptors, one rod and four types of cones, giving them tetrachromatic color vision. Mammals lost the ancestral RH2 and SWS2 classes and generally have dichromatic color vision, mediated by the SWS1 pigment (S-cones) and the LWS/MWS pigment (M-cones) [55]. There are, however, some significant deviations from this basic mammalian pattern. For example, some mammals appear to be monochromats, due to co-expression of SWS1 and LWS/MWS pigments within individual cones; the mouse is the best characterized representative of this group (see section IIIA3). In old world primates, on the other hand, the ancestral LWS gene was duplicated and subsequent changes in key amino acid residues separated the λ_{max} values of the two pigments, thus restoring trichromatic color vision in the human lineage [142,203].

Molecular phylogeny of the opsin genes provides unequivocal evidence that the last common ancestor of the vertebrate lineage had orthologs of all four classes of cone opsin genes (RH, SWS1, SWS2 and LWS), and since these ancient vertebrates lived in the rich spectral

[1] A detailed discussion of the numerous biochemical, morphological, and functional differences between rods and cones is beyond the scope of this review. We refer the interested reader to excellent classic and recent reviews on this topic [4,46,55,194].

[2] We use a terminology based on opsin gene families [203] because this designation is unambiguous and accurate across all vertebrate species. Opsins are commonly referred to by their color name, corresponding to the λ_{max} of the visual pigment (*e.g.* red, green, blue, *etc.*), but spectral sensitivity is subject to rapid and large evolutionary fluctuations within a group of genes that share a common ancestor, so experts recommend avoiding the use of color names [55].

[3] These λ_{max} values are for pigments with vitamin A1-based chromophores; aquatic vertebrates (teleost fish, amphibians and some reptiles) also use 3-dehydroretinal from vitamin A2, which shifts the λ_{max} of the pigment to longer wavelengths [22].

environment in the shallow seas of the early Cambrian, 540 million years ago, they almost certainly had photopic color vision [25]. The LWS and SWS1 genes, which are positioned at the extremes of the spectral range, are the most ancient, with SWS2 and RH genes appearing later as sister groups of SWS1 (Fig. 2). Rhodopsin and rod photoreceptors capable of scotopic vision were more recent evolutionary inventions based on duplication of the ancestral RH gene, an event that occurred close to the point of divergence of jawed (gnathostomes) vertebrates from the more primitive jawless (agnathan) vertebrates [49,159].

In several vertebrate species, the visual pigment expressed in a particular type of photoreceptor cell does not match other criteria for classifying rods versus cones

For example, the photoreceptors in jawless fishes (*e.g.* lampreys) have morphological and functional characteristics intermediate between classic rods and cones (Fig. 3). Some species of lamprey have photoreceptors that express RH1 (rhodopsin) but have outer segment discs that are open to the extracellular space (characteristic of typical cones, in contrast to rods, which have outer segment discs pinched off from the plasma membrane) [55]. Other species have morphologically cone-like photoreceptors with oil droplets in the inner segment, but with an RH1 pigment and the kinetics and sensitivity of rods [47,48]. Similarly, the retina of the nocturnal gecko has only rods (based on microscopic evaluation of the photoreceptors), but it contains both LWS/MWS and RH2 pigments and not the RH1 rhodopsin pigment [55]. Amphibians have two morphologically and physiologically distinct types of rods, the so-called 'red' and 'green' rods, and the latter use an SWS2 pigment (otherwise found in cones) but with the transduction machinery associated with rods [125]. Finally, the chameleon has an all-cone retina based on morphology, but all five opsin gene families are expressed, including an RH1 rhodopsin [55].

In summary, it is evident from the preceding considerations that a unified model of photoreceptor cell fate specification should encompass a comparative, "evo-devo" approach that acknowledges phenotypic variations in opsin gene expression and focuses instead on discovering the underlying developmental genetic mechanisms in the context of adaptive evolutionary changes that distinguish 'rod' and 'cone' photoreceptors in different animals.

III) COMPARISON OF PHOTORECEPTOR DEVELOPMENT AND PROPERTIES IN SELECTED SPECIES

This section will focus on a few experimental animal model systems, selected to illustrate the enormous range of observations that must be taken into account before we can develop a unified model of photoreceptor cell fate specification

A) Mice and Rats

Rodents have been and still are extensively used as experimental animals for biological research because they are small, easily housed and fed, breed readily, have a short gestation time, and mature rapidly (*cf*, [163]); their use for the study of photoreceptor lineage and differentiation, however, has changed considerably over time. We will present here a brief summary of those changes, which have had a significant impact on this field.

1) Pre-1990 period: descriptive studies—Use of rodent embryos for photoreceptor development research was fairly limited before 1990, possibly due to the small size of their eyes and *in utero* development, which makes them less amenable to experimental manipulation than those of chicks, fish or amphibians. Mouse and rat retinas have small photoreceptors, moreover, and their cones are scarce and difficult to identify by light microscopy [4]. Despite this relative neglect, the mouse was the subject of some classical studies of eye development during the pre-1990 period, including the pioneering study of cell "birth" in the retina

using ^{3}H-thymidine autoradiography [177], extended in great detail to the birthdating of rods and cones [35]. These studies provided an important framework for understanding photoreceptor lineage by demonstrating that cones are generated early, during a short period of embryonic development, whereas the period of rod genesis is much longer and extends into postnatal life. A similar timetable was documented more recently in the rat [99,163]. The pre-1990 era also included excellent descriptive studies of photoreceptor development by electron microscopy and lectin cytochemistry [19,89].

2) The 1990's: the golden era of transgenics and knockouts—The introduction of methodology allowing manipulation of the mouse genome *in vivo* had a profound impact on biological research in general, and on studies of photoreceptor development in particular [37]. Since the beginning of the 1990's, well over 400 papers using transgenic technology to study mouse photoreceptors have been published, many of them dealing with photoreceptor lineage and differentiation. Studies of photoreceptor development in the mouse expanded even further in the mid- 1990's with the introduction of methods for knocking out individual genes by homologous recombination. Together with the availability of well characterized lines of mutant mice, these methods made it possible to study important aspects of photoreceptor differentiation much better in the mouse than in other vertebrates, as exemplified by the investigation of the role of transcription factors such NRL, CRX, NR2E3 and the thyroid hormone receptor [41,43,52,72,80,117,120,133,145,155,171,182,183,202,205].

3) The post-1990's: a revisionist view

Similarities and differences between mouse photoreceptors and those from other species: The retina of the laboratory mouse (*Mus musculus)* in general, and the photoreceptors in particular, are different from other vertebrate species, including many rodents. This idiosyncratic nature was eloquently encapsulated in the title of a recent review article as "The uncommon retina of the common house mouse" [143], although these unusual features are likely not a result of domestication but of natural adaptations [176]. Comparative aspects of the structure, development and function of the mouse retina have been the subject of several recent and thorough reviews [4,12,23,25,50,92,123,142,158,203,204], so only a brief summary of the available information will be presented here.

As described in Section II, the presence of only two cone pigments in the mouse make it a less-than-ideal model for non-mammalian vertebrates (which have four) and for old world primates and humans (which have 'two plus', considering the tandem duplication of the LWS/MSW opsin gene). Additional idiosyncratic features of mouse cones that separate them from most mammals include the shift in the λ_{max} of their short-wave length (SWS1) pigment towards the UV [101,143], the regionalization of their S cones to the ventral (inferior) retina, and the co-expression of visual pigments within individual cones. Co-expression of cone pigments has been found during development in some species [143] and under experimental conditions in others [27], but it is generally not observed in adult mammalian retinas, in which the "one cone-one pigment" rule is prevalent [141,142]. Co-expression of SWS1 and MWS pigments in mouse cones is one of the few exceptions to this rule, and has been particularly well characterized since its discovery by Rohlich and colleagues [169] (reviewed in [123]). Co-expression of opsins was initially observed predominantly in the cones located at the equator of the eye [169], but a recent study with different antibodies showed that it is more prevalent than once thought, albeit with some topographical differences in the relative ratios of SWS1 and MWS pigments within individual cones [9].

SWS1 and MSW opsin gene expression in mouse cone photoreceptors is regulated by thyroid hormone. Mice with a deletion of the thyroid hormone receptor β2 (TRβ2) lack MWS-opsin and have a concomitant increase in SWS1-opsin immunoreactive cones, accompanied by an

expanded presence of SWS1-opsin expression from the ventral hemisphere to the entire retina [145]. Binding of endogenous thyroid hormone to this receptor is required to inhibit SWS1-opsin and to activate MWS-opsin, and the retinoid X receptor gamma (RXRγ) interacts with TRβ2 in this phenomenon [168]. This finely tuned regulation of MWS and SWS1 expression in mouse cones is striking, and may imply that the mouse only has one type of cone that expresses two different pigments in different proportions [9]. Although this interpretation has been disputed [143], it appears unlikely that mice could have dichromatic vision, which requires at least two distinct cone spectral subtypes. These considerations led to the suggestion that " ... cone-based performance in the mouse retina with its many dual pigment cones certainly is not representative for the mammalian retina..."[154].

In summary, it is unquestionable that mouse genetics had a profound impact on our understanding of photoreceptor development, but caution should be exercised in using that information as the basic foundation for a general model of photoreceptor cell fate specification in vertebrates.

Genetic manipulations are now possible in most vertebrate species: The sequencing of the genome of several frequently used experimental animal species has been completed or is fairly advanced; examples are the chick [32], *Xenopus* (http://www.ensembl.org/Xenopus_tropicalis/index.html) and zebrafish (http://www.sanger.ac.uk/Projects/D_rerio/). This has, of course, allowed comparative studies of the mouse *vis a vis* other species, and some differences relevant to photoreceptor development have begun to emerge. A significant example is the transcription factor Nrl, which plays a key role in rod differentiation in the mouse [133,182,183]; its orthologue has not been found in the chicken genome and the putative orthologue in fish is expressed in the lens in addition to photoreceptors [51]. Similarly, the Crx gene plays a role in cone and rod differentiation and maintenance in mouse retina [72,73], but in zebrafish crx has additional roles in early retinal patterning [175]. It is still uncertain whether an OTX5 family member present in the chick is a *bona fide* orthologue of mouse Crx [161]). Sections IIIB and IIIC discuss these and other differences in more detail.

Almost in parallel with the increased availability of genomic information, there was an increase in available methods for genetic manipulations in embryos of most commonly used experimental animals: Transgenesis, for example, is now extensively done in *Xenopus* [15,91,108] and zebrafish [8,16]. A variety of reagents and methods for genetic loss- and gain-of-function, such as anti-sense oligonucleotide Morpholinos, RNAi, electroporation, and viral vectors, have been introduced and applied to embryos of many species [33,85,111, 112,115]; the temporal and spatial resolution of these methods seems to be at least as good as what can be achieved with conditional transgenics and knock-outs in the mouse [34]. We anticipate that much research effort in the near future will be devoted to determining whether specific genetic mechanisms of photoreceptor determination and differentiation are conserved among species.

B) Teleost Fish

Most retinal researchers are familiar with the teleost fish that are widely used as experimental models to study photoreceptor specification and development - historically the goldfish, but now largely replaced by zebrafish and medaka fish. The latter two species are advantageous because they offer attractive genetic tools for developmental studies: forward genetic screens have uncovered mutations that produce photoreceptor defects [121,188] and transgenic lines are available in which expression of GFP (green fluorescent protein) is targeted to specific photoreceptor subtypes under the control of various opsin promoters [62,81,107,184]. Goldfish and zebrafish are closely related members of the carp/minnow family (*Cyprinidae)* and,

although medaka fish belong to a distant taxonomic group, all three species are small, freshwater teleost fish native to Asia.

Despite their extensive use and numerous experimental advantages, however, goldfish, zebrafish and medaka fish can hardly be considered representative of fishes in general—Roughly half of the extant vertebrate species are teleost fish, which are extremely diverse in morphology, ecology and behavior, and demonstrate an enormous range of adaptive specializations in photoreceptor structure and function [194]. One of the most unusual aspects of photoreceptors in teleost fish is their ability to undergo rapid and dramatic adaptations in response to the spectral and luminance properties of the aquatic photic environment. These include long-term evolutionary adaptations that reflect mechanisms of speciation and population variation [153], as well as life history changes in individual fish during metamorphosis or seasonal migrations that expose them to water of different qualities [6,96]. Most of these adaptations involve changes in expression of visual pigments and/or types and proportions of different photoreceptors.

1) Environmental adaptations and opsin gene regulation—The aquatic environment offers a wide range of spectral habitats, and as a consequence visual pigments and photoreceptors in teleost fishes show striking adaptive variations [22]. Especially interesting examples are the hundreds of species of cichlid fish that live in the spectrally diverse habitats of the East African Rift Lakes, which are closely related but have highly diverse color patterns, sexual dimorphism, and variations in cone subtypes. Gene duplications in the SWS2 and RH2 opsin classes produced seven different opsins in the recent common ancestor of these cichlids. Intriguingly, each species expresses only three of the available opsin genes, which gives these cichlid species spectrally distinct cone complements that correlate with the distinct habitats in which they live [153]. A similar situation is found in bluefin killifish in Florida, where populations living in clear spring water have increased numbers of the SWS1 and SWS2 cone opsins (sensitive to shorter wavelengths) compared with populations living in tannin-stained swamps, which have a higher frequency of RH2 and LWS cones (sensitive to longer wavelengths) [71]. Both genetic and environmental variations affect opsin expression in these fish [70]. Polymorphism in visual pigment expression associated with sexual dimorphism also occurs in the LWS cones in guppies, including co-expression of two different LWS genes in a single cone [11]. We know virtually nothing about the molecular mechanisms that control opsin gene regulation and cone cell fate specification in these fishes.

Opsin gene expression in teleosts is subject to developmental stage-dependent and position-dependent regulation: Zebrafish have two LWS genes and four RH2 genes that encode pigments with different λ_{max} values [44]. The LWS and RH2 genes are located in separate clusters that represent tandem duplications of the ancestral opsin classes, independent of the genome-wide duplication in the teleost lineage. The genomic organization of the LWS and RH2 gene clusters in zebrafish is highly reminiscent of the gene duplications that produced the LWS/MWS array on the human X-chromosome [141]. The LWS and RH2 genes exhibit dynamic expression patterns in the developing zebrafish retina, such that the genes that encode visual pigments with the shortest λ_{max} in their respective class (*i.e.* RH2-1, RH2-2 and LWS-2) are expressed in the embryonic retina, followed later in larval development by those with a longer λ_{max} (*i.e.* RH2-3, RH2-4 and LWS-1) [185]. In the adult retina expression of the LWS and RH2 opsins is patterned topographically, with longer wavelength subtypes in the ventral and peripheral regions and shorter wavelength subtypes in the central to dorsal area. The spatiotemporal growth patterns of the teleost retina [54,139,165] suggest that cones generated postembryonically must sequentially express different genes within the tandem array as the animal matures and the eye continues to grow. The functional significance and molecular

mechanisms underlying this plasticity in cone spectral properties and how it is accomplished through differential gene regulation are not yet known.

Opsin substitutions have been directly demonstrated in teleost rod photoreceptors as an adaptation to the restricted photic environment of deep water: In eels of the genus *Anguilla*, for example, larvae and juveniles live in the coastline surface waters and rivers, and after several years they become sexually mature and migrate into the deep ocean [10,97,196]. The rhodopsin in rod photoreceptors of young eels has a λ_{max} of 501 nm (for the A_1-based chromophore), but under the influence of gonadotrophins the rods begin to express a blue-shifted rhodopsin with λ_{max} of 482 nm, which is matched to the spectral quality of deep water. The 482 nm rhodopsin first appears in the newly synthesized discs at the base of the outer segments, as visualized by microspectrophotometry of individual photoreceptors [196]. Expression of the 482 nm RH1 mRNA can be detected within a few hours after hormonal injection and is complete within 4 weeks, at which time expression of the 501 nm RH1 is undetectable [10,97].

2) Life history changes: addition of photoreceptors—In addition to opsin switching, teleost fish also adapt to changes in their photic environment by adding new photoreceptors or removing those that are no longer well suited to the environment, and replacing them with new and better suited ones.

The simplest and most common example of this type of plasticity is the addition of rod photoreceptors in the postembryonic retina: In many species, larval forms have an all-cone retina and rod genesis occurs only at metamorphosis, which can be many months after hatching in some marine species [20,86,164]. This sequence of photoreceptor genesis - cones before rods - is a typical feature of retinal development in other vertebrates and reflects the evolutionary sequence of photoreceptor origins. What is distinct about fish is that the period of rod genesis is often developmentally late and very prolonged, somewhat reminiscent of rod genesis in the rodent retina [35,163]. In teleosts, rod photoreceptors are generated by mitotic progenitors located within the differentiated retina [164]. These rod progenitors are continuously generated by slowly proliferating, self-renewing retinal stem cells associated with radial Müller glia in the inner nuclear layer [94,166].

Metamorphosis in some species involves a dramatic transformation in body form that accompanies significant changes in habitat and visual capacities, including alterations in spectral subtypes of cones: Flatfish (*e.g.* halibut, flounder, sole) are an extreme example, in which the larva is a bilaterally symmetric, surface-dwelling animal, and during metamorphosis one eye rotates to the other side of the body, the fish turns on its side and descends to the ocean floor [58]. Pre-metamorphic fish express the RH2 opsin in single cones whereas, as the retina grows, post-metamorphic, adult animals add SWS2 and LWS cones, and rods with RH1 rhodopsin [86,126].

Microspectrophotometric measurements of photoreceptors from flounders offer no evidence for multiple opsin expression in individual cones [60], and ^{3}H-thymidine birthdating studies indicate that the post-metamorphic cones are produced at the peripheral (ciliary) margin of the retina in a circumferential germinal zone [96]. The larval flounder eye is only a few hundred micrometers in diameter, a small fraction of the adult eye, which is several millimeters in diameter [59]. As is typical of teleost fish, most of the adult retina is generated postembryonically by neurogenesis at the ciliary marginal zone (CMZ) [54,139,165,166], which is regulated by the growth hormone/insulin-like growth factor I axis [150]. In flatfish, as in amphibians, metamorphosis is controlled by thyroid hormone, and specification of the complete repertoire of cone subtypes in the post-metamorphic retina that is generated by the CMZ is dependent on thyroid hormone signaling [127].

Teleost cones are organized into a geometrically precise mosaic array of spectrally distinct double cones and single cones: The LWS and/or RH2 cones form closely apposed pairs of either the same or different spectral types in double cones, a morphological specialization found in all vertebrate groups except placental mammals [55]. The pairs of cones are held together by specialized adhesive junctions mediated by proteins of the epithelial apical polarity complex that includes Stardust/Crumbs/Par/atypical-PKC [195]. Teleost fish retinas are unique in the precision of the spatial arrangements of distinct cone subtypes, and these arrays are classified into two major patterns - row mosaics and square mosaics [57]. Row mosaics consist of alternating rows of single cones (SWS1 and/or SWS2) and double cones (RH2/LWS, RH2/RH2 or LWS/LWS pairs) that radiate outward from central retina like spokes on a wheel [147]. In square mosaics, double cones form the sides of the squares, with SWS2 cones in the center and SWS1 cones (if present) at each of the four corners [57]. Each of these spectral cone subtypes has a distinct morphology in addition to the opsin gene it expresses: in many species the SWS1 gene is selectively expressed in short, single cones, SWS2-expressing cones are also single cones but slightly longer in length, and the RH2 and LWS opsins are in either long single or paired cones [57]. We know almost nothing about the cellular and molecular mechanisms that generate cone mosaic patterns or that drive the fusion of single cones to form double cones or that cause rotation of double cone pairs into a grid pattern of repeating squares, although mathematical models based on differential adhesion have been created that reproduce in computer simulations the row mosaic pattern characteristic of zebrafish [136].

3) Life history changes: loss of photoreceptors

In the salmonid fishes, metamorphosis is accompanied by a loss, rather than a gain, of specific cone subtypes: The SWS1 cones (the “corner cones” in the square mosaic) have a UV-sensitive visual pigment, and these cones are lost through apoptosis as the young fish leave their natal streams and move into deeper lakes and/or marine waters [5,113]. As a consequence the adult retina has reduced sensitivity to UV light as measured by electroretinograms [5]. The loss of UV-sensitive cones is thought to represent an adaptation to the reduced amount of UV light in their new habitat, and this metamorphic change is again mediated by thyroid hormone (TH) [29]. Treatment of young fish with TH causes precocious, apoptotic loss of UV-sensitive corner cones and inhibits expression of SWS1 opsin [6,7,189]. However, when TH treatment is discontinued, the SWS1/UV cones reappear in their normal positions at the corners of the square mosaic and can be labeled with the thymidine-analogue, bromodeoxyuridine (BrdU), indicating that they regenerate from mitotic progenitors in central retina that normally generate rod photoreceptors [6].

These examples demonstrate that the role of TH in regulating cone photoreceptor cell fate specification must be complex, since in one case increased levels of TH signaling results in up-regulation of LSW and SWS2 opsins and the generation of LWS and SWS2 cones, and in the another case TH causes down-regulation of SWS1 opsin and apoptosis of the SWS1 cones. How these outcomes are related to the mouse retina, in which TRβ2 signaling is required for expression of the MWS opsin in mouse cones [168], is not yet clear.

4) Regeneration of cone photoreceptors after injury or light lesion—In the adult teleost retina, any injury that significantly depletes neurons stimulates robust neuronal regeneration and complete restoration of all retinal cell types [95]. In particular, selective loss of photoreceptors by exposure to intense light or heat triggers progenitors in the rod lineage to switch to production of cone photoreceptors; rods are generated after the cones are restored, that is to say, in the same temporal order observed in the embryonic retina [166,191,197]. The signals that modulate cell fate choices of the mitotic progenitors and that assign cones to the primary fate are unknown. Although cones are restored during regeneration, the spatial

regularity of the cone mosaic pattern is not recovered [181], implying that developmental mechanisms responsible for constructing the precise cone mosaic arrays are not recapitulated.

5) Additional intrinsic and extrinsic signals that regulate photoreceptor differentiation—The genetics of photoreceptor development is being investigated in zebrafish through forward mutagenesis screens [140,188]. About 50 different loci have been identified that affect photoreceptor development, and although to date only a few of these have been characterized and the affected genes identified, in most cases photoreceptors are initially specified but fail to differentiate normally and subsequently degenerate. Only one mutation described thus far, *pob (partial optokinetic response b)* shows a distinctive photoreceptor phenotype, in which LWS cones selectively degenerate [28]. The *pob* gene encodes a novel, conserved protein that appears to be involved in protein sorting or trafficking [186], but why it is essential only for the LWS cones is unclear.

Zebrafish have orthologues of a number of transcriptional regulators implicated in photoreceptor cell fate specification in mammals, including *Crx* [118], a putative *Nrl* [51], and *Nr2e3* [40,110], but the functional roles of these genes in fish cannot necessarily be predicted from mammalian studies. For example, *Crx* is expressed only in post-mitotic photoreceptor precursors in the mouse retina [72], but in zebrafish it is expressed in proliferating retinal progenitors where it plays a role in the establishment of the early retinal primordium [175]. Another paired-class homeodomain transcription factor, *Rx/Rax*, is required for early stages in formation of the optic primordium in mammals [131] and promotes Müller glial fate in the retina [74]. However, zebrafish and medaka fish have paralogous copies of the ancestral *Rx* gene: the *rx1* and *rx2* genes are expressed in cone progenitors and in differentiated cone photoreceptors [45], whereas the *rx3* gene is required for the initial stages of eye formation and mutations in *rx3* result in eyeless fish [106,122] and defects in the retinal pigmented epithelium (RPE) [170]. The function of *rx1* and *rx2* in cone photoreceptor development in fish is not yet understood, and any potential role for *Rx/Rax* in mouse photoreceptor development may have been missed because of the scarcity of cones and/or the pleiotropic functions of the single mammalian gene.

C) Chick

The chick embryo has been recognized for many years as an excellent experimental system for developmental biology studies, and its usefulness has increased even further with the sequencing of the chicken genome and the introduction of methods for functional genomics (see Section IIIC2). Many important contributions to our understanding of photoreceptor cell development have been derived from chick studies but,as in the case of mice and fish, it may not be always warranted to extrapolate them to other species.

1) Photoreceptors in the chick retina—The chick has at least 6 types of photoreceptors: RH1 rods, LWS/RH2 double cones, and LWS, RH2, SWS2 and SWS1 single cones. The distribution of the various types of photoreceptors does not follow a highly regular mosaic of the type that has been described in fish [30]. Cones represent over 75 % of the photoreceptors, and have a distinctive oil droplet in their inner segment; the oil droplet has a characteristic pigmentation in each cone subtype, and serves as a cut-off filter that contributes to wave length discrimination and color vision [24,26,53,77,83]. Oil droplets are observed in at least some species of all vertebrate classes except placental mammals. The carotenoids for oil droplet color are obtained from the diet, and must therefore be transported and/or incorporated in a highly selective manner to establish the precise correlation that exists between the color of the oil droplet and the visual pigment expressed in each type of cone.

2) Development of chick photoreceptors

Timing of photoreceptor differentiation in the chick retina: The chronology of cell generation and related developmental events in the chick retina has been described and reviewed in significant detail [17,69,79,104,134,135,137,162,178,179,192]. The postmitotic precursors that will eventually develop as photoreceptors remain in the same retinal layer where they undergo their terminal mitosis, adjacent to the RPE [76]. Although the incubation time of the chick and the gestation time of the mouse are very similar (*ca*. 20-21 days), photoreceptor generation in the chick retina occurs during a very compressed period (between embryonic day 3 and 6 in the fundus), a period when other retinal neurons are also being generated [17]; no temporal separation of rod and cone births has been described in the chick. Taken together, these results argue against the possibility that timing of terminal mitosis may be a determinant of the fate of retinal progenitor cells in the chick.

Transcription factors: Not all the transcription factors known to regulate photoreceptor differentiation in mice and other animal species are present in the chick. An important example is *Nrl*, a retina-specific gene encoding a basic motif/leucine zipper domain transcription factor, whose deletion in mice results in the complete loss of rod function and super-normal SWS1-cone function [133] reflecting the apparent differentiation of progenitors cells into SWS1 cones rather than into rods [133,146,182]. No *Nrl* chick orthologue has been identified, although the chick has an *L-Maf* gene that belongsto the same molecular family as *Nrl* and, like it, is expressed in rods [148]. A similar ambiguity applies to Crx, a member of the Otd/Otx family of paired-like homeodomain proteins [41,64,72]. *Crx* is expressed predominantly in adult mouse photoreceptors and pinealocytes, as well as in some inner retinal cells, and photoreceptors develop abnormally when it is knocked out in mice [73] An *Otx5*-related gene in chick may be a *Crx* orthologue, but its function has not been analyzed [161].

Additional transcription factors important for photoreceptor development in mammals are present, but have not been well characterized in the chick. An example is *Nr2e3*, whose mutations cause the enhanced S-cone syndrome in mouse and man [42,52,80]; the function of the chick orthologue of this gene has not been established. On the other hand, it has been reported that *NeuroD* plays a key role in photoreceptor differentiation in the chick [199-201] and fish [93] but, while *NeuroD i*s involved in various aspects of retinal development in mammals [3,84,93,100,138], its involvement in photoreceptor differentiation is less clear. Similarly, the inhibition of photoreceptor differentiation by the homeobox gene, Pax6, has been documented in the chick embryo [187], but a similar effect on photoreceptors was not observed in *Xenopus* [90], and to the best of our knowledge has not been specifically investigated in other vertebrates. A chick gene related to *Rx/Rax*, called *RaxL*, has been implicated in specification of retinal ganglion cells [173] and differentiation of cone photoreceptors [39, 148].

Microenvironmental regulation: The responses of developing photoreceptors to ciliary neurotrophic factor (CNTF) illustrate the substantial differences between the chick and other species regarding the role(s) of extracellular signals in photoreceptor development. The consensus view is that CNTF causes a decrease in the number of rhodopsin(+) cells in rat retina, although the effects have been interpreted in different ways [144,174], and more recent evidence suggests that CNTF actually causes death of developing photoreceptor cells in the mouse [56]. On the other hand, in the chick CNTF has the opposite effect, that is to say, it stimulates rather than inhibits photoreceptor differentiation [66-68,109]. Molecular analysis of these effects has shown that chick photoreceptor responses to CNTF include a dramatic (60 %) and specific increase in the frequency of cones expressing the RH2 cone pigment [27, 198], accompanied by co-expression of LWS and RH2 mRNA in approximately 25 % of the photoreceptors [27].

In summary, these examples demonstrate a lack of consistent results across species in both intrinsic and extrinsic regulation of photoreceptor specification and differentiation.

IV) METHODOLOGICAL LIMITATIONS

A) Cell-specific "markers" and the lineage relationships of photoreceptor subtypes

The widespread use of cell-specific "markers" is based on the expectation that the expression of a given gene in a cell is a reliable and valid proxy for the cell's identity and differentiated state. This assumption is frequently not warranted in developing tissues, as one of us recently noted [2]. Given the dynamic nature of gene expression during development, some putative "markers" of the specific differentiated state of a cell (*e.g.* islet-1) may be restricted to a single cell type early in development, but become more broadly expressed at later stages [88]. In contrast, it is not unusual for some genes (*e.g. Pax6* and *Chx10*) to be broadly expressed in all retinal progenitor cells, and to become restricted to one or more subpopulations of differentiating cells as development advances [18,63,132,149]. Furthermore, undifferentiated cells can express "differentiated cell markers" and/or markers corresponding to more than one lineage [98], and individual genes can be expressed in the "wrong cells" as a probabilistic event [152].

The use of cell-specific "markers" for the analysis of photoreceptor lineage is further hindered by the peculiar dynamics of photoreceptor development. Rods and cones are similar in many respects, but demonstrate a range of differentiated properties (Fig. 3). Their structural similarities include their elongated, compartmentalized, polarized organization, with a complex outer segment process, and a specialized ribbon-containing synaptic terminal. None of these structural features are discrete but instead exhibit a gradient of specializations among rods and cones from different vertebrate classes. By the same token, phototransduction is the basic function of both photoreceptor types, but the sensitivity and dynamic properties of their responses to light vary due to differences in visual pigments and other molecular components of their phototransduction machinery that are encoded by different genes [55,78,82,141,151]. Cone subtypes are generally more similar to each other than to rods, but they differ in the visual pigment they express and, in many species, in the color of their oil droplets [1]. As reviewed above, some transcription factors are common to all photoreceptors (*e.g. Crx* in mammals and teleost fish), while others are restricted to specific subtypes (*e.g. Nrl* and *Nr2e3* in mammalian rods, *rx1/rx2* in teleost cones, and *RaxL* in chick cones).

Mature photoreceptor subtypes are usually categorized by structural, functional and molecular criteria (with the caveats noted above), but there are currently no "markers" that would allow the identification of their respective undifferentiated progenitors. Most studies of photoreceptor cell lineage and specification have attempted to compensate for this limitation by using antibodies against molecules expressed during late stages of photoreceptor differentiation, in particular the opsins, with the expectation that their expression would be an indication of progenitor cell commitment (see below). This assumption, which has created considerable confusion in the field, would only be warranted if the entire process of photoreceptor differentiation were controlled *cell-autonomously* by intrinsic mechanisms set in motion in early photoreceptor progenitors; but it is not. Although cell-autonomous mechanisms do control some initial aspects of photoreceptor differentiation, including the expression of a small group of photoreceptor-specific genes [1,27,30,103,172,180], most aspects of the photoreceptor differentiation occur much later in development and appear to be regulated by microenvironmental factors rather than by cell-autonomous mechanisms. These late differentiation events include outer segment formation, synaptogenesis and the expression of most photoreceptor-specific genes [27,30,31,36,103]. Some specific examples illustrate the limitations of using markers of differentiated cells for evaluating perturbation experiments aimed at investigating photoreceptor specification and lineage. Thus, CNTF-dependent

decreases in the number of rhodopsin(+) cells in rat retinal cultures have been interpreted as *re-specification* of cells destined to become rods [61], *arrested differentiation* of cells already committed to the rod fate [144], or a *transient and reversible down-regulation* of rhodopsin expression [174]. Similarly, increases in rhodopsin-immunoreactive cells in retinoic acid-treated rat retinal cultures were first thought to reflect increases in *progenitor cell commitment to the photoreceptor fate* [105], but subsequent studies found no evidence of a fate switch, and showed that retinoic acid acts by *shortening the maximum time between terminal mitosis and detectable rhodopsin expression* [193].

In summary, the expression in a progenitor cell of a putative cell-specific "marker" whose expression is restricted to a given mature cell type in the adult retina should not be considered definitive proof of the commitment of the progenitor to a particular cell lineage. For these reasons, lineage relationships of rods and cones can be hypothesized (Fig. 1), but have not been amenable to detailed investigation.

B) Retrospective nature of tests of developmental potential and cell commitment

Another reason why it has been difficult to evaluate rod and cone lineage decisions is that "*cell commitment"* is still defined *operationally* and *retrospectively* [2]. The basic goal of experiments that test cell commitment is to challenge progenitor cells by altering their microenvironment through pharmacological treatments, and/or by cell and tissue transplantation, recombination, or culture. Cells that give rise to different derivatives under different conditions are considered *multipotential*, whereas those that give rise to the same derivatives regardless of their microenvironment are considered *committed progenitors*. Experiments of this type involving retinal cells have been extensively reviewed [37,38,65, 114,116,119,130,156,157,167,206]. These operational tests of cell commitment have provided useful information, but have so far failed to elucidate conclusively the lineage relationships of rods and cone subtypes. An important reason for their lack of success is their *retrospective* nature; in other words, by the time the experiments show what progenitors did under various conditions, they do not exist as progenitors any longer.

A second limitation of these experiments is that they investigate *cell populations*, rather than *individual cells;* therefore, the experiments can disclose whether a population contains progenitors committed to a particular fate, but do so without identifying those progenitors within the heterogeneous cell populations present in the developing retina. Although cell death and cell proliferation are routinely quantified in these experiments, their possible impact on experimental outcomes is not always clearly excluded due to the lack of accurate criteria for the identification of sub-populations of undifferentiated but otherwise "committed" progenitor cells. Consequently, if a treatment increases the frequency of rods with concomitant decreases in the frequency of cones in developing retinal cells grown in culture, it is difficult to distinguish conclusively between induction of uncommitted precursors to differentiate as rods rather than as cones, increased survival of rods, increased death of cones, increased proliferation of progenitors destined to become rods, decreased proliferation of progenitors destined to become cones, or various combinations of these mechanisms.

We suggest that further progress in the analysis of cell differentiation in the retina requires the development of methods for the evaluation of the commitment of *individual cells, prospectively*, and at the *molecular level*.

V) A UNIFIED MODEL OF PHOTORECEPTOR CELL FATE SPECIFICATION IN VERTEBRATES

In this review, we have attempted to highlight the diversity of developmental strategies and solutions that vertebrates use to create a retina with specific subtypes of photoreceptors adapted to the visual needs of the particular species. Due to limitations of space we have had to concentrate on a few experimental animal model systems while omitting many important and interesting studies that are relevant to this topic. Despite this lack of completeness it is clear that the answer to the question we posed in the title is “No”.

The first task in building a unified model of photoreceptor cell fate specification is to establish the endpoint - what types of photoreceptors must be specified?

Already at the outset we stumble, because we have not yet established a consistent set of criteria that define rods and cones as discrete photoreceptor subtypes. Although the visual pigment contained in a given photoreceptor cell may have seemed a reasonable criterion, especially since rhodopsin has long been the visual pigment associated with rods, RH1 (rhodopsin) expression is not a definitive marker of rods, since some photoreceptors that do not express RH1 function as rods and others have different rod-identities at different developmental stages, turning off one RH1 gene and turning on another. Furthermore, expression of the RH1 rhodopsin gene is a late event in photoreceptor differentiation, whose fate may have already been specified as a rod. These same ambiguities apply to cone photoreceptors. The challenge we face in understanding how different cone spectral subtypes are specified is especially difficult because of the evolutionary disappearance of two families of opsin genes in the mammalian lineage and the peculiarities (and fundamental degeneracy) of the cone system in the widely used mouse model. The single most important confounding factor in the experimental analysis of photoreceptor cell fate specification to date has been the failure to distinguish intrinsic and extrinsic regulators that affect levels of opsin gene expression, *per se*, from developmental mechanisms that direct a retinal progenitor along a cell fate lineage that leads to specification and differentiation of a specific type of photoreceptor cell. A potentially promising analytical tool to distinguish rods from cones is whether they express “rod” or “cone” versions of components of the visual transduction cascade (*e.g.*, transducin, arrestin, recoverin, phosphodiesterase, guanylate cyclase, cGMP-gated cation channel) [55]. However, this will not help distinguish among cone subtypes, which share components of the “cone” version of the cGMP cascade, despite obvious morphological differences (*e.g.*, in size, shape, oil droplet color, synaptic connectivity) that indicate they are distinct cell types. Like the visual pigments, moreover, most of these molecules are expressed at late stages of photoreceptor differentiation, and this limits their usefulness for studies of photoreceptor lineage.

A second consideration that must be built into a unified model is an explanation for the topographic distribution of different types of photoreceptors

These spatial patterns range from the hemispheric (dorsal-ventral) gradient in levels of expression of SWS1 and MWS opsin genes in the cone population in the mouse retina, to the regular geometric cone mosaic array in teleost fishes, in which each distinct spectral/ morphological cone subtype has a specific neighbor relationship with other spectral subtypes. In this review, we have not even discussed another important specialization in cone photoreceptor distribution present in retinas of some species of fish, birds and in some primates including humans, which is the fovea [194]. Photoreceptors in the primate fovea are subject to highly specialized developmental signaling mechanisms that regulate their identity (to exclude rods) and that alter the morphology of foveal cones [31,87]. These interesting and important

aspects of photoreceptor specification and differentiation are not easily studied due to the difficulty of working with primate models.

A third parameter we must take into account in a unified model is the duration of photoreceptor genesis and changes in the distribution of photoreceptor subtypes generated over developmental time

In birds and mammals, this window of photoreceptor genesis is limited to embryonic and early postembryonic development, but the period of photoreceptor production can be very short (as in chick) or quite prolonged (as in rodents and primates). Teleost fish represent an extreme, in that photoreceptor production continues throughout postembryonic life as the eye enlarges and the retina grows by adding new neurons at the ciliary margin. In many fish rod photoreceptors are generated continuously by retinal stem cells in the central regions of differentiated retina, and in some species, the type and distribution of photoreceptors can change dramatically as an adaptation to changing visual environments. In some cases, differentiated photoreceptors switch the specific opsin genes they express, and in other cases, metamorphosis is accompanied by the loss and/or addition of specific cone subtypes.

The most prevalent current view of lineage and cell fate specification in vertebrate retinas is that the competence of retinal progenitors to generate different types of retinal cells changes with developmental stage, such that the photoreceptors produced by early progenitors differentiate as cones and later photoreceptor progenitors become rods [119]. Although this order of photoreceptor genesis is generally true, there are many exceptions and a progressive lineage scheme cannot account for the diversity of temporal and spatial regulation of photoreceptor genesis among vertebrates. A one-way developmental clock mechanism also cannot account for injury-induced regeneration of cone photoreceptors in the differentiated retina of adult fish from progenitors that otherwise would generate only rods. The evidence reviewed here indicates that a consensus model of photoreceptor cell fate specification should emphasize the importance of extrinsic signaling mechanisms derived from hormonal and microenvironmental factors that modulate intrinsic regulators of photoreceptor cell fate and thereby determine the type of photoreceptors produced by retinal progenitors at any given place and time.

In conclusion, is it realistic to expect that a unified model of photoreceptor cell fate specification can be constructed?

The data reviewed in the preceding sections indicate that a ‘one-size-fits-all’ model is not possible, and that it would be unrealistic to seek single answers to each of the twelve questions posed in the Introduction. On the other hand, if the many nuances and complexities of molecular mechanisms that generate photoreceptor diversity in different animal model systems are taken into account, a consensus model should be achievable within the context of the fundamental principle of evolution: *descent with modification from a common origin*. Vertebrate photoreceptors are evolutionarily derived from an ancient prototype cell, which was present in the common ancestor of all animals, both vertebrates and invertebrates [12,13,160]. From molecular phylogeny we know that this ancestral photodetector cell had a visual pigment composed of a retinal-based chromophore bound to an opsin apoprotein, which was located in a ciliated appendage (precursor of the outer segment) and which had a G-protein transduction cascade activated by light stimulation. Starting from this common heritage, different types of vertebrate photoreceptors evolved by multiplication and diversification, beginning with a photopic (“cone”) detector, which then evolved into a scotopic (“rod”) photoreceptor to expand the range of the habitable photic environment. This evolutionary perspective provides a logical definition of photoreceptor specification, as stated succinctly by Arendt, “A cell type is a homogeneous population of cells expressing the same set of orthologous genes for specification and differentiation to implement a defined cellular phenotype”, and “*Sister cell types evolve*

from one common precursor by cell type diversification" [12]. With this framework in mind, developmental biologists can expect to find unique combinatorial molecular codes that generate different subtypes of vertebrate photoreceptor cells, within a hierarchical structure in which subsets of molecular mechanisms are shared by sister cell types (*e.g.*, photoreceptors adapted for photopic vision would have a common "cone-type" developmental code). Our challenge is to discover the sets of orthologous genes that define photoreceptor cell types and then to unravel the code.

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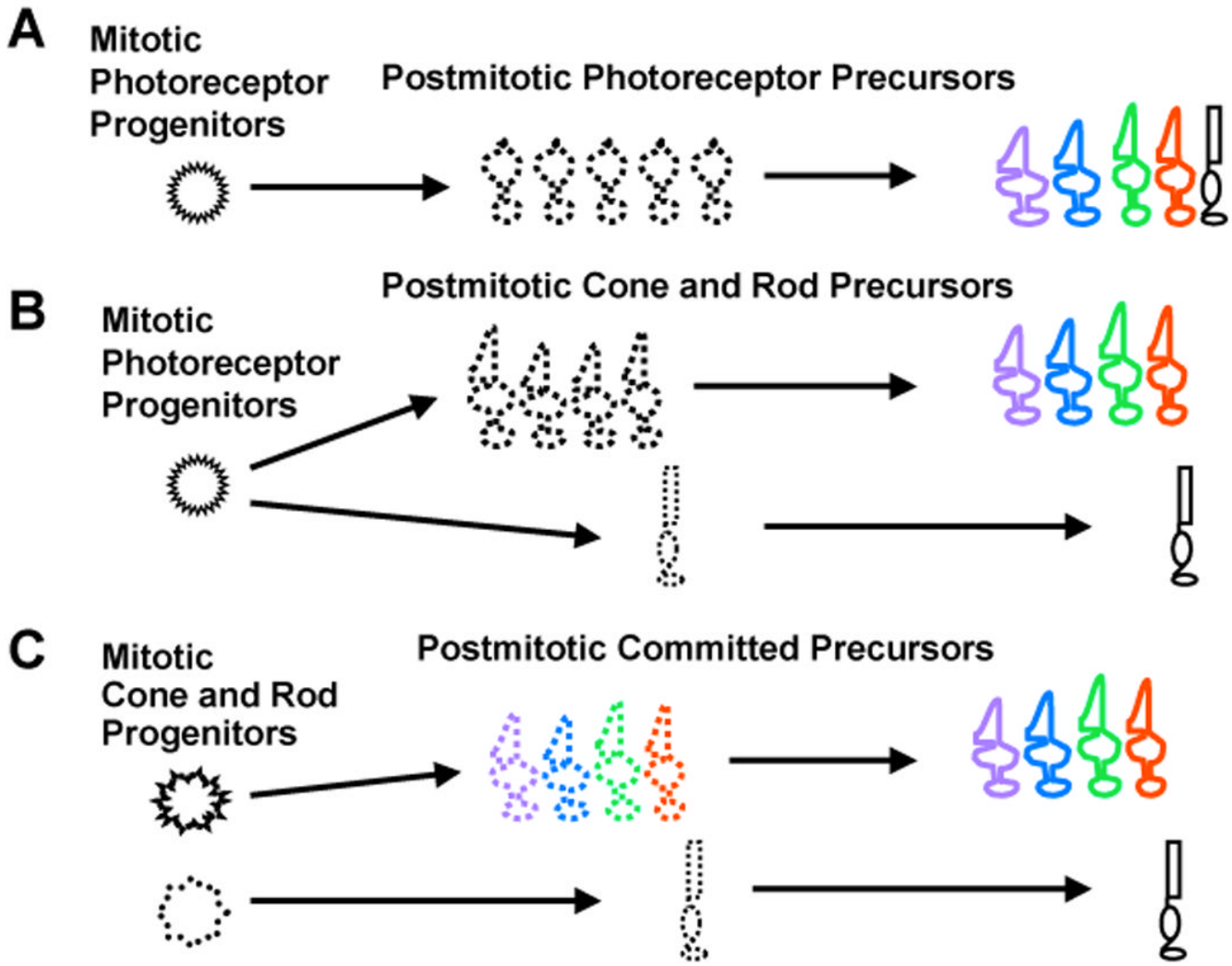

Figure 1.

Hypothetical models of lineage relationships between rods (RH1 opsin) and cones expressing LWS opsin (red), RH2 opsin (green), SWS2 opsin (blue) and SWS1 opsin (violet). The *homogeneity* of the outer nuclear layer (ONL) in the early embryo would be consistent with the existence of an initial population of *uncommitted photoreceptor progenitors* (**A**). Heterogeneity associated with the *segregation of rod and cone lineages* could emerge at late stages of photoreceptor differentiation (**A, *a1***), or during the long interval between photoreceptor generation and their advanced ("terminal") differentiation (**A, *a2, a3***). The *heterogeneity* of the early ONL would be consistent with a model that postulates early segregation of rods and cones (**B**), or even of rods and each separate cone subtype (**C**).

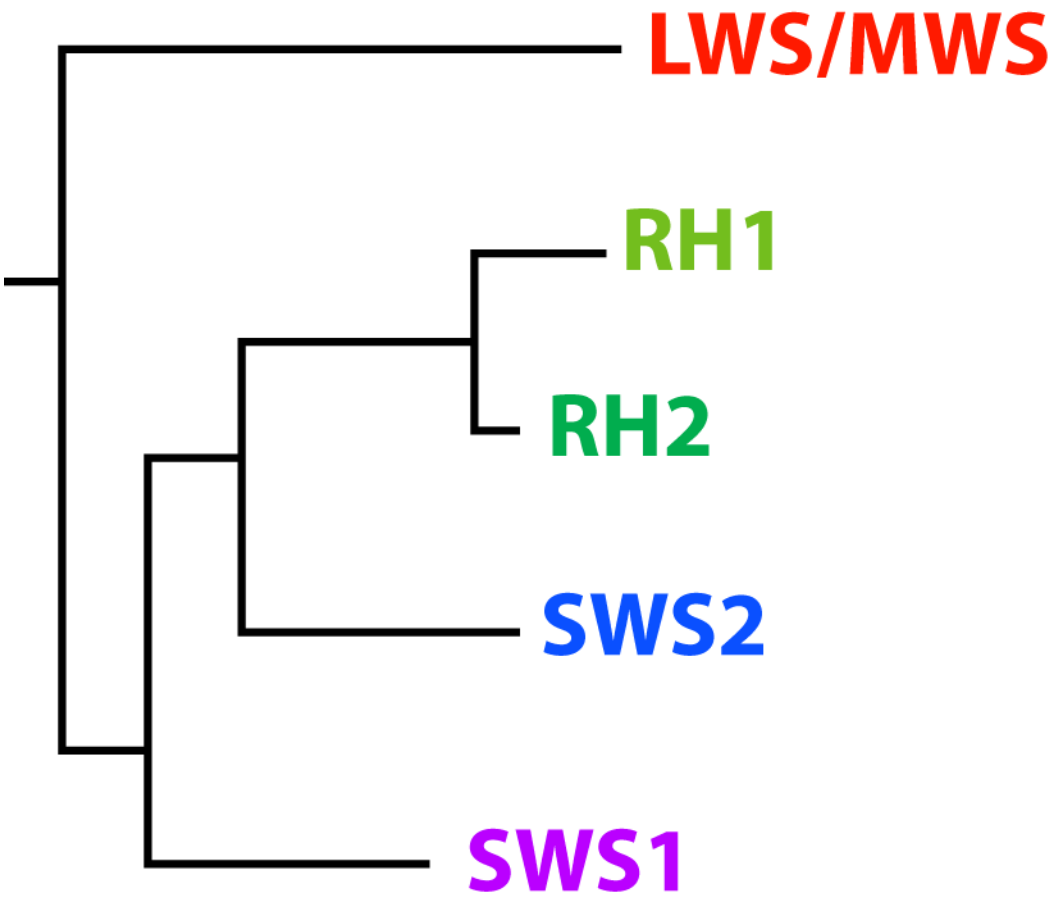

Figure 2.

Molecular phylogeny of vertebrate photoreceptor opsin genes. Beginning with an ancestral opsin gene approximately 700 million years ago, a series of four gene duplication events with subsequent diversification produced five opsin gene families with spectral sensitivities ranging from longest (red) to shortest (ultraviolet) wavelengths: LWS/MWS, RH1, RH2, SWS2, and SWS1. The position of the branch point along the horizontal axis from left to right indicates the relative evolutionary age, from oldest to youngest, respectively. The most closely related are RH1 (rhodopsin) and RH2, which diverged approximately 500 million years ago. The LWS/MWS family is the oldest and most divergent of the five opsin gene families.

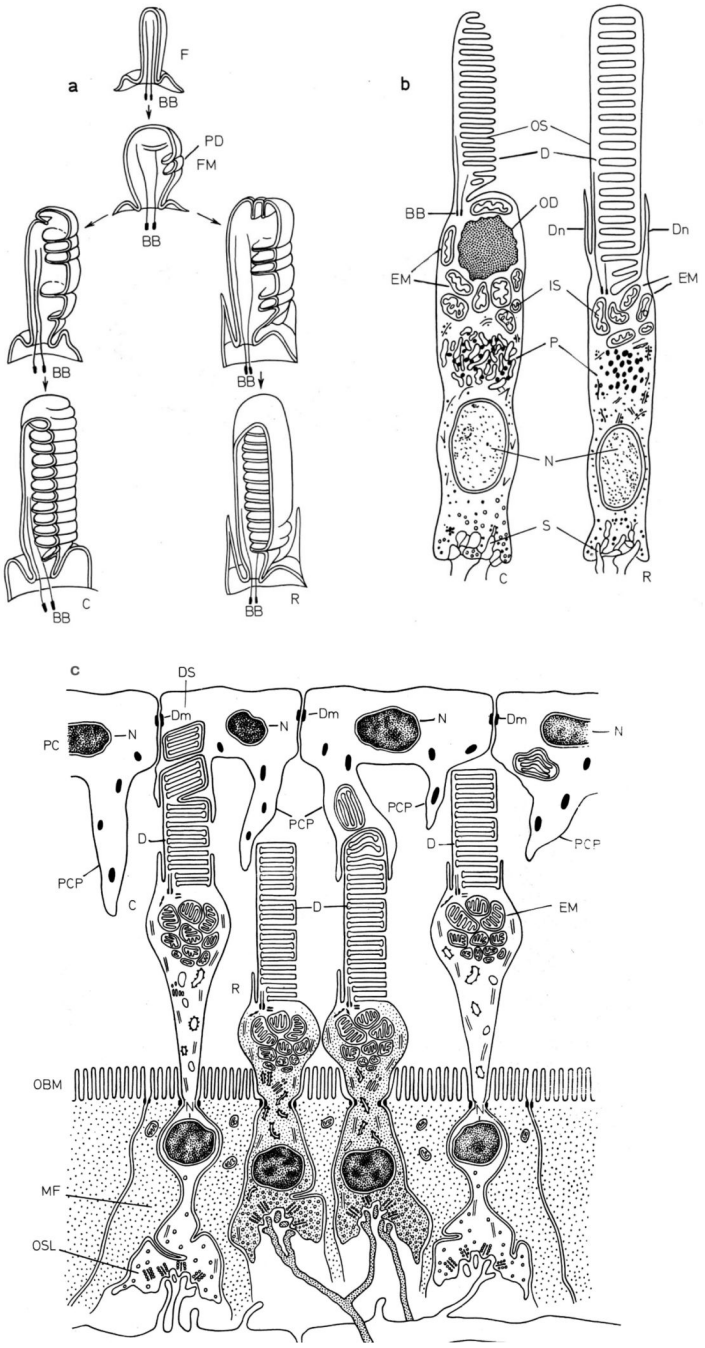

Figure 3.

Morphology of cone and rod photoreceptors. The development [**a**] and structure [**b**] of a "typical" vertebrate cone (C) and rod (R) photoreceptor. [**a**] Development of the outer segment. The visual pigment is in membranous discs of the outer segment, which derive from infoldings of the plasma membrane of a microtubule-based, ciliated appendage. In rods, the discs pinch off completely from the plasma membrane, but in cones the discs are open to the extracellular environment. [**b**] Differentiated rod and cone photoreceptors. Note that the synaptic terminals of cones and rods are usually more distinct than in this illustration, in that cone pedicles are larger with multiple ribbon synapses and rod spherules are smaller with a single ribbon synapse. Abbreviations: F, flagellum (more accurately, cilium); FM, folds of the flagellar (*i.e.*, ciliary)

membrane; BB, basal body; PD, presumptive disc; OS, outer segment; D, disc; OD, oil droplet; Dn, dendrite (more accurately, calycal process); EM, ellipsoid mitochondria; IS, inner segment; P, paraboloid; N, nucleus; S, synapses. (**c**) The lamprey retina offers examples of 'hybrid' photoreceptor types. All photoreceptors have cone-like morphology, with open outer segment discs and synaptic terminals with the morphology typical of cone pedicles. However, the shorter type of photoreceptor has rod-like properties (rhodopsin visual pigment and scotopic sensitivity and kinetics). Abbreviations: PC, pigment cells; Dm, desmosome; Ds, disc stratification; PCP, pigment cell process; OBM, outer boundary membrane (outer limiting membrane); MF, Müller fiber; OSL, outer synaptic layer. Reprinted with permission from Vinnikov, Y.A. 1982. Evolution of Receptor Cells. Cytological, Membranous and Molecular Levels. (Translated from Russian by Nicholas Bobrov). Molecular Biology, Biochemistry and Biophysics, vol. 34, Springer-Verlag, Berlin Heidelberg New York, 141 pp.