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Review

Photopigment coexpression in mammals: comparative and developmental aspects

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Summary. In mammals, each cone had been thought to contain only one single type of photopigment. It was not until the early 1990s that photopigment coexpression was reported. In the house mouse, the distribution of color cones shows a characteristic division. Whereas in the upper retinal field the ratio of short wave to middle-to-long wave cones falls in the usual range (1:10), in the ventral retinal field M/L-pigment expression is completely missing. In the transitional zone, numerous dual cones are detectable (*spatial coexpression*). In other species without retinal division, dual cones appear during development, suggesting that M/L-cones develop from S-cones. Dual elements represent a transitory stage in M/L-cone differentiation that disappear with maturation (*transitory coexpression*). These two phenomena seem to be mutually exclusive in the species studied so far.

In the comparative part of this report the retinal cone distribution of eight rodent species is reported. In two species dual cones appear in adult specimens without retinal division, and dual elements either occupy the dorsal peripheral retina, or make up the entire cone population. This is the first observation proving that all cones of a retina are of dual nature. These species are good models for the study of molecular control of opsin expression and renders them suitable sources of dual cones for investigations on the role and neural connections of this peculiar cone type.

In the developmental part, the retinal maturation of other species is examined to test the hypothesis of transitory coexpression. In these species S-pigment expression precedes that of the M/L-pigment, but dual cones are either identified in a small number or they are completely missing from the developing retina. These results exclude a common mechanism for M/L-cone maturation: they either transdifferentiate from S-cones or develop independently.

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Introduction

The process of visual perception begins in the retinal photoreceptor cells: the rods and the cones. Each of these cells has a peripheral process that penetrates the outer limiting membrane and reaches up to the pigment epithelium. This process is divided into an outer and inner segment by an incisure that represents the connecting cilium. The outer segments are the primary sites of photoreception and are full of membrane discs housing the photoreceptor molecule, also called visual pigment. The visual pigment consists of the integral membrane protein, opsin and of a chromophore that is covalently linked to it. The structure of the opsin molecule highly resembles other G-protein coupled membrane receptors but its function is slightly different. The opsin in itself is not light sensitive and for functioning as a light-sensitive receptor it needs to be coupled to the chromophore, 11-cis retinal (or 11-cis 3,4 dehydro-retinal). Upon photon absorption the chromophore changes its conformation to all-trans retinal, which initiates a cascade of biochemical events. The light-induced conformational change of the retinal leads to a set of conformational changes of the opsin; at the end of which a semi-stable form, called metarhodopsin II, is produced. This activates its G-protein, the transducin, which in turn activates the cGMP-phosphodiesterase enzyme causing a decrease in the intracellular level of cGMP. The signal molecule cGMP is responsible for keeping the sodium ion channels in the plasma membrane open, that mediate an ion current through the membrane in the dark (dark current). Upon illumination, the concentration of cGMP drops abruptly, leading to the closure of these channels and to the hyperpolarization of the cells, by which the photoelectric transduction is completed.

From the receptor cells, the impulse progresses to the different types of bipolar cells and finally to ganglion

cells representing the main output of the retina. At least two types of interneurons, the horizontal and the amacrine cells modulate synaptic transmission in the external and internal plexiform layers, respectively. These cells and their complex synapses ensure that the signal is not simply transmitted to the higher levels of the central nervous system, but the first steps of image processing start already within the retina. Due to its good accessibility both with anatomical and physiological methods, and its relatively simple connections compared to cortical areas, a great number of attempts have been made to get a better insight into these primary steps of information processing. The retina became an excellent model organ to study the central nervous system.

Understanding retinal functions, especially color discrimination, is impossible without knowing the organization of the receptor cells. How do different receptor types populate retinal areas? Are there any general distribution pattern for the rods and cones? How do these cells develop? How do cells with different properties and functions work together in forming an image that can be processed by higher centers?

Properties of rods and cones

It is well known that all mammalian visual pigments contain the same chromophore, and it is the protein part (opsin) that is responsible for their different spectral characteristics. Substitutions of amino acids at critical sites can change the sensitivity and color preference of opsins.

The rhodopsin and thus the rods have lower threshold sensitivity; they amplify light signals more than cones. As shown by Dennis Baylor and his colleagues (1979), even a single photon reaching a single rod can produce a detectable electrical response. These cells are therefore responsible for twilight (scotopic) vision. High sensitivity on the other hand requires a sensitive signal transduction system with spatial and temporal summation of impulses. Averaging out the signals of several receptors also means that the resolution of the image (both spatial and temporal) is of poor quality.

Cone opsins mediate daylight (photopic) vision. They have a high threshold sensitivity, but once cones are activated, the signal travels to the visual cortex without significant summation, thus with great resolution. This is especially true in case of the foveal cones of primates, where loss of information is literally zero: each ganglion cell receives excitatory input from only one single cone.

Color discrimination is also a cone-based function. In mammals three different types of cones have been discovered so far. Each of these three types contains a slightly different visual pigment designed to maximize absorption of light in different part of the visible spectrum. Short wave sensitive cones (S-cones) carry opsin molecules with peak sensitivity in the blue part of the spectrum (λ_{max}=420 nm). A few exceptions have recently been reported amongst rodent species where the maximum sensitivity of S-pigment was found to be shifted towards UV frequencies (Jacobs et al., 1991; Jacobs and Deegan, 1994). Middle wave sensitive cones (M-cones) are excited most by green light (λ_{max}=530 nm), while long wave sensitive cones (L-cones) are red-orange sensitive (λ_{max}=560 nm) (Dartnall et al., 1983; Schnapf et al., 1987).

Only some diurnal primates have trichromatic vision (Nathans et al., 1986; Yokoyama and Yokoyama, 1989; Jacobs et al., 1996). As shown by electrophysiological examinations, most mammals are dichromats possessing only two types of cones. One of them is sensitive to the short wavelengths (blue- or UV-sensitive) whereas the peak sensitivity of the second cone system is either in the green or in the red part of the spectrum (Jacobs, 1993; Yokoyama, 2000, Szél at al., 2000). This second population is often referred to as middle-to-long wave sensitive (M/L) cones.

Discrimination of photoreceptor types

Traditionally, rods and cones have been discriminated by characteristics based purely on morphological features. Rods usually possess longer, more gracile outer segments and, at the ultrastructural level, the photoreceptor discs are totally detached from the plasma membrane. The nuclei of rods are placed more vitreally, usually occupying several rows, and the synaptic apparatus has a sphere-like appearance (rod spherules). Cones, on the other hand, have thicker but shorter outer segments where all discs retain their connections with the cell membrane. The nuclei usually form a single row immediately under the external limiting membrane; the synaptic apparatus is more complex, often referred to as cone pedicle. In fact, the duplicity theory, stating that the vertebrate retina contains two types of receptors, rods and cones, was put forward based purely on such morphological observations (Schultze, 1866). Also the total exclusion of rods from the primate fovea was reported as early as 1935 without the use of more sophisticated methodology (Østenberg, 1935).

In contrast, unequivocal distinction of cone-subtypes proved to be practically impossible by morphological means. There are some characteristics of S-cones that may differentiate them from other populations: their slightly longer and slightly wider inner segments in some species and their smaller pedicles (synaptic apparatus) penetrating deeper into the outer plexiform layer are some of these. S-cone nuclei are positioned closer to the outer limiting membrane than M- and L-cones and they are also more sensitive to degeneration, noxious agents and poor fixation. Unfortunately all of these characters are unreliable, subject to fixation and species differences and therefore are not of much practical value. Also, ultrastructural investigations could rarely be extended to larger retinal areas.

Several other methods have been tried to identify

color sensitive cones. Among electrophysiological techniques, the electroretinogramm (ERG) can reveal the spectral sensitivity curves of cones (Jacobs et al., 1991; Calderone and Jacobs, 1995, 1999). Indeed, the presence of three different cone types in primates was discovered with such measurements. One drawback of this technique is that it is unable to give the sensitivity curve of a single cone, though it works well on larger retinal regions or even on whole retinas. The topography of cone types in relation to one another can not be studied this way.

Microspectrophotometry (MSP) on the other hand, can measure the color sensitivity of a single cone, or even a few cones in close vicinity, but the study of large retinal regions is practically limited (Dartnall et al., 1983; Nunn et al., 1984; Mollon and Bowmaker, 1992; Parry and Bowmaker, 2002). The use of both MSP and ERG techniques is further complicated by the fact that they all require surviving retinal preparations or even living subjects.

Histological techniques, on the other hand, have the advantage that they can be performed on fixed material, both on sections and on larger retinal pieces, even on whole retinas (whole mounts). These methods usually do not need special instrumentation and are relatively easy to reproduce.

The introduction of methods, based on the selective uptake of dyes was the first successful attempt for the discrimination of spectrally different cone types on fixed materials (Harwerth and Sperling, 1975; De Monasterio et al., 1981; Ahnelt, 1985; Ahnelt et al., 1987).

Lectin cytochemistry was, and still is, widely used to distinguish certain types of photoreceptors. Lectins bind to the carbohydrate components of the interphotoreceptor matrix (IPM: Röhlich, 1970) surrounding the outer segments and attaching them to the pigment epithelium. Some components of the IPM are general, surrounding all elements, but some of them are specific to a certain type of receptor (Blanks and Johnson, 1984; Sameshima et al., 1987; Mieziewska et al., 1991; Tien et al., 1992). Two lectins have been used widely in photoreceptor research. Wheat germ agglutinin (WGA) is specific to N-acetyl-D-glucosamine and selectively labels rods. The other type of lectin, most frequently used is the Arachis hypogaea lectin or known popularly as peanut agglutinin (PNA) lectin. It binds to all cones in most mammals by recognizing the D-galactose-beta-N-acetyl-D-galactosamine group. In a few species, it shows some selectivity in that labeling of S-cones is more intense than that of M/L-cones. So far this selectivity was only reported in some cone-dominant retinas (Szél et al., 1993a) and the fovea of some primate species (Röhlich, personal communication). In all other mammals, PNA lectin is considered as a non-specific all-cone marker without selectivity. Besides the interphotoreceptor matrix, PNA also binds weakly to some carbohydrates of integral membrane proteins in the outer segments of cones (Hageman and Johnson, 1986; Johnson and Hageman, 1987, Blanks et al., 1988; Röhlich et al., 1989) but the former usually masks the labeling of the latter.

Immunocytochemistry

The breakthrough in studying photoreceptor distribution came with the production of color cone specific antibodies in the ‘80s (Szél et al., 1988; Wang et al., 1992; Vissers and DeGrip, 1996). Up to now, several series of both mono- and polyclonal antibodies have been produced against those components of the receptors that allow discrimination. Besides the visual pigments (Szél et al., 1988; Wang et al., 1992; Vissers and DeGrip, 1996; Applebury et al., 2000), other elements of the visual cascade can also show minor differences in their protein structure that can be detected by monoclonal antibodies. Examples include arrestin (S-antigen: Long and Aguirre, 1987; Müller et al., 1989; Nork et al., 1993; Hendrickson and Hicks, 2002) carbonic anhydrase (Nork et al., 1990) and NADH-diaphorase (von Schantz et al., 1994).

Our laboratory was the first in producing pigment-specific monoclonal antibodies that reliably differentiated rods, the S- and the M/L-sensitive cones (Szél et al., 1988). Three of the antibodies were proven to be practically useful in the study of photoreceptor distribution. Anti rhodopsin (AO), a rat polyclonal antiserum produced against bovine rhodopsin, reliably detects rods in all mammalian species studied so far (Röhlich and Szél, 1993; Szél et al., 2000); and two more monoclonals, produced against a crude mixture of chicken photoreceptor membranes. Mab OS-2 labels the short wave sensitive mammalian cones: its specificity was proven by light- and electron microscopic investigations as well as with immunoblotting (Szél et al., 1986, 1988; Szél and Röhlich, 1988). As shown by blocking the binding sites with synthetic peptides, OS-2 is specific to the last 12 amino acids of the C-terminal of S-pigment. Mab COS-1, the other antibody frequently used, detects both the M- and the L-pigments, by binding to the last 6 amino acids of the C-terminals (Röhlich and Szél, 1993). The green- and red-sensitive pigments of mammals are almost identical; with the homology in amino acid sequence estimated to be 96% in humans (Nathans et al., 1986)! No antibodies have ever been produced that could reliably discriminate these two populations. As already mentioned, primates are the only mammals with a retina containing both green- and red-sensitive visual pigments (Jacobs et al., 1996). The vision of all other mammals is dichromatic with one pigment exhibiting a peak sensitivity in the short, and a second one in either the middle or the long wave part of the spectrum (M/L-cones) (Yokoyama, 2000). Thus, the distinction of M- and L-pigments is a relevant task only in primates, where exclusively physiological methods were successful so far.

Antibodies produced by other laboratories have similar characteristics (Wang et al., 1992; Vissers and DeGrip, 1996, Applebury et al., 2000). As we can see,

besides the clear advantages, the method also has its limitations. It is the epitope of the visual pigment, and not the spectral characteristic that is detected. Due to homology, visual pigments of the same subfamily usually can not be distinguished by immunoreactions. OS-2 for example binds both to the blue- and to the UV-sensitive S-cones. This feature however does not cause any practical difficulty because S-cone expresses only one of the two pigments (Szél et al., 2000). As discussed previously, except for the primate retina, the same argument also holds true for the discrimination of M- and L-cones. The actual spectral characteristics, if needed, can only be measured by physiological methods.

Interestingly, recent reports have shown that there is at least one antiserum that can possibly differentiate between blue- and UV-sensitive cones. An antibody against protein kinase C produced labeling of the outer segments of S-cones only in those species (such as the mouse and the rat), that possess ultraviolet sensitive opsin (Wikler et al., 1998). In other species it failed to label the S-cone population. This antibody however is not specific to cones: besides UV-cones it also labels a population of bipolar cells.

Recently, new techniques have also been introduced to mapping photoreceptor distributions. In some reports in situ hybridization with probes identifying the mRNA-s of various pigments were used (Raymond et al., 1995; Bumsted et al., 1999; Applebury et al., 2000; Rey et al., 2002). It has the clear advantage that in developmental studies it can identify a certain cone type before its pigment is actually synthesized, days before immonocytochemistry could produce a detectable signal. The method basically has the same limitations as immunocytochemistry: it also fails to differentiate between M- and L-pigments as well as between blue- and UV-sensitive cones.

General distribution of color specific cones

Using both physiological and histological methods a relatively simple view has been formulated on the receptor distribution of the mammalian retina by the early 1990s (Jacobs, 1993; Szél et al., 1996). Most species were shown to possess a rod dominant retina. The percentage of cones was usually low with values falling in the range of 1% to 14% (rat 1% (Szél and Röhlich, 1992), Mongolian gerbil 14% (Govardovskii et al., 1992)). In most mammalian species, two types of cones have been detected. One of them was proven to be short wave sensitive, while the other one was sensitive to either the middle or to the long wavelengths. Immunocytochemistry showed that the ratio of these two types of cones was rather constant with S cones comprising the minority (5-10%) of the cones and M/L-cones dominating the retinas. Each cone contained only one visual pigment and both types were distributed evenly. This means that no region being devoid of any of the two cone types was detected; and the ratio of the two elements was also practically invariant with retinal location. In many species studied, there was a central region with high local cone density values that decreased regularly towards the periphery (centro-peripheral gradient in the distribution of cones). This central region was either elongated around the retinal representation of the horizon or was rather circular. The former is commonly referred to as visual streak, while the latter is known as area centralis. Species with forestal life-styles usually possess area centralis, while animals living in open areas can better use the elongated streak. The central region could be considered as being equivalent to, but less specified than the primate fovea.

Characteristic of the "usual" rod dominant retina is thus the presence of two cone types populating the retina homogenously in a relatively constant 1:10 ratio with higher central and lower peripheral values. The schematic drawing of this type of distribution is shown in Figure 1A.

Cone-dominant retina

In a few species with strictly diurnal life-style, the ratio of rods to cones was reported to be reversed. Cones make up the majority of receptors; rods comprise only 5-10% of all elements. Examples of such retinal distributions include the tree shrew (Müller and Peichl, 1989) and the ground squirrel (Szél and Röhlich, 1988; Szél et al., 1993a; Kryger et al., 1997). The ratio of S- to M/L-cones however still remains in the same interval as in rod dominant retinas (1:10), and the homogenous distribution of cone-types holds true for these species as well.

A few special characteristics, however, were reported. As we already mentioned, PNA, otherwise

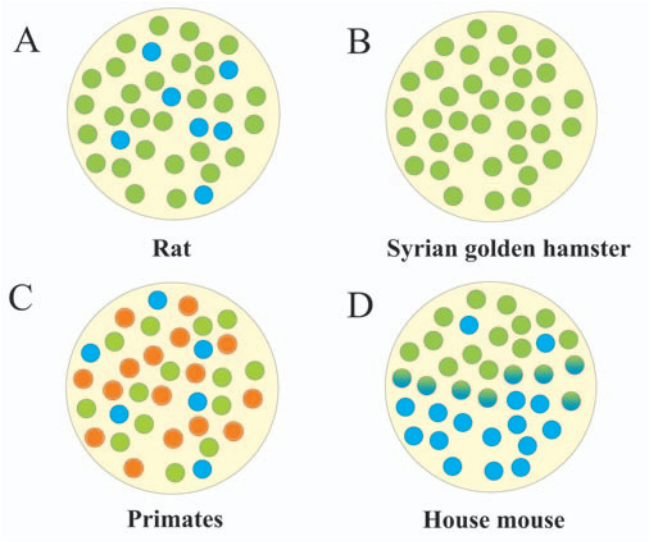

Fig. 1. Schematic drawing showing the retinal cone distribution patterns of different mammalian species. Color sensitive cones are represented by circles of the appropriate color.

considered a general cone marker, could discriminate between cone types in the ground squirrel retina (Szél et al., 1993a). Furthermore, when labeling with one of the S-cone specific antiserum, OS-2, an interesting weak cross-reactivity with rods was reported (Szél and Röhlich, 1988). In mammalian species with cone dominant retinas, some or all rods are also detectably labeled by OS-2. This reaction is not unprecedented in non-mammalian species and is probably due to the similarities of the two photopigments, S cone opsin and rhodopsin. Interestingly, other S-cone like traits (transducin isoforms) of rods were also reported by von Schantz et al. (1994). Further studies are needed to explore the possible functional significance of this phenomenon and to assess weather it concerns other (or all) cone dominant retinas.

Retinas devoid of S-cones

In some nocturnal species, the complete lack of cones carrying S-pigment was detected. Some rodents (Syrian golden hamster (von Schantz et al., 1997; Calderon and Jacobs, 1999), Apodemus species (Szél et al., 1994b), African giant rat (Peichl and Moutairou, 1998)) and some nocturnal primates (Wikler and Rakic, 1990; Jacobs and Deegan, 1992; Jacobs et al., 1996) represent the most well known examples. The retina of these species is typically rod dominant; cones comprise only a few percentage of the population and are all sensitive to the middle to long wavelengths (Fig. 1B). Recently, some underwater mammals were also reported to possess similar retinas proving that the lack of blue sensitivity is not confined only to nocturnal species (bottlenose dolphin: Fasick et al., 1998). As was shown, the dolphin possesses a gene homologous to other short-wavelength sensitive opsins but it is not expressed in vivo and has accumulated a number of genetic deletions. The dolphin therefore also lacks the common dichromatic form of color vision typical of most terrestrial mammals.

Retinal cone distribution in primates

The retinal photoreceptor distribution in primates always attracted special attention and was found to show some peculiarities. Due to medical implications most information has been accumulated concerning the human retina (Curcio et al., 1990, 1991; Gouras, 1991; Tessier-Lavigne, 1991; Djamgoz et al., 1995). Humans, like most primates, possess a special retinal region called the fovea. From a comparative point of view, it could be regarded as an area very similar to the area centralis of other mammals, but showing much more specialization. The image of an object in focus is normally projected onto the fovea where resolution of fine details is maximal. This region is completely devoid of rods, only cones with much thinner cell bodies are present and are very tightly packed (foveal cones) thus reaching high density values (Curcio et al., 1990, 1991; Djamgoz et al., 1995). Another characteristic of this region that also contributes to the high visual acuity is that light does not have to traverse all inner layers of the retina. Bipolar and ganglion cells bend away from the central fovea leaving a small pit in retinal architecture. Also, as already mentioned, summation of information coming from this region is literally zero: input to the center of the receptive field of each ganglion cell in the fovea is supplied by one single cone (no convergence occurs).

Interestingly, in some adult primates, including humans, not only rods, but blue-sensitive cones were also reported to be missing from the very center of the fovea (foveal dichromacy or foveal tritanopy: Marc and Sperling, 1977; Williams et al., 1981; Ahnelt et al., 1987; Szél et al., 1988; Curcio et al., 1991; Martin et al., 2000). The possible role of this central dichromacy is controversial.

One suggestion concerns chromatic aberration. The optical resolution of the short wavelength system is inheritably limited. As lights of different wavelengths pass through the lens, they bend differentially. There is a discrepancy between the images formed by the blue, the green and the red part of the spectrum that could seriously blur the image formed by the optical system. The difference is the largest between the short and the middle-to-long wavelengths; and it is negligible between green and red lights. In order to eliminate this problem, blue light is excluded from the image formation in the site of the greatest accuracy, the fovea. This assumption could explain why the S-cone free area might develop but how it is created is still a question to be answered.

Foveal dichromacy is not a general characteristic of all primates. In some species no region devoid of immunopositive blue-cones was detected (Bumsted and Hendrickson, 1999). Even in those species whose retina exhibits this peculiarity in adults, S-cones still seem to evenly populate the fovea during retinal development (Röhlich et al., 1994a). Also, in 4-6 week-old human infants the blue cone system is still present and reported to be functional in the foveal center by Volbrecht and Werner (1987). Cornish and co-workers (2004b) however in a recent article argue that this central S-cone free region is indeed present in prenatal human retina. The question thus remains unresolved whether this partial dichromacy can be considered as a type of degeneration, or it really has some functional role, as suggested by other authors.

Outside the fovea, the primate retina resembles that of other mammals (Jacobs et al., 1996). In the centro-peripheral direction, the percentage of cones soon falls and rods represent the dominating type of photoreceptors in the majority of the retina. A typical primate retina thus could be considered a mixture combining the characteristics of both rod- and cone-dominant retinas. Interestingly, the cone density shows a slight increase again at the far periphery, where a cone-rich ring was reported (Mollon et al., 1998). The function of this ring is yet unclear and might be responsible for detecting dangerous or interesting objects at the edge of the visual

field, but other possible functions have also been proposed.

Humans with normal color vision are trichromats with three different cone types sensitive to the blue, green and yellowish-red part of the visible spectrum, respectively (Nathans et al., 1986; Schnapf et al., 1987; Jacobs et al., 1996). Immunocytochemistry shows that outside of the human central fovea S-cones make up about 10% of all cones (Szél et al., 1988; Curcio et al., 1991; Jacobs et al., 1996; Bumsted et al., 1997; Bumsted and Hendrickson, 1999; Cornish et al., 2004a, b). The percentages of M- and L-cones however could not be evaluated by antibodies due to the 96% identity of the M and L cone opsin. Physiological methods using adaptive optics in living subjects and microspectrophotometry revealed that the ratio of these two elements showed great personal variations. Either of the two could make up the majority or they could be present in an almost identical ratio without any detectable consequences on the color vision of the subjects (Mollon and Bowmaker, 1992; Roorda and Williams, 1999; Carrol et al., 2002). Variations with retinal eccentricity were also proposed by the authors. The possible importance of these changes in M/L-cone distributions is not known. A schematic and simplified drawing of the primate retina is shown in Figure 1C.

A significant amount of information has been accumulated on the position and inheritance of photoreceptor genes as well (Nathans and Hogness, 1984; Nathans et al., 1986; Yokoyama and Yokoyama, 1989; Nathans, 1999). Rhodopsin gene is found on the 3rd chromosome. The S-pigment gene is situated on the 7th chromosome; any anomaly concerning blue color vision shows an autosomal type of inheritance. The middle as well as the long wave sensitive pigments are encoded on the q arm of X chromosome and thus inherited as sex-linked traits. The locus containing the red pigment is situated proximally, followed by one or more loci (2-5 or even more possible copies!) for the green-sensitive pigment. A locus control region, situated upstream from the pigment genes directs their expression in such a way that one and only one of these genes is expressed in a single cell (Smallwood et al., 2002; McMahon et al., 2004).

Originally, there was probably one single locus on each X chromosome encoding the original M/L-pigment, which showed great polymorphism. The final separation of these two pigments was the result of a gene duplication (Dulai et al., 1999). Polymorphism still is an important factor that needs to be taken into consideration even when discussing human color vision. As mentioned, the amino acid sequence of M- and L-pigments are almost identical, the homology is estimated to be around 96% (Nathans et al., 1986). This homology shows great individual variations, which can alter the actual absorbance of the pigments. If the absorption maxima of the red and green opsins are shifted closer to each other, the color discrimination capacity decreases. This state is usually referred to as anomal trichromacy.

Polymorphism could also be responsible for increased color discrimination capacity as reported recently (Jameson et al., 2001). Since there can be more than one copies of the green-sensitive pigment in humans, and either of them could be expressed in a single cell, the possibility is given in some individuals to possess more than three different cone types! Subjects having a single S- and one L-cone population, but more than one different M-pigments, have been identified! Indeed, if the color perception of such individuals were accessed by physiological methods, they were found to perceive significantly more chromatic appearances in comparison with trichromatic controls (Jameson et al., 2001).

Not all primates are trichromats; this property seems to be confined to diurnal Old World primates and to humans. Apart from one exception, New World primates show a high degree of polymorphism. They could be either di- or trichromats. In these species color vision was reported to be dependent on sex. All males are dichromats having only one X chromosome with a single copy of the M/L-gene. Females can either be dichromats also, if they are homozygotes, or trichromats, if they are heterozygotes to the M/L-pigment gene on the two X chromosomes (sex-linked polymorphism: Jacobs et al., 1996; Nei et al., 1997; Bumsted and Hendrickson, 1999; Dulai et al., 1999). Humans (and other Old World primates) are thus all trichromats because there are at least two copies of M/L-pigment on each X chromosome, with one of them being sensitive to middle, the other one to long wavelengths.

Some nocturnal primates were reported to possess only a single green-sensitive population of cones, accordingly they are monochromats (Wikler and Rakic, 1990; Jacobs and Deegan, 1992; Jacobs et al., 1996).

Dogmas concerning cone distribution

Based on early observations, a few general rules were created on the retinal distribution of photoreceptor types and for a long time they were thought to be valid for all mammalian species (Jacobs, 1993; Szél et al., 1996).

One cone-one pigment theory

Each cone outer segment necessarily contains one and only one visual pigment. The coexpression of different cone opsins described in submammalian species (Archer and Lythgoe, 1990; Makino and Dodd, 1996; Cheng et al., 2004) is not present in mammals.

S:M/L cone ratio equals 1:10

If the retina of a certain species contains more than one cone type, the ratio of S- and M/L-cones is relatively constant with S-cones comprising about 10% of the total cone population. This ratio is independent of the density of elements, and applies both to rod- and to cone-

dominant retinas.

Homogenous distribution of cone types with centro-peripheral gradient

Apart from the general centro-peripheral photoreceptor gradient, the two cone types populate the retina homogenously. Higher density values for both cone types are visible at the central than at the peripheral retina. There are no regions from which any of the cone types would be excluded, except for the primate fovea, where the lack of S-cones could probably be regarded as a type of degeneration.

Even today the retina of the majority of species examined still seems to obey the general rules set forth by these early observations. It was only recently that considerable exceptions from the above mentioned dogmas were discovered.

Heterogeneity in the distribution of cone types – divided retinas

A widely used laboratory animal, the house mouse (*Mus musculus*) was the first to prove that there are deviations from the general dogma, when a striking separation of color sensitive cones was reported by Szél and co-workers (1992). The mouse possesses a rod dominant retina with cones comprising about 3% of all photoreceptors. Labeling all cones with PNA, a simple centro-peripheral gradient and a roughly uniform overall

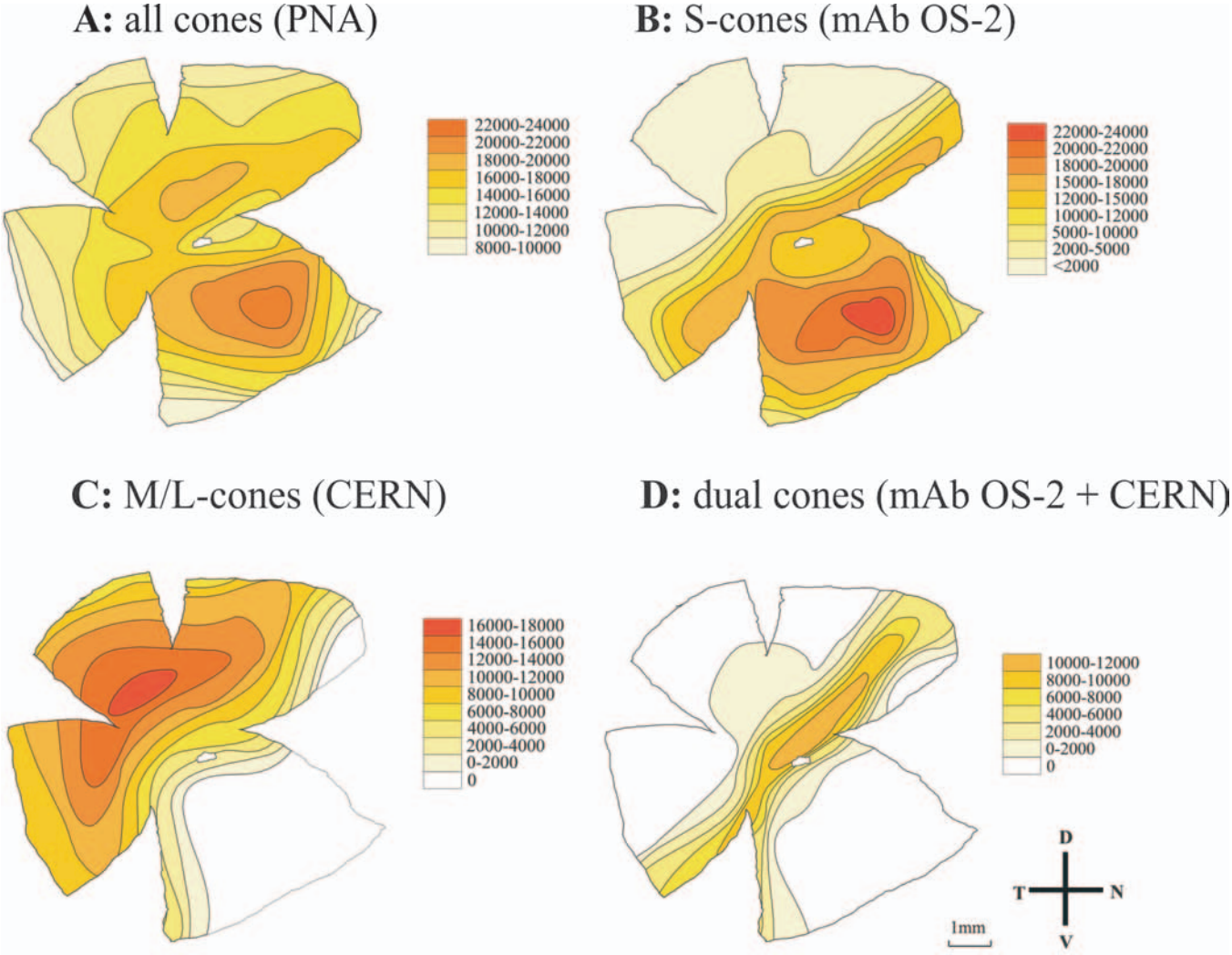

Fig. 2. Isodensity maps illustrating the spatial separation of cone types in the divided retina of the house mouse. The maps, derived from the same left retina show the color-specific cone distribution as revealed by pigment specific antibodies and PNA lectin. Whereas the overall cone distribution is roughly uniform **(A)**, blue-sensitive cones occupy mostly the ventral retinal half **(B)**, called the blue-field. This ventral region is completely devoid of the M/L-cone population **(C)**. At the transitional zone of the two retinal fields, most cones coexpress both pigments **(D)**.

cone density was observed (Fig. 2A), however, immunocytochemistry revealed that COS-1 positive M/L-cones were restricted to the superior retinal half and blue-sensitive cones were present in much higher number in the ventral retinal regions (Fig. 2B,C). The two cone systems thus occupy opposite retinal halves. The dorsal part rather resembles the usual cone distribution with M/L-cones making up the majority, and S-cones comprising the minority of elements. In the ventral half only blue-sensitive cones were present. This peculiar M/L cone opsin free region is usually referred to as blue-field. Another striking discovery was made when attention was turned towards the border of the two retinal fields. In this transitional zone, the majority (about 90%!) of cones coexpress both cone opsins (Röhlich et al., 1994b) (Fig. 2D).

Two different pigments in one cone cell: dual cones

Thus in mice there is 1) a dorso-ventral separation in color cone distribution and 2) there are cones expressing two different visual pigments simultaneously (Fig. 1D). Recently the regional separation of cone types as well as the coexpression of opsins was demonstrated with electrophysiological methods (Calderone and Jacobs, 1995) proving that immunocytochemistry could reliably detect cone cells with two different opsins.

Comparative studies showed that this special division is not restricted to mouse species. A similar distribution was later reported in the rabbit (Juliusson et al., 1994; Famiglietti and Sharpe, 1995), guinea pig (Röhlich et al., 1994b) and vole (Lukáts and Cooper, 1999, unpublished). A few slight differences, however, were present in the extension of the blue-field and in the ratio of double labeled elements. In the rabbit, the M/L-cone free area occupies only a small ventralmost crescent of the retina; whereas in the guinea pig all cones of the blue-field were also shown to coexpress both opsins. In all species studied however, if there was any sign of retinal division, the region of high S-cone density was always situated in the ventral half of the retina.

Recent histological and electrophysiological studies furthered our view on photopigment coexpression (Glösmann and Ahnelt, 1998; Lyubarsky et al., 1999; Applebury et al., 2000). The transitional zone is probably much larger than indicated by the first reports. The majority of the cones of the mouse retina coexpress both pigments, albeit at various rates depending on retinal location. About 10% of all cones in all retinal location express only the S-opsin. All other cones could probably coexpress both pigments with regional variation. In the dorsal region the M/L-pigment, in the ventral region the S-opsin expression dominates.

Biological significance of topographic separation

The role of such retinal division in the vision of these animals is far from being fully understood. A reasonable idea would be that in the ventral region that screens the sky, the presence of higher S-cone density would be advantageous for the animal with giving a better contrast against the blue background. The superior part focusing towards the green vegetation would have a similar role (Szél et al., 1992, 1998, 2000). Unfortunately, up to now, there is no evidence to support this hypothesis.

The phenomenon is rather widespread amongst rodents. Several mouse species, both pigmented and albino, were studied. In some European species (*M. spicilegus, M. spretus, M. macedonicus*) similar distribution was reported, whereas in all non- European species studied to date (*M. caroli, M. cooki, M. plathythrix, M. pahari*), the retina was shown to be "usual" without a dorso-ventral gradient; with *M. plathythrix* and *M. pahari* being devoid of S-cones as well. In other mouse species belonging to the Apodemus genus (*A. sylvaticus*, *A. microps*), there was no sign of division either. Especially noteworthy is the fact that two species, Mus spicilegus and Apodemus sylvaticus live practically in the same habitat, with similar morphological, physiological features and life-styles. Even their feeding habits are identical, yet their retinal cone distribution differs significantly: the former possesses a divided, the latter a non-divided retina. The fact that both species live together in the same region strongly argues against any possible advantage of the regional separation of cone types (Szél et al., 1994b).

Another idea proposed was that this unusual division was a type of degeneration of laboratory animals due to living in artificial illumination for generations. However, other laboratory animals, like the Norwegian rat, do not possess divided retinas, whereas mice living outside of laboratory do. Consequently, this distribution is species-dependent and probably not influenced by environmental factors. Only slight within-species variations have been observed, not affecting the validity of the above statement (Lukáts and Cooper, unpublished).

Another interesting suggestion that needs consideration is whether these species could possess true color discrimination. In the ventral region, practically only S-pigment is present, thus color discrimination seems unlikely. On the other hand, the dorsal region possesses a cone distribution similar to that of other mammalian species with the possibility of having a normal color vision. The important question that needs to be answered is: what happens in the transitional zone? This is especially important when we take into account that this zone is much more widespread than was previously thought. In this region all cones contain the S-opsin and the majority of them the M/L-opsin as well. ERG studies on double labeled cones employing the UV and green part of the spectrum showed that any answer to the UV test stimuli could be suppressed by applying green background illumination and vice versa (Calderone and Jacobs, 1999; Lyubarsky et al., 1999). Studies using chromatic adaptation thus failed to show the independence of the two - blue and green - systems, they both probably use the same intracellular signal

pathways. Light of any of the two wavelengths would consequently produce the same signal, making color discrimination unlikely. Jacobs and co-workers (2004), however, argue in a recent article, that if the ratio of the two pigments present in the same cone cell vary with different retinal locations, and thus have different spectral absorption properties, then color vision is theoretically possible. Indeed, using behavioral testing they proved that mice could be trained to discriminate light stimuli of different wavelengths. To what extent the three retinal regions are involved in color discrimination remains to be evaluated.

Added to the arguments, we also have to mention that monochromacy, even the total lack of a second cone population, does not completely exclude the possibility that some color-discrimination capacity exists. Rods, together with the remaining single cone population, can provide the basis of dichromacy at dusk or at dawn when both populations are functional. Ophthalmologic examinations carried out on human individuals who suffer from blue-cone monochromacy, with the total lack of both the green- and the red-sensitive visual pigments, showed that some comparisons between quantal absorption rates in rods and S-cones could also be made. (Reitner et al., 1991) The question as to whether this comparison is a general characteristic applying to all mammals and, as proposed, for the inferior mouse retina; (Yamamoto and Gouras, 1993) or just some compensation due to the reorganization of synaptic input in the deficiency of the receptor types needs further investigation.

Dual cones in non-divided retinas

Up to the present past, the occurrence of cones expressing two different visual pigments (dual cones) was considered exceptional, a phenomenon restricted to a small number of “exotic” mammalian species. In a few adult mammals, whose retina possesses a topographic separation of cone types, elements coexpressing the S- and M/L-pigments were visible at the transitional zone. Although the actual size of the transitional zone is questionable and also subject to interspecies variations, authors agree that when division of the retina is present, dual cones are also detectable (Szél et al., 1992; Juliusson et al., 1994; Röhlich et al., 1994b; Famiglietti and Sharpe, 1995). Is there any cone distribution pattern, other than the divided retina, that also incorporates double labeled (dual) cones?

Recently, a similar retinal cone pattern was described in insectivore species by Peichl et al. (2000). Immunocytochemistry revealed that in the dorsal retina the S- to M/L-cone ratio was in the normal range (1:10), whereas in the ventral retinal half, blue-sensitive elements were present in a surprisingly high ratio (50-70%)! This distribution is thus similar to that observed in divided retinas, but M/L-cones were never completely missing from the S-cone rich field, and no transitional zone was identified. Further, cones exhibiting photopigment coexpression (dual cones) - that are the general characteristics of the mouse and other mammals with topographic separation of cone types - were present only in small numbers in insectivores.

Up to now, the presence of division in retinal cone distribution seemed to be restricted to European mouse species. In all non-European species studied, a homologous distribution of cone types was reported with two (S- and M/L-cones) or one single (only M/L-cones) population (Szél et al., 1994b, 2000). Therefore, in the last years we investigated several South African rodent species in cooperation with the South African University of Pretoria (Department of Zoology and Entomology) and the French Institute INSERM (Unité 371 Lyon). The question was whether we could detect dual cones in any of these mouse-like rodents of African origin, and if so, would they occupy the transitional zone, or is there any other type of distribution pattern that incorporates such elements? Another reason for the species selection was that we could obtain first-hand information on the life style of all (both diurnal and nocturnal) animals. The possibility is thus given to match the reported type of retinal distribution with the habits of the animals. The assumption was that it might give clues to understanding the possible role of coexpression in the vision of mammals. Details of this study will be published elsewhere.

Most animals used in this comparative study were collected in Pretoria, South Africa. Three diurnal species were chosen: *Otomys unisulcatus* (Karoo bush rat), *Rhabdomys pumilio* (Striped field mouse), and *Parotomys brantsii* (Brants' whistling rat). *Aethomys namaquensis* (Namaqua rock mouse), *Dendromys melanotis* (Grey climbing mouse), *Mastomys natalensis* (Multimammate mouse) and *Saccostomus campestris* (Pouched mouse) represented the nocturnal African rodents (Skinner and Smithers, 1990). For purposes described in detail later, the investigation was also extended to another, non-African rodent, the Siberian hamster (*Phodopus sungorus*).

Dual cones in the dorsal peripheral retina

In the three diurnal species, *Otomys unisulcatus, Rhabdomys pumilio* and *Parotomys brantsii*, extremely high cone densities were found, with peak densities close to 70.000 cones/mm^2. This value is surprisingly high and comparable to the ones reported in species with cone dominant retinas (Szél and Röhlich, 1988; Müller and Peichl, 1989; Müller et al., 1989; Petry et al., 1993; Szél et al., 1993a, 1998, 2000; Kryger et al., 1997). Still, an important difference was revealed between the two types of distributions. Anti-rhodopsin (AO) labeling showed that in the South African diurnal species rods are abundant and cones make up only about 15-30% of all photoreceptors, in contrast to the tree shrew, and in other cone dominant retinas, where rods represent only the minority (5-10%) of the total population. Consequently, the retina of these diurnal species is characterized by the

presence of both high rod and cone densities (under publication).

No sign of uneven distribution of cone types were found in these diurnal species, but interestingly, in *Otomys unisulcatus* we could detect the presence of double labeled elements in small number restricted to the superior peripheral retina. These elements caused a slight, but detectable local increase in the number and relative percentage of cones labeled by the S-specific antibody (Fig. 3). On other regions of the retina, the ratio of blue- to green-to-red-sensitive cones fell in the usual range (1:10). All double labeling methods used reliably detected dual cones in all adult specimens examined. This double stained population locally comprised about 2-5% of all cones, and morphologically they highly resembled cones in developing retinas (Fig. 4). Great differences were detected both in the size and in the staining intensity of cone outer segments, with a significant population of dual elements, similar to the samples from early postnatal days.

This S-cone rich region is clearly different from the blue-field observed in the mouse retina. The percentage of cones, immunoreactive with anti-S probes is only slightly higher than elsewhere, both cone types are present and dual elements comprise only a small percentage of the population (5-10%). In contrast, in the transitional zone of the mouse most elements (90%) are double labeled. Further, in species with retinal division, the blue-field always occupies the ventralmost part of the retina (Szél et al., 1992; Juliusson et al., 1994; Röhlich et al., 1994b; Famiglietti and Sharpe, 1995).

Another peculiarity observed in the *Otomys* retina was that dual elements morphologically resembled cones seen during development (Szél et al., 1993b, 1994a, 1998, 2000; Bumsted and Hendrickson, 1999; Szepessy

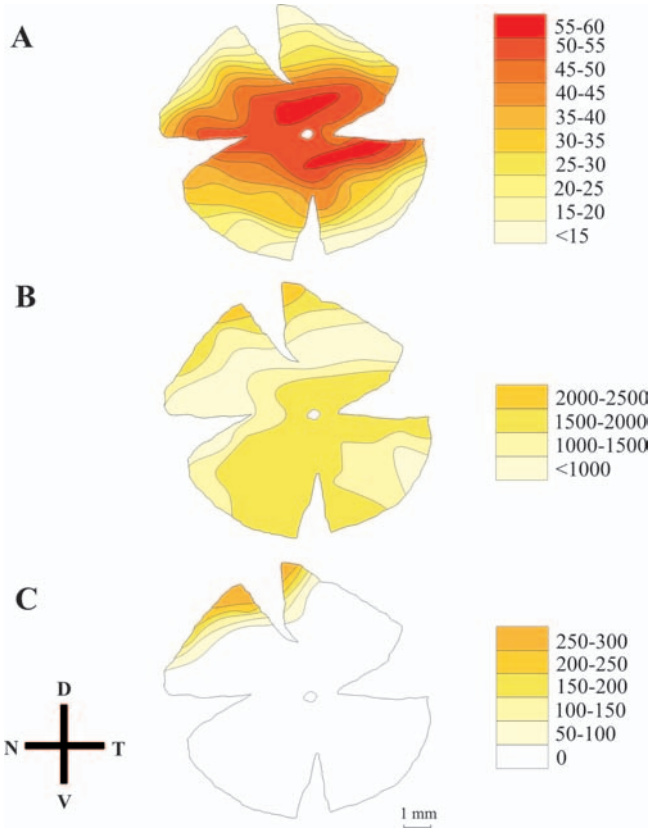

Fig. 3. Isodensity maps showing the distribution of M/L- **(A)**, S-cone **(B)** and double labeled cone populations **(C)** in the *Otomys* retina as indicated by immunocytochemistry. Note the high cone densities and that all elements with coexpression of different pigments are confined exclusively to the superior peripheral retina. Density values are given in 1000 cones/mm^3 in figure 3A, and in cones/mm^3 in figure 3B and C.

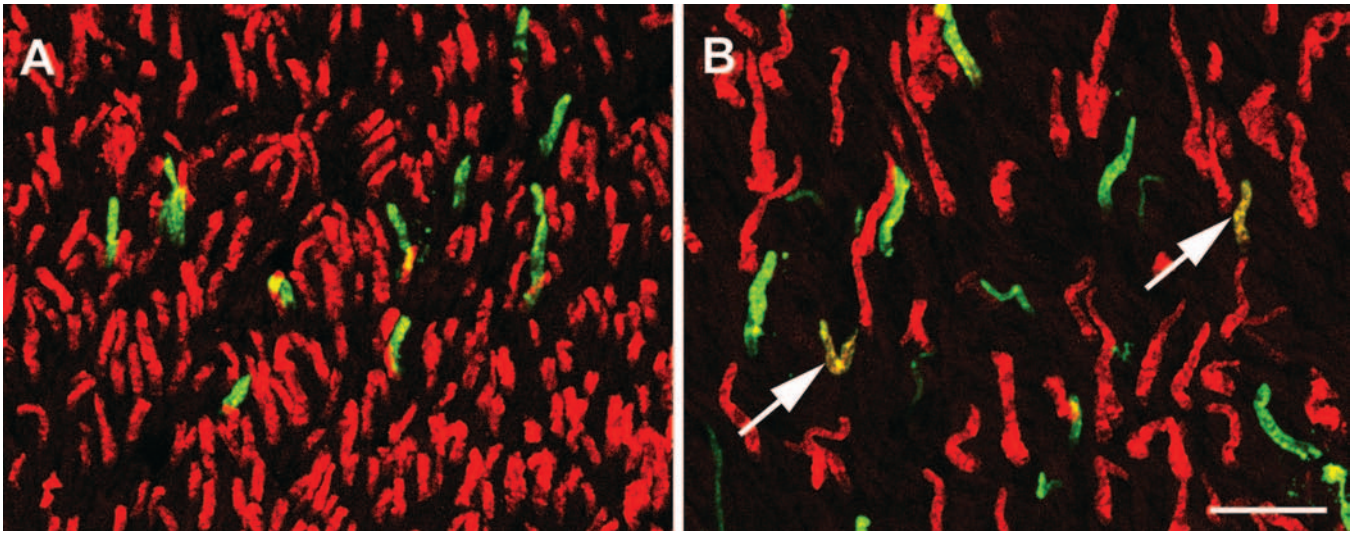

Figure 4. Confocal micrographs showing the comparison of the central- **(A)** and peripheral **(B)** retinal regions in *Otomys unisulcatus*. Blue-sensitive cones are labeled green, the green-sensitive elements in red. Note the morphological heterogeneity and the relative excess of blue cones in the periphery. Some peripheral elements are recognized by both markers (arrows). Bar: 20 µm.

et al., 2000). Cone outer segments of different sizes are visible and the staining intensity also shows extreme variations. This raises the question as to whether these cones are postmitotic cells in the phase of differentiation. In the developing retina, the peripheral region is the last to cease maturation (centro-peripheral gradient: Szél et al., 1993b, 1994a, 1998, 2000; Bumsted and Hendrickson, 1999; Szepessy et al., 2000), consequently, if some undifferentiated stem cells could survive till adulthood, the logical site to expect them would be the extreme periphery. Regeneration process based on surviving multipotent cells is not unknown in certain sub-mammalians, e.g.: in some adult fish, where the retina continues to grow throughout the whole life span. Furthermore, in some of these species, cell division and differentiation were also shown to be restricted to the peripheral regions of the retina (Cid et al., 2002). Unfortunately, reports describing a similar phenomenon in mammals are missing, though putative stem cells were identified in the pars ciliaris retinae - again close to the far periphery (Tropepe et al., 2000). These cells were not reported to be functional in vivo, however, they could be induced to differentiate into several different retinal cell-types in vitro. Also, a recent report by Kicic et al. (2003) raises the possibility that under certain conditions (e.g.: induction by activin A, taurin, EGF) CD90+ - presumably pluripotent - cells derived from the bone marrow, could differentiate to form retinal cells. Furthermore, as shown, after experimental damage to the retinal structure, these cells could definitely integrate into the retina! Thus the possibility for regeneration is given. Taking our results into account, it is possible that some stem cells indeed function continuously in the peripheral retina, and the developing receptor cells integrate into the retinal mosaic. Intensive studies are under way to prove or exclude this possibility in the adult mammalian retina.

Interestingly, recent reports in literature also raise the possibility that *Otomys* is not the only species that possesses this type of cone distribution. Similar local peripheral increase in S-cone density was reported in the cat and ground squirrel retina (Kryger et al., 1997; Lindberg et al., 2001). Unfortunately, none of these authors report on any double staining in their methodology.

Even more intriguing is the fact that after experimental retinal detachment in the cat, regeneration was found to be the strongest in this dorsal region (Lindbergt et al., 2001). Most of the regenerating cones express the S-pigment, thus blue cone density reaches extreme values, higher than the ones measured prior to detachment, just like during the development of the rat retina. To some extent, this experiment also argues in favor of the existence of mitotic cells and highlights the possible connection between developmental events and double stained elements in adults.

The presence of both visual pigments in all cones

In the four nocturnal species studied (*Saccostomus campestris*, *Aethomys namaquensis*, *Dendromys melanotis* and *Mastomys natalensis*) cone densities were found to be much lower, comprising 1-10% of the total receptor population. This is in agreement with reports on other species with similar lifestyles (Jacobs, 1993; Szél et al., 1996, 1998, 2000). No sign of dorso-ventral gradient or compartmentalization of color specific cones was detected.

However, in one of the above-mentioned nocturnal species, the pouched mouse (*Saccostomus campestris*), we came across a surprising phenomenon (Lukáts et al., 2002). We found that the number of cones, stained by the M/L-specific antibody was approximately equal to that labeled by the S-specific antibody (Fig. 5). This was

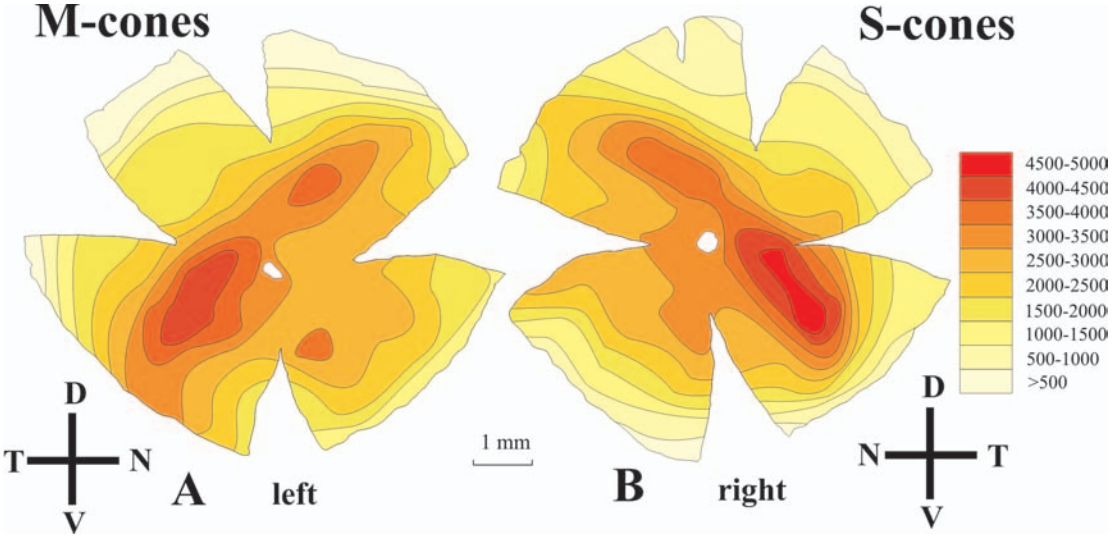

Fig. 5. Isodensity maps derived from the left **(A)** and the right retina **(B)** of the same specimen of the pouched mouse. On the right eye JH455, a polyclonal antibody specific for S-cones, on the left retina mAb COS-1, selectively labeling M/L-cones, respectively, was used. Note the symmetrical distribution of the two pigments, with a nearly horizontally aligned visual streak and peak densities in the infero-temporal region. Density values are given in cones/mm^2.

an unexpected finding, since in most mammals studied up to now; M/L-cones outnumber S-cones by a factor of about 10 (Jacobs, 1993; Szél et al., 1996, 2000). This unusual 1:1 ratio between apparently M/L- and S-cones could be explained by assuming the presence of either 1) two distinct M/L- and S-cone populations of equal densities or 2) a single cone population expressing both M/L- and S-opsins.

At this stage of the study an interesting report was published by Calderon and Jacobs (1999). Their immunocytochemical examinations on the Siberian hamster revealed that the densities of the cones expressing the M/L- and the S-cone opsin, respectively, were also present in a 1:1 ratio over the entire retina. Even more important was the fact that ERG studies in the Siberian hamster confirmed the presence of two visual pigments with peaks in the region of 370 and 500 nm, while behavioral discrimination using chromatic adaptation failed to show independence of the two systems. This surprising similarity with the Saccostomus prompted us to perform a comparative immunocytochemical analysis on both the pouched mouse and the Siberian hamster in order to reveal, whether the 1:1 ratio represents two independent cone populations or rather, as was expected, one cone population expressing both visual pigments (dual cones). In both rodent species, the pouched mouse and the Siberian hamster, a striking agreement between S- and M/L-cone densities was found at all retinal locations (Fig. 5). There was no sign of a dorso-ventral gradient in the expression of the two pigments, and there was no region identified where any of the antisera failed to produce a reaction.

PNA lectin is a well-known marker of all cones. If antibodies against S- and M/L-cone pigments recognized distinct cone types in these species, isodensity maps of PNA-positive cones should show roughly twice as high densities as those found with either of the two color cone specific antibodies alone. Such a labeling however showed that the density values derived by using an S-cone or M/L-cone specific marker and PNA were practically identical in both species examined (Fig. 6). These findings clearly demonstrate the presence of a uniform cone population with similar affinity to both M/L- and S-specific antibodies.

To prove that the same elements are indeed stained by all cone-markers used, double label studies on retinal whole mounts, as well as on consecutive sections, were performed. Each and every cone outer segment was labeled by both antibodies, irrespectively of the area of sampling. Picture pairs obtained with a confocal laser scanning microscope in the Siberian hamster (Fig. 7A,B) and the pouched mouse (Fig. 7C) show that the cone populations recognized by M/L- and S-pigment specific antibodies, respectively, are identical. The colocalization of both markers in these studies as well as in those where the two markers were applied on consecutive semithin sections provides irrefutable proof for the existence of dual cones all over the retina of these species.

The pouched mouse and Siberian hamster thus were proven to be specific for cone distributions never reported before (Lukáts et al., 2002). When comparing this expression pattern of cone opsins with that of other rodents, it becomes clear, that the uniform expression of both M- and S-opsins in all cones of the Siberian hamster and the pouched mouse is also novel and unique. It is not restricted to a small transitional zone, as described in divided retinas, and no dorso-ventral difference in the expression of cone opsins was

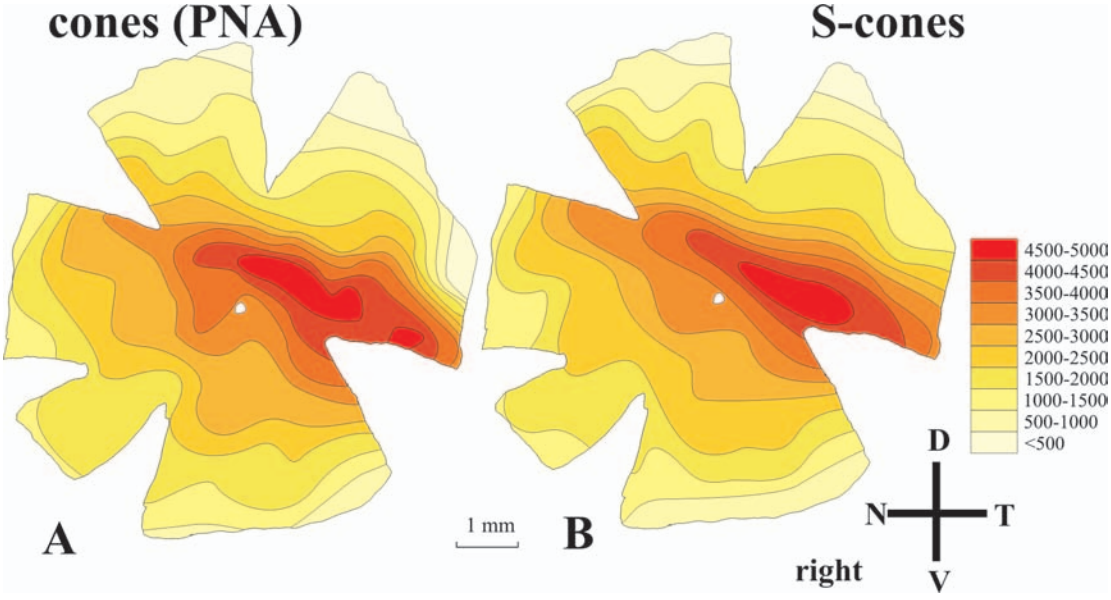

Fig. 6. Isodensity maps derived from the same right retina of *Saccostomus campestris* (pouched mouse), showing density values obtained using an all cone marker PNA **(A)** and a blue-cone specific antibody JH455 **(B)**. The two maps are practically identical. Density values are given in cone/mm^2.

observed.

When comparing the Syrian hamster with the Siberian hamster, (von Schantz et al., 1994; Calderone and Jacobs, 1999; Lukáts et al., 2002) it becomes clear that in both species the pure S-cone population is missing (no genuine S-cones occur). Both species have a single cone population that expresses only the M-opsin in the Syrian hamster and both M- and S-opsins in the Siberian hamster. Since there is only one receptor population, a real color vision is probably not present. While in the Syrian hamster the photopic sensitivity is confined to the green range of the spectrum, in the Siberian hamster this range is extended towards the blue and UV part (Calderone and Jacobs, 1999). Whether the broadening of the spectral sensitivity of this single cone population provides any advantage for the Siberian hamster or not, remains to be elucidated. A further question is whether the hamster can take advantage of this photopic sensitivity at all, in addition to the dominating scotopic sensitivity, when collecting additional visual information. As both species are considered strictly nocturnal, this is at least questionable (Ferraro, 1988; Skinner and Smithers, 1990; Ellison et al., 1994).

When comparing the life styles of these two peculiar species, the Siberian hamster and the pouched mouse, striking similarities could be found (Ferraro, 1988; Ellison et al., 1994; Skinner and Smithers, 1990; Bennett personal communication). Both of them have a "hamster-like" appearance, with short tail, pouches connected to the mouth in which food is collected and stored, and their feeding habits are also similar. Further, the pouched mouse is also referred to as African hamster, though there is only little taxonomical connection between these two species. The question thus logically arises as to whether it is this surprising similarity in life-style that is reflected in their similar retinal cone distributions? Indeed, this may give us the first clue to better understanding the functions of this peculiar retinal pattern.

Patterns of dual cone distribution in mammals

The experiments presented above demonstrate unequivocally that the problem of dual cones is not restricted to a relatively small population of exotic mammalian species. At least five different types of distributions are now known with smaller or larger dual cone contingent in adult mammalian species.

1) Divided retinas (mouse-like pattern) – there are at least two different populations of cones, with one of them containing exclusively the S-opsin (genuine S-cones – 10% of all cones), while the other type (90%) probably expresses both pigments with a dorso-ventral gradient. In the dorsal cones M/L-, in the ventral cones S-opsin production dominates (Szél et al., 1992; Juliusson et al., 1994; Röhlich et al., 1994b; Famiglietti and Sharpe 1995; Applebury et al., 2000) (Fig. 8A).

2) Retina of insectivores – with cone distribution pattern similar to the ones reported in the mouse. The insectivore retina contains S-cones in surprisingly high ratio on the ventral retinal field. Unlike in the mouse, M/L-cones are not entirely missing from this region and dual elements could only be identified in small number (Peichl et al., 2000) (Fig. 8B).

3) Dual cones situated exclusively in the dorsal peripheral retina – presently only one species, *Otomys unisulcatus* is reported with this type of retinal cone distribution. The function as well as the frequency of this retinal pattern is under extensive examination (Fig. 8C)

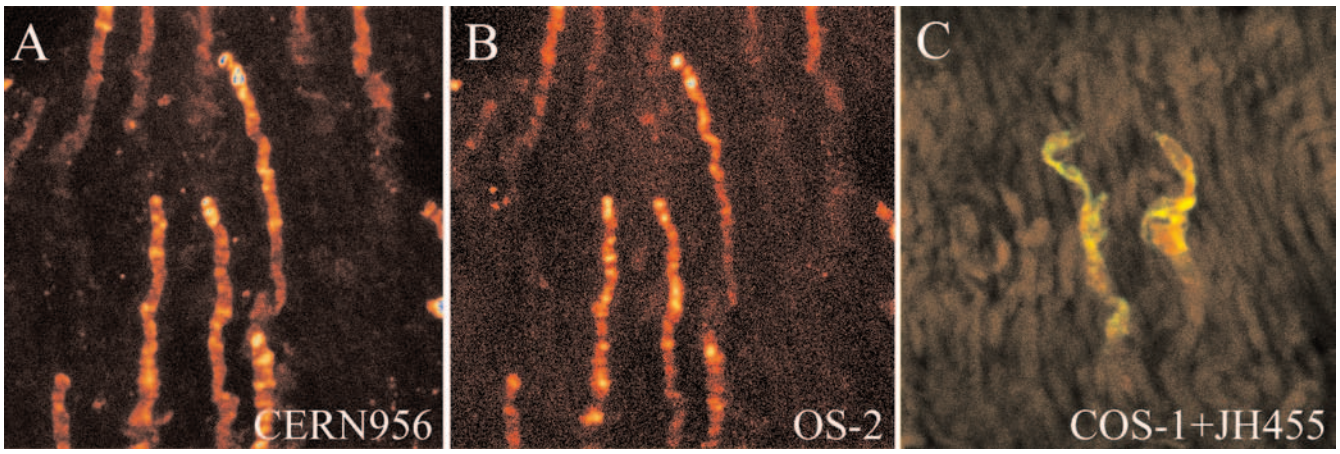

Fig. 7. Confocal images demonstrating the distribution of M- **(A)** and S-pigments **(B)** in the cone outer segments of the Siberian hamster as shown by opsin specific antisera. **C.** Combined confocal image of two cone outer segments as labeled by antibodies COS-1 (in red) and JH455 (in green) in *Saccostomus campestris*. All cones exhibit a homologous distribution of both pigments and no proximo-distal gradients could be observed. x 2000

4) All cones coexpressing both opsins – at least two species, the Siberian hamster and the pouched mouse, possess retinas comprised exclusively of dual cones without the genuine S-cone population and the dorso-ventral gradient in the expression of S- and M/L-opsins (Lukáts et al., 2002). These retinas thus contain only one cone type, which however coexpresses both pigments without any spatial gradient (Fig. 8D)

5) Recent reports by Cornish and co-workers (2004a) on human retina state that dual cones are present, though in extremely small percentage, in adult human retina. These elements are seemingly evenly distributed on the whole retina, without any gradient, or peripheral preference. The possible function of these cones, if there is any at all, remains to be elucidated.

These data show that the presence of dual cones is much more widespread than previously reported. Since they are present in large numbers, even comprising the whole cone population in two species, the fact that they are functional, can not be questioned. The function however is still unclear. ERG studies on the Siberian hamster showed that this system can respond to both the UV and green part of the spectra, therefore these cones are probably unable to sense color contrasts and take part in color discrimination (Calderone and Jacobs, 1999). It is still unknown at present what could be the advantage of expressing both opsins and extending the spectrum of cones at the cost of giving up color discrimination in the vision of these animals. However, the widespread character of this phenomenon in itself stimulates further studies on the subject.

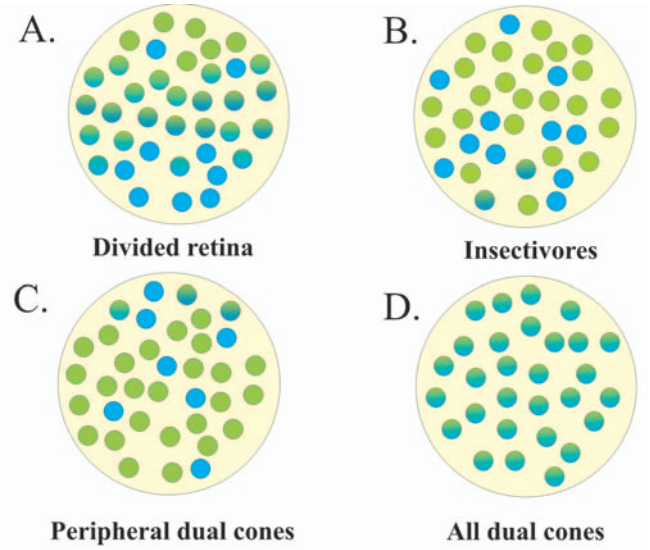

Fig. 8. Different types of color specific cone distributions incorporating dual cones. In a divided retina dual elements are restricted to the transitional zone **(A)**. In the retina of insectivores a cone distribution similar to that of the mouse was detected, but S-cones are not exclusive constituents of the blue-field and dual elements appear only in small numbers **(B)**. Two more newly discovered distributions are described in detail in this report: in the Otomys some cones coexpress both opsins in the far peripheral retina **(C)** whereas in some rodents the retina is made up exclusively of dual elements **(D)**.

The development of color specific cone patterns

In the last few years, attention has also turned towards the development of retinal cone distributions. How do these cells with very similar characteristics, but with markedly different functions develop? Do cone types develop independently or is there some connection between the S- and M/L-opsin production? Is there a general cone precursor that can differentiate in either direction or does the retina contain distinct sets of S- and M/L-precursor cells? Is there a connection between the presence of double labeled (dual) cones and developmental events? For proper color discrimination, the cones with different sensitivity must be in close vicinity to each other. What organizes this topographic arrangement? How and why is a specific distribution pattern created? Using antibodies against visual pigments or in situ hybridization, several attempts have been made to study the spatial and temporal organization of photoreceptor differentiation. The advantage of these probes is that positivity indicates opsin expression, thus the commitment of a given receptor type, even before it would become functional and could be detected by physiological methods (Sears et al., 2000).

In order to interpret developmental studies correctly, one has to consider that the order in which the photoreceptors are created (become post mitotic) is not necessarily the same as the order of opsin expression. Using radioactive thymidin in mice, Carter-Dawson and La Vail (1979) showed that cones become post mitotic well before rods; yet it is the latter that first expresses pigments (Szél et al., 1993b, 1996, 1998, 2000; Szepessy et al., 2000). Furthermore, opsin expression does not necessary mean that the receptor is functional. Other elements of the phototransduction, indispensable for proper functioning, were shown to develop only later (e.g. in S-cones they appeared 1-3 weeks after the presence of blue opsin was first detectable (Sears et al., 2000)). A similar degree of expressional gap is likely to be present in other types of receptors as well. Synapse formation too could be completed several days after opsin expression has started (Sears et al., 2000).

Both *in vivo* and *in vitro* studies have been performed to get a better insight into photoreceptor differentiation and the organization of retinal fields. Whereas some of the basic processes seem to run parallel in all developing retinas, a series of unexpected differences have also been reported. Developmental studies so far are thus frequently full of contradictions.

In most mammals photopigment expression begins postnatally (Szél et al., 1993b, 1994a, 1998, 2000; Szepessy et al., 2000), it is only in primates where opsin production could be detected in utero (Curcio and Hendrickson, 1991; Bumsted et al., 1997; Bumsted and

Hendrickson, 1999; Röhlich et al., 1994a; Cornish et al 2004a,b). The first immunopositive elements appear in the center and the development progresses towards the periphery. This centro-peripheral gradient is a general characteristic of all immature retinas; a few days of discrepancy between the developmental stages is retained throughout the whole process.

In all species studied, the order of immunologically detectable pigment expression has been proven to be the same (mouse, rat (Szél et al., 1993b, 1994a), rabbit, tree shrew (Szél et al., 1998, 2000, Szepessy et al., 2000), and primates (Curcio and Hendrickson, 1991; Bumsted et al., 1997; Bumsted and Hendrickson, 1999; Röhlich et al., 1994a; Cornish et al 2004a, b)). The first detectable elements, the rods, usually appear centrally, and rhodopsin expression is then quickly extended to the peripheral regions. The first cones following the rods are blue-sensitive; M/L-cone phenotypes are the last to be detected. The order is thus rods – S-cones – M/L-cones. There is only one record describing a developmental sequence different from this general scheme and is not without some contradiction. In the fovea of primates, which is the first region showing any differentiation M- and L-cones were detected earlier than S-cones by Wikler and Rakic (1991). Other authors on the other hand report the parallel appearance of S-, M- and L-pigments in this region. Outside the fovea, in the peripheral retina of primates S-cone expression is always in advance of the other two pigments as in all other species (Curcio and Hendrickson, 1991; Bumsted et al., 1997; Bumsted and Hendrickson, 1999; Röhlich et al., 1994a; Cornish et al., 2004a).

Transitory photopigment coexpression

A peculiar phenomenon was reported when studying the kinetics of the development of cone types in the rat retina (Szél et al., 1994a). As in all other mammals, the first detectable elements were the rods, followed by blue- and later by the green-sensitive cones. When the density of immunopositive receptor types was plotted as a function of time, it became evident that the number of cones carrying blue-opsin reached values far superior to the ones considered normal in adult specimens. A few days after being detected, S-cone density increases to about 8-9000 cone/mm^2, which is about one order of magnitude higher than the adult level. After postnatal day 12, blue-cones begin to disappear, density values fall extensively. M-cones on the other hand appear only later, at postnatal day 8; their number increases sharply and stabilizes without a sharp peak at values comparable to the ones measured in adults. Only a slight decrease is visible afterwards, that is by no means as intensive as that of S-cones. As seen, within the same time interval, the density of S- and M/L-cones changes in an opposite way. Green-cones increase their density, whereas S-cones are reduced in number. This kinetics could not be explained by the selective death of blue-sensitive elements: apoptosis was never detected to that extent. Also, the growing of the retinal area, which is estimated to be about 10%, is negligible when compared to the decrease in the number of S-opsin positive elements. In search of alternative explanations a new idea of photoreceptor development was proposed with S-cones transdifferentiating into M/L-cones (Szél et al., 1994a, 1998, 2000). The prediction was that some – the majority – of the detected S-cones switch to produce the M-pigment.

If it actually happened so, there must have been elements that simultaneously contained both opsins. Indeed such double labeled cones were identified in great numbers (Szél et al., 1994a). According to this proposition, all cones first express only the blue-sensitive pigment, which is the default pathway in differentiation, and about 10% of all cones continue to do so till adulthood (genuine S-cones). The rest, comprising the majority, will transdifferentiate to form the definitive M/L-population. They start to express the green-sensitive pigment too, which is distributed evenly in the outer segments thus intermixed with the S-pigment since the removal of apical discs by the pigmented epithelium takes a few days to complete. Most elements are thus double labeled because they already produce the M-opsin but have not yet removed their S-pigment content. After the differentiation has completed, not a single cone exhibiting photopigment coexpression was observed in adults (Fig. 9).

Default pathway in cone differentiation

Other experiments also indicate that the default pathway in cone differentiation is probably the S-pigment expression. Some signal is presumably needed to turn the majority of the elements to switch on the M/L-pigment production. If the signal is missing, differentiation could be retarded or never completed. In vitro experiments with explantation and transplantation of retinal pieces supplied evidence supporting this idea. In mouse retinal explants from newborn pups, the complete lack of M/L-pigment was reported; only S-cones were present in supernormal numbers (Söderpalm

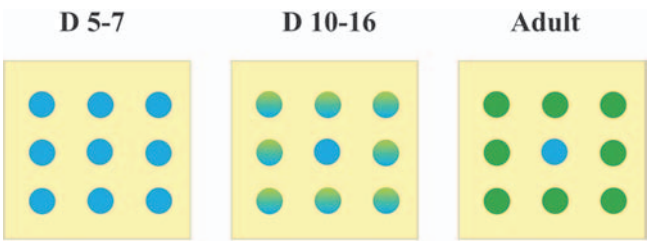

Fig. 9. Schematic model of cone development with transdifferentiation. At first, all cones express the S-opsin only, and some of them continue to do so in the adult (genuine S-cones). The majority of elements later turn on the M/L-opsin production and transdifferentiate into the definitive M/L-cone population. For a limited time interval, these cones contain both pigments.

et al., 1994). Extending the time of culturing did not have any significant effect on incomplete development. The crucial factor for green-cone differentiation was thus missing from the culture medium. In transplants, derived from older ages (postnatal day 3 to 10), some green-sensitive cones could be detected, but their number was always significantly lower than that of the control retinas (age-matched littermates). The appearance of M/L-cones in the latter case could probably be explained by the fact that some, but not all presumptive M/L-cones were already committed at the time of the explantation (Wikler et al., 1996). Recent reports on rat and rabbit retinal explants showed that in a modified culturing medium supplemented with hormones and vitamins, M/L-cones indeed differentiate (Pinzón-Duarte et al., 2002; Mack et al., 2003). Comparing the differences in the media used in the experiments may give us the possibility of identifying the factors indispensable for green-cone differentiation.

Results of transplantation experiments carried out on rabbit retinas also confirmed the same putative mechanism (Bergström et al., 1994; Szél et al., 1994c). When embryonic rabbit retinal pieces were explanted subretinally into the eye of adult specimen, the presence of some M/L-pigment positive cones could usually be detected, but their number was well under the normal values and the differentiation was significantly retarded. Thus the adult organ specific environment was more effective than the conventional tissue culture medium, but not quite matched the characteristics of the normal developing retinas.

The fact that dual cones were identified both in the developing retinas and in some adult animals like the ones with divided retinas (e.g.: the mouse), raises another crucial question. Is there a possible connection between the two types of coexpressions? A logical assumption comes from comparing the retinal cone distribution pattern of the mouse with the retinal developmental sequences reported in the rat (Szél et al., 1992, 1994a; Röhlich et al., 1994). The stages that *follow each other in time* (temporal sequence) during maturation in the rat correspond to the *spatial separation* of different retinal regions in the mouse. The inferior retinal field contains exclusively the blue-sensitive pigment just as seen in a 4-8 day-old rat. The transitional zone corresponds to the excess of dual cones visible in postnatal week 2, whereas the superior retinal half resembles the adult rat distribution pattern (Fig. 10). It was supposed that the cone differentiation starting all over the retina is interrupted at different stages on different retinal regions (Szél et al., 1996, 2000). When compared to the retinal development of the rat, the main difference could be that the signal, which triggers the shift from S- to M/L-pigment production is thus topographically determined. In the dorsal retinal half, the transdifferentiation is completed, incomplete in the transitional zone, and frozen at the earliest stage in the ventral retina. Even some of the possible factors were identified that could be responsible for supplying this positional information during development. The different levels of retinoic acid production coincide with the dorsal and ventral territorial division, and other molecules were also shown to be distributed along a dorso-ventral gradient (Constantine-Paton et al., 1986;

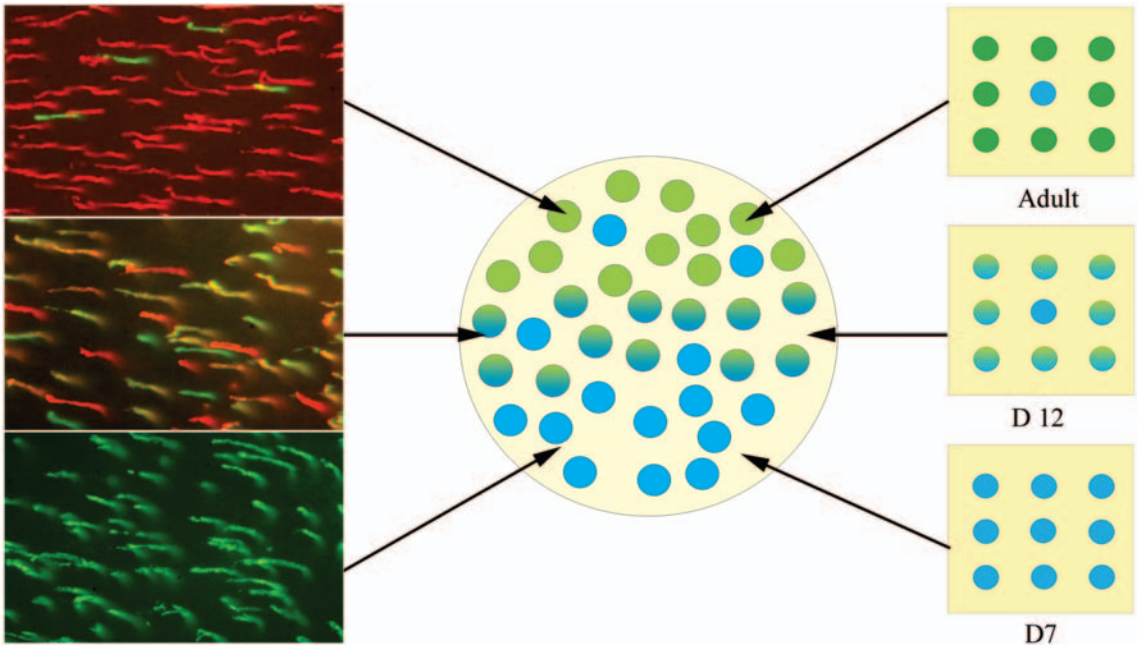

Fig.10. Comparison of transitory and spatial photopigment coexpressions in mammals. The staining patterns visible in different retinal regions in the mouse correspond to the stages that follow each other in time during the development of the rat retina as demonstrated by the schematic drawings and fluorescent double labeling. On the micrographs, green-cones were labeled in red, S-cones in green. Most of the elements of the transitional zone coexpress both opsins.

McCaffery et al., 1993, 1999).

When the development of the mouse retina was examined in detail, surprisingly no dual cones were identified at the dorsal retinal field at any stage of maturation (Szél et al., 1993b). Yet again blue-cones were the first identifiable cone types at the ventral retinal field. They later appeared also on the dorsal part, but in that field their number was never significantly higher than at adult stages. The M/L-pigment appeared only later; its presence was restricted exclusively to the dorsal field. No cone already producing the green pigment contained the blue-opsin in detectable amount, except for cones in the transitional zone (Szél et al., 1993b). This fact strongly argues against the common mechanism postulated for the development of dual cones.

When the experiments were extended to other species, a confusing view emerged. In the gerbil (*Meriones unguiculatus*) developmental events seemed to follow the kinetics described in the rat (Szél et al., 1994a), whereas in other species (mouse (Szél et al., 1993b), bovine (Szél et al., 1998, 2000), some primates (Bumsted et al., 1997; Wikler and Rakic, 1991)) another type of development was found. The sharp increase in S-opsin positive elements were missing, values higher than in adults were never reached. Further, no cones labeled by more than one antisera were identified. Even more confusing is the data presented by Bumsted and Hendrickson (1999) on the retinal development of primates. Differences were reported in cone differentiation of the Macaque and human retina! In the developing Macaque eye no sign indicated transdifferentiation, while in humans a set of immature cones were shown to coexpress the S- and the M- or L-opsins. Is it possible that photoreceptor development could follow two different strategies? In some species M/L-cones develop from the S-cones, in other species are they created independently? Or the techniques applied were just not sensitive enough to detect those ephemeral dual cones?

In search of answers to these questions the retinal maturation of two species (the common rabbit and the tree shrew) was examined in detail. Both possess a retina with a special cone distribution. The question to be answered was: do transitional dual cones appear during development of the photoreceptor cells in these species? Present techniques using highly sensitive immunocytochemistry and computerized image analysis would probably be able to detect the presence of cone opsins in extremely small quantities. Studying the retinal development in these species, we expected to obtain a general view of retinal cone development in mammals.

The common rabbit, often used in ophthalmologic studies, has a modified divided retina (Juliusson et al., 1994; Famiglietti, 1995). The blue-field occupies only a small ventralmost crescent of the retina ("blue streak"), the transitional zone is relatively narrow, and thus all M/L-cones of the dorsal mid-periphery practically contain only one single pigment in adults. This situation is advantageous if one aims to detect the possible presence of transitory photopigment coexpression. Since the results of retinal transplantations were also derived from the use of this species (Bergström et al., 1994; Szél et al., 1994c), any result obtained by our study could lead to a deeper understanding of the transplantation and explantation experiments.

The other species, the tree shrew (*Tupaia belangeri* obtained from the breeding colony of Heywood M. Petry, University of Louisville, USA) is also an interesting model, since it possesses a cone dominant retina with characteristics similar to those of the ground squirrel (Müller and Peichl, 1989; Petry et al., 1993). Cones make up the majority of elements, with rods comprising less than 5-10% of the population. The two cone types are arranged homogenously with higher central and lower peripheral values. No cones labeled by more than one antisera were identified in adult specimens.

Further, the tree shrew also has interesting taxonomical connections: some authors propose a link between them and primates (Kirsch et al., 1983). Undoubtedly, the retinal structure of tree shrew does resemble the primate fovea in several aspects. Conclusions drawn on cone development of the tree shrew might well be applied to primates as well.

The development of the rabbit retina

The order in which the immunocytochemically distinguishable photoreceptors appear in the developing rabbit retina proved to be the same as reported previously in other mammals. As shown on whole mounts reacted by visual pigment specific antibodies mAb OS-2, mAb COS-1, AO, the first elements starting to express opsin were the rods. They could be identified using anti-rhodopsin specific antisera even by postnatal day 1 or 2 in the central-superior part of the retina. S-cones followed rods in the sequence of development. The blue-pigment expression was first detectable by postnatal day 2 or 3. No immunopositive M/L-cones were seen before the end of the fist week (P 6-7). The order of photopigment expression was thus rods – S-cones – M/L-cones. This sequence seems to be generally true for all mammals, whether they have a retina with homogenous cone distribution or with separation of the different cone types (Bumsted and Hendrickson, 1999; Szél et al., 1993b, 1994a, 1998, 2000; Szepessy et al., 2000).

In order to estimate the density change of cone subtypes with different color sensitivity, immunopositive outer segments were counted, and the tendency in the change of immunopositive elements was plotted (Szepessy et al., 2000). S-cones appeared as early as postnatal day 2, and increased their density very regularly. By postnatal day 14 a plateau was visible with a peak density close to 2000 cones/mm^2, followed by a gradual decrease in number reaching adult values approximately by postnatal day 24. M/L-cones appeared

much later, not before day 6; their number increased sharply to about 12000 cones/mm2 by the end of the week 2. A slight decrease was clearly detectable in the following days and the density finally reached adult levels (9000 cones/mm^2) soon after.

It is clear that the values obtained in developing rabbit retinas are different from the ones found in mammalian species with transitory photopigment coexpresssion (Szél et al., 1994a, 1998, 2000). In contrast to the rat, rabbit S-cone density values exceeding the ones detected in adults, were never observed. There was also no sign of the opposite change in the density of S- and M/L-cones within the same time interval; instead, there was a slight decrease in the number of both cone types after the second week. This could probably be attributed to the moderate increase in retinal area. According to our measurements, the area of the developing rabbit retina increased by about 10% without the concomitant change in the number of immunopositive elements (Szepessy et al., 2000). At least partially, programmed cell death (apoptosis) can also be responsible for this decrease of densities. It has been shown to play an important role in the development and organization of all neural structures (Reme et al., 2000).

Although the kinetics of development of color specific cones strongly argued against a transitory photopigment coexpression, we regularly performed double labeling using the very sensitive consecutive tangential semithin sectioning method. Studying the reconstructed images of more than 100 section-pairs of 30 specimens of several different ages proved without doubt that not a single cone in the dorsal part of the developing rabbit retina contains more than one type of photopigment (Szepessy et al., 2000). There was one exception however: the transition zone between the superior retinal region and the blue streak, where cones coexpress both pigments even at adult stage.

Thus, in the rabbit retinal development, the kinetics of cone opsin expression and series of double labeling experiments showed that transitory photopigment coexpression does not occur.

The development of the tree shrew retina

In agreement with previous reports (Müller and Peichl, 1989; Müller et al., 1989; Petry et al., 1993) the tree shrew possesses a cone-dominant retina. Rods comprise only 7-8% of all receptor populations depending on retinal location. Amongst cones two populations could be identified. 4-10% of all cones are blue-sensitive, the rest, as shown by electrophysiological examinations, responds best to long wavelength stimulation (Jacobs and Neitz, 1986). As demonstrated both in whole mount immunocytochemistry and on semithin cross and tangentional sections, each cone in adults contains only one type of photopigment: no double staining with mAb COS-1 and mAb OS-2 was detectable in any region examined (Szél et al., 1998). The reconstructed image summarizing the staining pattern in the adult tree shrew is shown in Figure 11.

An interesting feature of cone dominant retinas was however revealed by these experiments: like in the ground squirrel, all rods were reactive not only to AO but also to OS-2, an antibody recognizing only the blue-sensitive cone opsin. This type of cross reactivity is not unprecedented in sub-mammalian retinas, where OS-2 usually stains all the rods. It could also be provoked in other mammals by using considerably higher concentrations of the antisera, but with normal application it seems to be restricted to species with cone dominant retinas (Szél and Röhlich, 1988).

Binding of OS-2 to rods could seriously confuse any conclusion that might be drawn on the development of photoreceptors, so we first checked if the double staining of rods also occurs in younger ages. This cross-reaction remained undetectable on all samples before postnatal day 28, probably due to the lower amount of opsin accumulation in the outer segments. Since rod outer segments are positioned slightly more vitreal, approximately level with cone inner segments, each receptor was easily identifiable.

In the developmental series the appearance of immunopositive elements were the same as in previous studies (Szél et al., 1993b, 1994a, 1998, 2000; Bumsted and Hendrickson, 1999; Szepessy et al., 2000). Rods were the first elements to start opsin production, soon followed by the S- and later by the M/L-cones. As seen in Figure 12A, the first blue-sensitive cones were

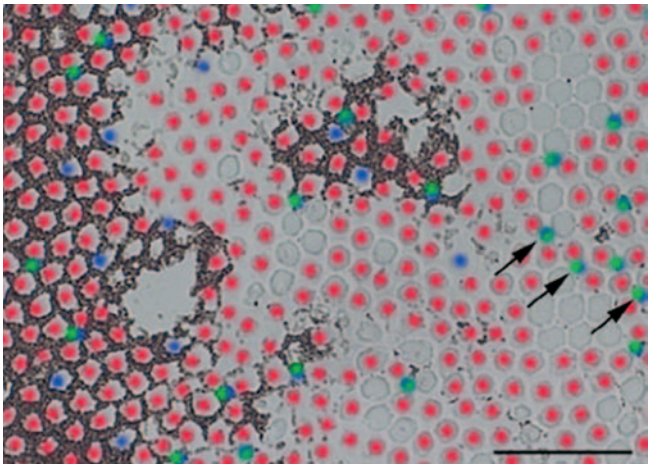

Fig. 11. Reconstructed image of the adult tree shrew retina. Consecutive tangential semithin sections were reacted with COS-1 (M/L-cones), OS-2 (S-cones) and AO (rods) respectively. Identical areas of the sections were photographed, the positive elements were digitally selected, colored, and finally all positive elements were projected onto the same picture. Red and blue cones are represented by the corresponding colors. Rods that absorb best in the green part of the spectrum, are coded by green dots. It is obvious that there are no cones synthesizing two opsins, but, similarly to the ground squirrel retina, rods bind both AO and OS-2 antibodies as indicated by the intermixing green and blue colors (arrows). Bar: 10 μm.

detected at postnatal day 6 and their number increased gradually, reaching adult values soon. As in case of the rabbit (Szepessy et al., 2000), we never detected values significantly greater than in adults, and there was no sharp decrease in the number of immunopositive S-cones. M/L-cones were not detected before postnatal day

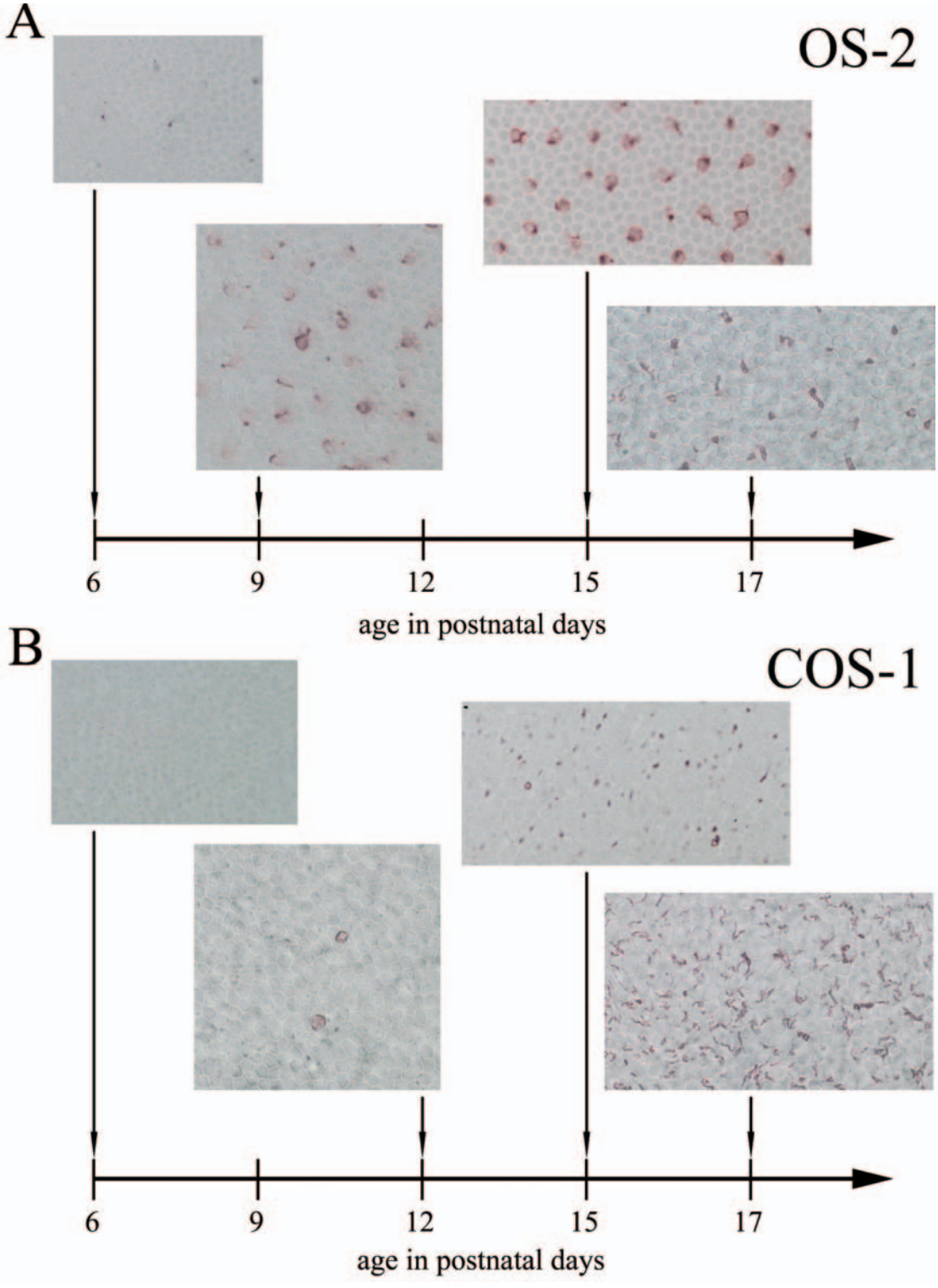

Fig. 12. Representative photomicrographs showing the developing S- **(A)** and L-cones **(B)** in the immature tree shrew retina as detected by antibodies OS-2 and COS-1, respectively.

12 (Fig. 12B). With increasing numbers they soon reached adult density values without any significant fluctuation.

The kinetics described here is very similar to what has been reported in the rabbit and in other species without transitory photopigment coexpression (Szél et al., 1998, 2000; Szepessy et al., 2000). However, to make sure that no small populations of double labeled elements are overlooked, several series of consecutive semithin tangential sections were treated alternately with OS-2 and COS-1. In most sections examined by computerized reconstruction we did not detect any cone outer segment reactive with more than one antisera. In a few retinal samples however the reconstruction showed different results (Szél et al., 1998). Some elements, making up about 5% of all cones, bind both antibodies and therefore contain both pigments (Fig. 13).

Thus in the tree shrew, although the kinetics of opsin expression showed that transitory photopigment coexpression was unlikely, the combination of sensitive techniques like computerized reconstruction and semithin tangential sectioning helped the detection of the presence of a small population of cones coexpressing two types of opsin proteins.

This population is relatively small as compared to the transitional zone (Röhlich et al., 1994b) or even to the developing retina of rats where the majority of cones were stained by both antisera (Szél et al., 1994). Also it is restricted spatially to a small concentric ring of the retina. This observation suggests that the switch of pigment production from S- to M/L-cone opsin takes much shorter time in the tree shrew than in other species. At any time interval only a small percentage of the total population expresses both pigments, which makes their identification far more difficult.

This differences reported here raise a crucial question. We originally assumed, that cone differentiation follows one way in all mammalian species, and using a more sensitive methodology, we may be able to identify transitory dual cones in all species examined. Indeed, we could only identify cones labeled by more than one antisera in the tree shrew in very low quantity (Szél et al., 1998, 2000; Szepessy et al., 2000). The small number of dual elements in this species could be explained by two assumptions: either 1) only some of the cones develop with transdifferentiation (there are M/L-cones that never produce the S-pigment), or 2) the switch from S- to M/L-pigment expression is rapid, so that only a limited number of cones coexpress both pigments at a given time interval. Although one of these alternatives might be true for the tree shrew, it can not be applied to the rabbit. The careful examination of the developing rabbit retina both on double labeled whole mounts or consecutive semithin sections never revealed dual cones during cone development. Thus, although we successfully proved coexpression in the tree shrew, the rabbit retina most probably develops in another way.

As we expected, a recent report by Cornish and co-workers (2004a) proved that the tree shrew can indeed be used as a model of primate cone maturation. When studying the pre- and postnatal development of human cones, a situation very similar to the tree shrew was reported. A small percentage of human cones, dominantly at the "front" of M/L-cone differentiation coexpress both pigments. The only difference is that these dual cones are not entirely missing from the rest of the retina; even being present in adults though in an extremely small percentage. The question why these transitory dual cones are missing from the retina of other primates (Bumsted and Hendrickson, 1999) remains to be answered.

Thus it seems that in some species, including the rat, gerbil, the tree shrew and humans, cones develop with transdifferentiation (Szél et al., 1994a, 1998, 2000; Bumsted and Hendrickson, 1999; Cornish et al 2004a). All cones first express the S-pigment only, and the majority of them switch to produce the M/L-opsin. In the

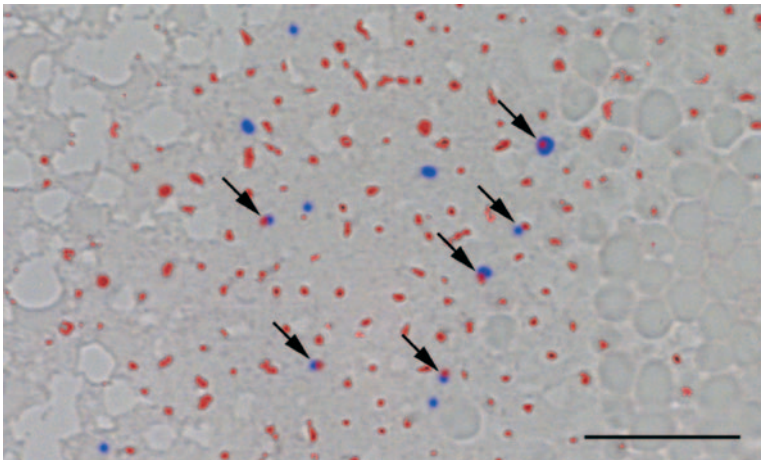

Fig. 13. Superimposed pictures of two consecutive tangential semithin sections of the retina of the developing tree shrew (postnatal day 19). Each positive element was selected, and digitally colored. S-cones were assigned the blue, L-cones the red color. Note that a significant number of cones contain both opsins, as indicated by the overlapping colored dots. Some of the dual elements are marked with arrows. Bar: 10 μm.

transitory phase of this switch cones containing more than one pigment could be identified in great numbers. In contrast, in the other group of mammals cone types probably follow individual differentiation lines, which means that all cones express only one of the two pigments, and dual cones are not present (Wikler and Rakic, 1991; Szél et al., 1993b; Bumsted et al., 1997; Bumsted and Hendrickson, 1999; Szepessy et al., 2000).

Possible regulation of cone differentiation

Even though these new experiments cast doubt that a common way of cone differentiation exists, it is still worthwhile to speculate on the possible regulation of differentiation. Early transplantation and explantation experiments (Bergström et al., 1994; Söderpalm et al., 1994; Szél et al., 1994c; Wikler et al., 1996) indicated, that the default pathway is the S-pigment expression. A signal is supposedly needed to induce the majority of the cones to express M/L-pigment, and if the signal is missing, maturation remains incomplete. It was only recently that M/L-pigment production was first achieved in retinal tissue cultures from neonatal animals (Pinzón-Duarte et al., 2002; Mack et al., 2003), and this encourages to find the hypothetical signal that deviates cones towards the M/L-pigment expression. A number of different candidates were assumed to regulate M/L-cone formation, but the clear function of these factors usually remained unidentified. Thyroid hormone and especially its ß2 receptor is probably the only candidate factor with an identified function. Mutant mice lacking this receptor fail to synthesize green-opsin, all cones express only the UV-pigment. Also, S-cones are higher in number than in normal retina (Ng et al., 2001; Yanagi et al., 2002). Retinal nuclear receptor (Yanagi et al., 2002), retinoic acid (Mori et al., 2001), different growth factors with appropriate receptors and some other molecules were also mentioned in recent studies (Nag and Wadhwa, 1999; Cornish et al., 2004c; Szabó et al., in preparation). Supposing photopigment coexpression and transdifferentiation in the M/L-cone type, one of the last steps in development seems to be the turning on of the M/L-opsin gene with turning off of the S-opsin production. If this control mechanism is not working, both M/L- and S-opsins are expressed or the expression of the opsins is shifted towards the blue (or UV) range (Applebury, 2001; Neitz and Neitz, 2001). From the evolutionary point of view it would be interesting to reveal the control factors that govern the opsin switch in cones. In this respect the Siberian hamster (together with the pouched mouse) deserves special attention as a suitable model, since it possesses a single cone population that coexpresses both M- and S-opsins in adult animals (Lukáts et al., 2002).

The molecular genetic comparison with the Syrian hamster in which this single cone population expresses the M-opsin only, offers a favorable model to study molecular control mechanisms in a pure system and to understand developmental, genetic and evolutionary aspects of opsin coexpression.

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