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Alix Darden
University of Oklahoma Health Sciences Center

Photoreceptor Development in Premetamorphic and Metamorphic *Xenopus laevis*

RYAN O. PARKER,[1*] BRENT MCCARRAGHER,[2] ROSALIE CROUCH,[3] AND ALIX G. DARDEN[2]

[1]Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina

[2]Department of Biology, The Citadel, Charleston, South Carolina

[3]Department of Ophthalmology, Medical University of South Carolina, Charleston, South Carolina

ABSTRACT

Transgenic *Xenopus laevis* are commonly used to study gene expression in photoreceptors, but only red rods and red cones are known to exist in the pre-metamorphic stages commonly used in transgenic studies. Using RT-PCR, this study shows that violet cones develop in early premetamorphic stages (Stage 35) with the red rods and red cones. Green rod development began in Stage 53 with the onset of metamorphosis. Anat Rec, 293:383–387, 2010. © 2010 Wiley-Liss, Inc.

Key words: photoreceptor; opsin; green rod; *Xenopus*; development

Xenopus laevis is an important model in vision research, and transgenic *Xenopus* are commonly used in photoreceptor studies (Amaya and Kroll, 1999; Hutcheson and Vetter, 2002; Moritz et al., 2002; Jin et al., 2003). One advantage of using the transgenic model is that photoreceptors develop rapidly in premetamorphic tadpoles, and transgenes can be analyzed in a matter of days. While the mature (postmetamorphic) *Xenopus* retina contains five types of photoreceptors (Rohlich and Szel, 2000), only two have been identified in premetamorphic tadpoles. Thus, the majority of *Xenopus* photoreceptors have not been characterized developmentally, and it is unclear if they are present in the early developmental stages commonly used for transgenic studies.

Adult *Xenopus* possess two types of rods and three types of cones. The rods are historically named by the color they reflect under a light microscope and consist of red rods and a small population of green rods (Denton and Pirenne, 1952; Rohlich et al., 1989; Rohlich and Szel, 2000). The cones are named by their spectral sensitivities, and red and violet sensitive cones have been identified with spectral studies (Chang and Harris, 1998; Starace and Knox, 1998). A blue-sensitive cone is thought to exist based on morphological findings (Wilhelm and Gabriel, 1999; Rohlich and Szel, 2000). The visual pigments in *Xenopus* photoreceptors are composed of a light-sensitive chromophore (11-*cis* dehydroretinal) covalently bound to an opsin protein distinct for each of the photoreceptors. While no opsin for the blue cone has been found, the opsins for the red rod (Saha and Grainger, 1993), red cone (Chang and Harris, 1998), violet cone (Starace and Knox, 1998), and green rod (Darden et al., 2003) have been identified in adult *Xenopus*, and can be used to characterize the development of these four photoreceptors.

Because many transgenic studies target specific photoreceptor classes (Moritz et al., 2002; Kefalov et al., 2003; Babu et al., 2006), our goal was to determine which photoreceptors are present in premetamorphic tadpoles. In developmental studies, only the red rods and red cones are known to exist in premetamorphic tadpoles (Chang

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*Correspondence to: Ryan O. Parker, Storm Eye Institute, Rm. 708, 167 Ashley Avenue, Charleston, SC 29403. Fax: (843) 762-2613. E-mail: parkerry@musc.edu

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TABLE 1. Primer sets for analysis of opsin development

Target	Accession number	Forward primer	Reverse primer	T_m (°C)	Amplicon
Green rod opsin	AY177405	AGGGAAACGCATGCGGCTCTA	ACATTCGTAGGTCAAACAAC	55.4	417 bp
Red rod opsin	L07770	TTGGTGGTGAAGTGGCCCTC	CCGCATGAGCATTGCATTCCC	61.0	207 bp
Red cone opsin	U90895	CAACCTGCGGTATCAGTGCTCTG	CCTGCAGACCAAACCCAAGAGAAA	61.0	144 bp
Violet cone opsin	U23463	CATTCACCCTGCAAGCAATA	CAACAGCCAAAGCATGAGAA	52.8	154 bp
EF-1α	X55324	GCTGGTGACAGCAAGAATGA	AGAAGCTCTCCACGCACATT	55.4	268 bp

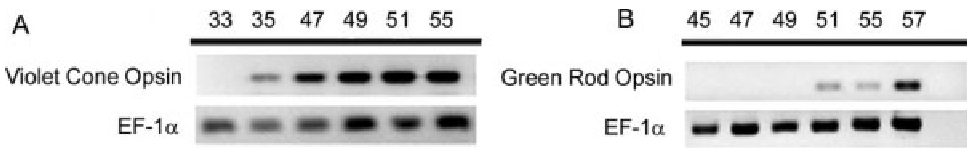

Fig. 1. Opsin mRNA expression in developing *Xenopus*. To characterize photoreceptor development, RT-PCR was used to identify opsin mRNA from total retina RNA. **A**: The violet cone opsin mRNA is present early in development at stage 35. **B**: The green rod opsin mRNA is absent in early developmental stages and first appears near the onset of metamorphosis at stage 51. Elongation Factor-1α (EF-1α) was used as an internal control.

and Harris, 1998). Reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry were used to characterize photoreceptor development (see Supporting Information for more details). Animal care was in strict compliance with the Association for Research and Vision in Ophthalmology statement for the *Use of Animals in Ophthalmologic and Vision Research*, and with the guidelines for the *Care and Use of Laboratory Animals* at the Medical University of South Carolina.

RESULTS AND DISCUSSION

Detection of Opsin mRNA Using RT-PCR

Photoreceptors are considered terminally differentiated when they express opsin (Cepko, 1996), and the opsins of the red rod, green rod, red cone, and violet cone have been identified in *Xenopus*. The red rod and red cone opsins are reliably detectable as early as stage 33 with *in situ* hybridization (Chang and Harris, 1998), but the violet cones and green rods have not been characterized developmentally. We used RT-PCR to determine which opsins were present in premetamorphic tadpoles (Table 1). In close agreement with previous studies (Chang and Harris, 1998), opsin mRNA from the red rods and red cones was detected as early as Stage 35 with RT-PCR. Similarly, violet cone opsin mRNA was detected in premetamorphic tadpoles at Stage 35 and persisted throughout the development (Fig. 1A). The early development of violet cones in *Xenopus* is consistent with the developmental studies in salmanid fishes (Cheng et al., 2007) and mammalian (rodents, primates) models (Szel et al., 1994; Bumsted et al., 1997; Xiao and Hendrickson, 2000), where analogous short-wavelength sensitive cones are among the earliest photoreceptors to develop.

Green rod opsin mRNA was not detected in early premetamorphic stages, where the other three opsins were readily identified. mRNA from the green rod opsin first appeared in Stage 51 tadpoles ($n = 2$ of 8 samples) and was present in all samples by stage 53 ($n = 5$) (Fig. 1B). Characterization of opsin mRNA expression with RT-PCR revealed that the red rods, red cones, and violet cones develop early in premetamorphic tadpoles, but the green rods are not consistently present until stage 53. Thus, the green rod has unique developmental characteristics and its development is separated temporally from the other photoreceptors.

Expression of Green Rod Opsin in Developing *Xenopus*

To confirm the delay in green rod development, immunohistochemistry was used to identify the green rod opsin in developing tadpoles. Retina cross sections were stained with an antibody against the red rod opsin (4D2) and with a recently developed antibody against the green rod opsin (XGN) (Darden et al., 2003). The specificity of the XGN antibody was determined in western blots and its staining pattern was characterized in retina cross-sections from adult *Xenopus*. The XGN antibody stained the thin, short outer segments of a sparse population of rod photoreceptors, presumably the *Xenopus* green rods (Fig. 2A). The 4D2 antibody stained the outer segments of a dense population of large rod photoreceptors (Fig. 2B), consistent with the red rods. Image overlays showed no overlap in the staining patterns and demonstrated that the two antibodies stained separate rod populations (Fig. 2C).

Immunohistochemistry was then used to determine if the green rod opsin was present in premetamorphic, metamorphic, and adult *Xenopus* retinal samples. No green rods were identified in Stages 35–49 ($n = 20$ per stage), and a single green rod was detected in stage 51 tadpoles ($n = 20$). Green rods appeared in approximately half of the stage 53 samples ($n = 19$), and by stage 55, all samples were positive for green rod opsin ($n = 5$). Figure 3 shows confocal images of *Xenopus* retinae from metamorphic (Stages 55 and 57) and adult samples. In agreement with our findings by RT-PCR,

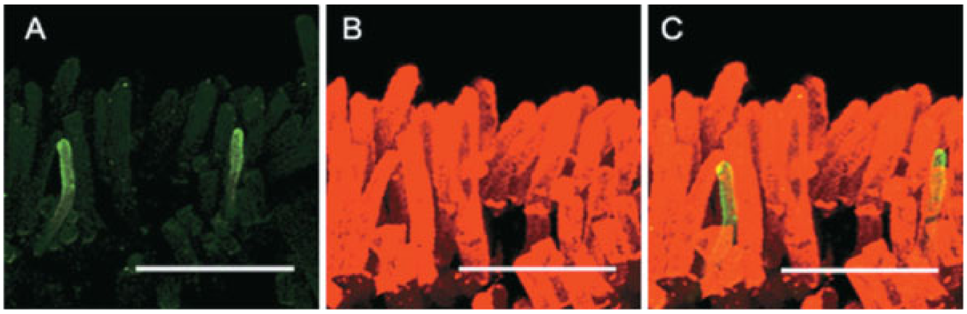

Fig. 2. Confocal images (630×) of paraffin cross sections (10 μm) from an adult *Xenopus* retina. **A**: Green rods were labeled with the XGN antibody (1:1,000), visualized with an Alexa 594 secondary antibody (1:500; Molecular Probes, Eugene, OR), and pseudocolored green. **B**: Red rods were labeled with the 4D2 antibody (1:1,000), visualized with an Alexa 488 antibody (Molecular Probes), and psuedocolored red. **C**: An overlay demonstrates the distinct staining patterns and morphology of the green and red rods. Green rods and red rods were psuedocolored green and red, respectively, in accordance with the historical nomenclature. Coloring and overlays were performed on Leica confocal software. Scale bars represent 40 μm.

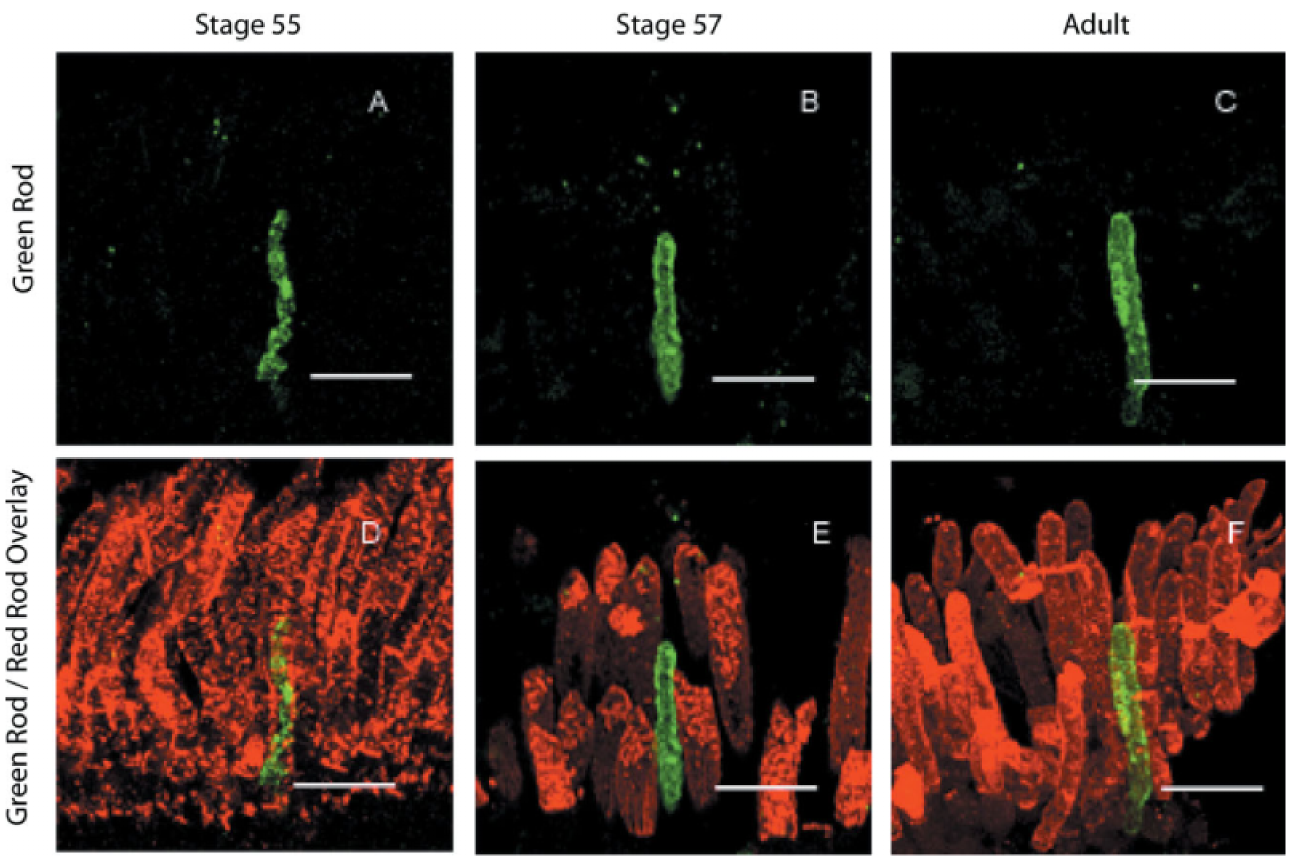

Fig. 3. Confocal images (630×) of *Xenopus* green rods in developing *Xenopus*. Green rods were first found consistently in stage 55 tadpoles. Here, we show images of green rods (**A**-**C**), and overlays (**D**-**F**) from stage 55, stage 57, and adult *Xenopus* eyes. Scale bars represent 30 μm.

immunohistochemistry confirmed that green rod development is delayed relative to the other photoreceptors.

Photoreceptor Development in *Xenopus*

The red rods and red cones in *Xenopus* are known to be present early in development (Saha and Grainger, 1993; Chang and Harris, 1998). Likewise, we found that the violet cone is also present in early developmental stages. Unlike the other photoreceptors, the *Xenopus* green rod was not detected by RT-PCR or immunohistochemistry in premetamorphic tadpoles. mRNA was first detected consistently in stage 53 tadpoles, and the opsin was first detected consistently in Stage 55 tadpoles.

Recent work shows that another amphibian, the tiger salamander (*Ambystoma tigrinum*), exhibits a delayed proliferation of green rods that is associated with metamorphosis (Chen et al., 2008). Similarly, it appears that the *Xenopus* green rod develops in association with the onset of metamorphosis.

Functional Significance of Green Rod Development

The functional significance of delayed green rod development in *Xenopus* remains unknown, but behavioral studies in several amphibian species offer a possible explanation. While green rods are named for their histological appearance, they are sensitive to blue light, and the development of blue-sensitive photoreceptors at metamorphosis is consistent with behavioral findings in other amphibians. Many Anuran (tailless) amphibians develop a preference for shorter wave-lengths (blue) of light as they mature. Specifically, young tadpoles are attracted to green light but develop a preference for blue light as they progress through metamorphosis (Muntz, 1963). Muntz (1963) hypothesized that the attraction to green light drew filter feeding tadpoles to the food and shelter of algae-rich shallows, and the attraction to blue light was a possible escape mechanism to draw mature animals into the safety of deep water. Furthermore, it was suggested that this response likely originated from the green rods. While the behavioral significance remains unclear, our finding that the green rod develops in association with metamorphosis offers convincing evidence that the blue-sensitive green rod is responsible for the growing preference for blue light in amphibians.

Possible Implications of Thyroid Hormone

The molecular cues that trigger green rod development remain to be determined, but thyroid hormone (TH) is likely to contribute. Between Stages 52 and 59, there is a 5-fold increase in TSHβ expression and associated increases in TH that drive the onset of metamorphosis (Manzon and Denver, 2004). Based on our findings, green rod development coincides with this TH surge at the onset of metamorphosis. Metamorphosis is a time when amphibians undergo drastic remodeling of all organ systems, and numerous changes have been defined in the *Xenopus* visual system (Marsh-Armstrong et al., 1999; Mann and Holt, 2001). Although no changes in opsin expression have been identified to date, recent studies in multiple vertebrate models have shown that TH can modulate cone opsin expression (Yanagi et al., 2002; Roberts et al., 2006; Veldhoen et al., 2006). Interestingly, the green rods are unique photoreceptors that function as rods but express an opsin that is categorized as a cone opsin (Ebrey and Koutalos, 2001). Thus, the cone-like opsin expressed in green rods could be under the developmental control of TH. While this study was purely descriptive and did not address mechanisms that induce opsin expression, the green rod's appearance at the time of increasing TH levels is consistent with findings in other vertebrate models (Yanagi et al., 2002; Roberts et al., 2006; Veldhoen et al., 2006) and suggests that TH could mediate green rod development in *Xenopus*.

SUMMARY

Xenopus metamorphosis is associated with dramatic changes in the visual system (Nagy and Witkovsky, 1981; Witkovsky and Powell, 1981). While the red rods, red cones, and violet cones are all present early in development, green rod development is delayed and begins in stages associated with the onset of metamorphosis. Development of the green rod at the onset of metamorphosis adds to the list of visual system changes associated with metamorphosis and explains the development of blue-sensitivity in amphibians at metamorphosis.

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