A Role for Cytoskeletal Elements in the Light-Driven Translocation of Proteins in Rod Photoreceptors

James J. Peterson,1 Wilda Orisme,1 Jonathan Fellows,1 J. Hugh McDowell,1 Charles L. Shelamer,1 Donald R. Dugger,1 and W. Clay Smith1,2

PURPOSE. Light-driven protein translocation is responsible for the dramatic redistribution of some proteins in vertebrate rod photoreceptors. In this study, the involvement of microtubules and microfilaments in the light-driven translocation of arrestin and transducin was investigated.

METHODS. Pharmacologic reagents were applied to native and transgenic *Xenopus* tadpoles, to disrupt the microtubules (thiabendazole) and microfilaments (cytochalasin D and latrunculin B) of the rod photoreceptors. Quantitative confocal imaging was used to assess the impact of these treatments on arrestin and transducin translocation. A series of transgenic tadpoles expressing arrestin truncations were also created to identify portions of arrestin that enable arrestin to translocate.

RESULTS. Application of cytochalasin D or latrunculin B to disrupt the microfilament organization selectively slowed only transducin movement from the inner to the outer segments. Perturbation of the microtubule cytoskeleton with thiabendazole slowed the translocation of both arrestin and transducin, but only in moving from the outer to the inner segments. Transgenic *Xenopus* expressing fusions of green fluorescent protein (GFP) with portions of arrestin implicates the C terminal domain of arrestin as an important portion of the molecule for promoting translocation. This C-terminal region can be used independently to promote translocation of GFP in response to light.

CONCLUSIONS. The results show that disruption of the cytoskeletal network in rod photoreceptors has specific effects on the translocation of arrestin and transducin. These effects suggest that the light-driven translocation of visual proteins at least partially relies on an active motor-driven mechanism for complete movement of arrestin and transducin. (*Invest Ophthalmol Vis Sci. 2005;46:3988–3998*) DOI:10.1167/iovs.05-0567

TRANSPOR'T of molecules is critical to the proper functioning of cells, but even more so for polarized cells. In neurons, perhaps the epitome of polarized cells, molecular transport uses both fast and slow components to renew membrane lipids and protein elements in the membrane and cytosol. Rod photoreceptors are arguably one of the most highly polarized cells with regard to both structure and function. Structurally, rods are divided into two segments, the rod outer segment (ROS) and the rod inner segment (RIS), joined by a narrow connecting cilium. The ROS contains a highly elaborate system of stacked, disc-shaped membranes that are densely packed with the visual pigment rhodopsin, whereas the RIS is more typical of the cell body region of a neuron, with the exception that it contains very densely packed mitochondria to supply the enormous energy needs of the photoreceptor.

Functionally, the ROS is primarily responsible for phototransduction, the process of absorbing a photon and converting it to a change in membrane potential. The function of the RIS is essentially to provide the energy demands and cellular building blocks needed to maintain the function of the photoreceptors. However, the line demarcating the functions of the ROS and RIS is somewhat blurred because some components are rapidly translocated between the two segments in a light-dependent manner. Nearly two decades ago, several studies showed that both arrestin and transducin almost completely change their respective compartments in response to light.1–5 In the dark-adapted retina, transducin localizes almost exclusively to the outer segment and arrestin to the inner segment. In response to an adapting light, these proteins translocate in opposite directions, with arrestin moving almost exclusively to the ROS and transducin to the RIS over the course of several minutes.

Studies that first identified the light-driven translocation of arrestin suggested that arrestin translocates to the ROS in the light as a consequence of its binding to light-activated phosphorhodopsin and is thus drawn to the outer segments by mass action.2 However, recent investigations have suggested otherwise. Using transgenic mice that are deficient in rhodopsin phosphorylation (either rhodopsin kinase is knocked out or the C-terminal serine and threonines in rhodopsin are replaced with alanines), researchers showed that arrestin translocates normally to the ROS in response to light, even in rods, where phosphorylation of rhodopsin is blocked, and thus the high-affinity binding partner for arrestin is lacking.6,7 These results suggest that the light-driven translocation of arrestin (and possibly transducin) may use an active motor-driven mechanism, perhaps using the cytoskeleton as molecular “train tracks” on which to move between the RIS and ROS.

In this study, we used *Xenopus* to investigate more fully the potential involvement of cytoskeletal elements in the light-driven translocation of arrestin and transducin. Using pharmacologic agents and transgenic animals expressing fusions of green fluorescent protein (GFP) to arrestin and portions of arrestin, we present evidence linking the translocation of arrestin to microtubules and the translocation of transducin to both microfilaments and microtubules.

METHODS

Preparation of Transgenes

Fusion of GFP cDNA to the 5’ end of the *Xenopus* rod arrestin cDNA open reading frame was as previously described.6,7 Ten, 20, and 30-

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amino-acid truncations at the C terminus of arrestin were prepared and fused with GFP as follows. The Xenopus arrestin cDNA was amplified by PCR with *Pfu* polymerase with a 5′ primer containing a *XhoI* restriction site immediately before the initiating ATG of the arrestin open reading frame (Table 1; primer 1), and with a 3′ primer that incorporated an *NheI* restriction site immediately after the codon for Glu-386 (10-amino-acid truncation, *Ar(c-10)-GFP*, primer 3), Arg-376 (20-amino-acid truncation, *Ar(c-20)+NheI*, primer 4), or Glu-366 (30-amino-acid truncation, *Ar(c-30)+GFP*, primer 5; see Table 1 for primer sequences). This PCR product was digested with *XhoI* and *NheI* and then used to replace the arrestin cDNA in XOPS-Ar-GFP plasmid8 that was removed by *XhoI*/*NheI* restriction. The resultant transgene fuses GFP to the C terminus of the truncated arrestin with an Ala-Ser linker that results from the reformed *NheI* restriction site. The XOPS plasmid contains a 1.3-kb fragment of the Xenopus rod opsin promoter to drive expression of the transgene in rod photoreceptors.9

Fusion of the C-terminal 45 amino acids of arrestin to the N terminus of GFP was accomplished in a similar fashion. A 5′ primer was designed that incorporated an *XhoI* restriction site immediately before the ATG codon of Met-352 (Table 1; primer 6). This primer was paired with a primer containing a *NheI* restriction site immediately after the last codon in the open reading frame (primer 2). The resultant PCR product was used to replace the arrestin cDNA in the XOPS-Ar-GFP plasmid vector at the *XhoI* and *NheI* sites. All plasmid constructs were sequenced to confirm the proper cloning of the desired cDNA and to confirm that no unintentional mutations were introduced by PCR. Plasmids were linearized with *SfiI* or *ApaLI*, and purified (Gene-CleanII; Bio 101, Vista, CA) in preparation for producing transgenic tadpoles.

**Preparation of Transgenic Animals**

Transgenic tadpoles were prepared by nuclear transplantation, according to the methodology of Kroll and Amaya,10 with modifications.9,11 Resultant tadpoles were kept in tadpole Ringer’s solution (10 mM NaCl, 0.15 mM KCl, 0.2 mM CaCl2, and 0.1 mM MgCl2) for 2 to 8 weeks, at which point they were screened visually through the eye to identify tadpoles that were expressing GFP, using blue light to excite emission from the GFP.

**Treatment of Tadpoles with Pharmacologic Reagents**

Wild-type tadpoles (stages 50–54) were obtained from Xenopus Express (Plant City, FL). Tadpoles were placed in tadpole Ringer’s solution (10 mM NaCl, 0.15 mM KCl, 0.2 mM CaCl2, and 0.1 mM MgCl2) for 2 to 8 weeks, at which point they were screened visually through the eye to identify tadpoles that were expressing GFP, using blue light to excite emission from the GFP.

Image Quantitation

Fluorescence intensity in photoreceptor compartments was quantified as follows. Individual color channels from the confocal TIF images were gray-scaled with image analysis software (Photoshop 6.0; Adobe Systems, Mountain View, CA) and imported into Scion Image (Scion Image 4.0.2; Scion Corporation, Frederick, MD). Single rod inner and outer segments were then outlined (Fig. 1), and total fluorescence for

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**Table 1. Sequence of Oligonucleotide Primers Used for PCR Amplification of Xenopus Rod Arrestin in the Preparation of Transgenes**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>1. Arrestin 5′+ XhoI</td>
<td>5′-GCCTCGAGATGGTGGAGAAAGAATCCGAG</td>
</tr>
<tr>
<td>2. Arrestin 3′+ NheI</td>
<td>5′-GGTCGGGCGGCTTACATCGATTCTGCTCCTTT</td>
</tr>
<tr>
<td>3. Ar(c-10)+NheI</td>
<td>5′-GCGCTAGCTTCTGGCTGAGTTCACCCTTGAG</td>
</tr>
<tr>
<td>4. Ar(c-20)+NheI</td>
<td>5′-GCGCTAGCGGCGGAAATCTTCAAAACACCAT</td>
</tr>
<tr>
<td>5. Ar(c-30)+NheI</td>
<td>5′-GCGCTAGCTTCTGGCTGATCTTCTTGCTCC</td>
</tr>
<tr>
<td>6. C45+XhoI</td>
<td>5′-GACTCGAGATGGTGCAATGACGTCAGCCAGGCG</td>
</tr>
</tbody>
</table>
adapted wild-type *Xenopus* tadpoles with 25 μM cytochalasin D, a concentration that can depolymerize microfilaments. Figure 2 shows that in untreated animals (Fig. 2A–C), arrestin localized to the RIS and axoneme in dark-adapted animals, translocates to the ROS after a 45-minute light adaptation, and then returned to the RIS and axoneme during dark adaptation (120 minutes) after the light-adaptation period. In animals that were treated with 25 μM cytochalasin D (Figs. 2D–F), the arrestin translocation was qualitatively indistinguishable from the untreated tadpoles. Quantitation of the fraction of total immunofluorescence in the ROS showed that the arrestin translocation was also significantly identical (P > 0.1) in treated and untreated tadpoles (Fig. 2G).

Translocation of transducin was tested in these same animals, immunostaining for the α-subunit of transducin (Fig. 3). In untreated tadpoles (Figs. 3A–C), transducin localized to the ROS of dark-adapted animals. After a 45-minute light exposure, transducin was visible, filling the RIS. If the animals were subsequently returned to the dark, transducin returned to the ROS, largely evacuating the RIS. In tadpoles treated with cytochalasin D (Figs. 3D, 3F), the localization of transducin was indistinguishable from untreated animals during overnight dark adaptation and 45-minute light adaptation. However, in tadpoles that were returned to the dark after a period of light adaptation, the translocation of transducin from RIS to ROS was significantly slowed (Fig. 3F). Quantitation of the transducin immunofluorescence shows that this difference is statistically significant (P < 0.01; Fig. 3G).

In an effort to rule out any nonspecific effects of cytochalasin D on rod photoreceptors, we used a second unrelated reagent, latrunculin B, which also targets microfilaments. This poison, isolated from sponges, has a different mode of action, blocking the nucleotide-binding site of actin.14,15 Like cytochalasin D, latrunculin B had no effect on arrestin translocation (Fig. 2H), but significantly slowed the return of transducin to the ROS during dark adaptation after light adaptation (Fig. 3H). The confluence of the results from these two disparate pharmacologic reagents strongly suggests an involvement of the microfilaments in the translocation of transducin, but only in the movement of transducin from the RIS to the ROS. At the same time, the lack of an effect on arrestin translocation suggests that arrestin does not appear to use microfilaments in its light-driven movement.

To demonstrate that application of cytochalasin D in the tank water of the tadpoles is an effective method for supplying the reagent, we immunostained retinal sections with anti-actin, to reveal the microfilaments that surround the ROS. Figure 4 shows that the microfilaments that are normally present in the calycal processes that surround the ROS are absent in the cytochalasin D-treated tadpoles.

### Role of Microtubules

Because the microfilament poisons used in the study were ineffective at perturbing arrestin translocation, we next tested whether there is participation of microtubules in arrestin and transducin translocation. Because rod microtubules contain acetylated tubulin, tadpoles were treated with thiabendazole, a benzamidole-class microtubule poison that can depolymerize acetylated microtubules.16,17 For these experiments, transgenic tadpoles expressing a fusion of GFP at the C terminus of arrestin were used. The addition of GFP to the C terminus of arrestin has been previously demonstrated not to affect arrestin’s affinity for light-activated phosphorylated rhodopsin nor to affect the translocation of the fusion protein,18 and those results were recapitulated in this study (Figs. 5A–C). In transgenic tadpoles treated with 500 μM thiabendazole (Figs. 5D, 5F), arrestin translocated from RIS to ROS in response to light, but was significantly slowed (Fig. 5F). Quantitation of the arrestin immunofluorescence shows that this difference is statistically significant (P < 0.01; Fig. 5G).

For quantitation along the length of a photoreceptor, a narrow rectangle was drawn that spanned the length of the photoreceptor, and the average fluorescence intensity was measured along this rectangle (Fig. 1). This measurement was performed on a minimum of two photoreceptors per image collected from each eye of three animals.

### Results

#### Role of Microfilaments

To test the potential involvement of microfilaments in the translocation of arrestin and transducin, we treated dark-
but was significantly slowed in its return from the ROS to the RIS during dark adaptation after exposure to light (compare Fig. 5C with 5F). This difference is quantitatively shown in Figure 5G. In addition to this major effect, it was also noted that the distribution of arrestin in the ROS during light adaptation in the treated tadpoles was different, having a much higher basal accumulation of arrestin than the more uniform distribution of arrestin in untreated control specimens (compare Fig. 5B with 5E). This difference was readily revealed by quantifying the fluorescence emission from GFP along the length of the rod (Fig. 5H), showing a strong basal-to-apical gradient for the thiabendazole-treated animals compared with a more homogeneous distribution in the ROS for untreated animals. Although not conclusive, this pharmacologic study suggests that arrestin translocation utilizes microtubules, particularly in the movement of protein from ROS to RIS during DA, but also in the more rapid dispersal of arrestin throughout the ROS during light adaptation.

Thiabendazole also affects transducin translocation. In tadpoles treated with thiabendazole (Fig. 6), transducin movement from ROS to RIS during light adaptation was distinctly slowed (compare Fig. 6B with 6E). In the untreated tadpole, approximately 70% of the transducin moved to the RIS, whereas only 40% of the transducin in thiabendazole-treated animals translocated during this same 50-minute light adaptation (Fig. 6G). The difference during light adaptation is statistically significant ($P < 0.01$). The distribution of transducin in treated tadpoles was indistinguishable from that in untreated animals under the other lighting conditions.

Effective penetration of the thiabendazole and an effect on the microtubules are shown in Figure 7. In untreated tadpoles, anti-acetylated tubulin immunostaining revealed the axoneme in the ROS and an obvious network of tubules in the RIS. Exposing the tadpoles to thiabendazole resulted in a clear loss of organization in the RIS microtubules (Fig. 7B), and a more diffuse appearance of the axoneme.

Translocation Domain in Arrestin

The results from previous studies showing that arrestin movement is independent from binding to light-activated phosphor-hodopsin$^{6,7}$ and our results indicating an involvement of microtubules in the translocation of arrestin suggest that arrestin may couple to one or more components that actively translocate arrestin. In an effort to identify what portion of arrestin might be coupling to the translocation machinery, we per-
formed serial truncations on the arrestin C terminus at 10-amino-acid intervals, fusing GFP with the new C terminus of the truncated protein. Our rationale for starting at the C terminus was based on a study of the immunolocalization of a splice variant of arrestin, p44. This splice variant is identical with full-length arrestin, except that the C-terminal 35 amino acids are replaced by a single alanine. Despite this similarity, p44 localizes to the ROS in dark-adapted bovine retinas, whereas full-length arrestin is in the RIS.\textsuperscript{18}

In Figure 8 we show the effects of removing 10, 20, or 30-amino acids from the C terminus of arrestin in our Ar-GFP fusion. Unlike the previous experiments, after the initial 60-minute light adaptation, tadpoles were kept in the light for an additional 3 hours (4 hours total light adaptation). In Xenopus this lighting condition also promoted translocation of arrestin from ROS to RIS, similar to dark adaptation, as previously documented\textsuperscript{8} and as demonstrated in Figure 8C. Removing 10 amino acids from the C terminus of arrestin had no effect on the translocation of arrestin for any of the three light conditions (compare Figs. 8A–C with 8D–F). Removing 20 amino acids also had no statistically significant effect on the translocation in either direction, although there appeared to be a

**Figure 3.** Effect of microfilament poisons on transducin translocation. Transducin (blue channel) was immunolocalized in wild-type tadpoles that were untreated (A–C) or treated with 25 μM cytochalasin D for 8.75 hours (D–F). Dark and light adaptation and lighting conditions are signified as in Figure 2. Fluorescence intensity in the outer segments was quantified for each group of animals (G). Tadpoles were also treated with latrunculin B, and fluorescence intensity quantified (H). Error bars represent SEM for 12 retinal sections taken from three tadpoles for each time point. Nuclei are stained with green fluorescent reagent (green channel; Sytox green; Molecular Probes, Eugene, OR). (A, inset) Fluorescence staining in the absence of the primary anti-transducin antibody. Scale bar, 20 μm.
trend toward a slowed return of the truncated Ar-GFP to the RIS during DA (Figs. 8G–I). The loss of 30 amino acids has a dramatic effect on the localization of arrestin (Figs. 8J–L). In the dark-adapted tadpole, Ar(c-30)-GFP is proportionally more localized to the ROS than the RIS. There is some translocation of this truncated arrestin in response to light, but the translocation of this shortened arrestin is considerably slowed, if not halted, in its return to the RIS during extended light adaptation. Replicates of these qualitative observations are also shown quantitatively (Fig. 8M).

In addition, in the truncated arrestin lacking 30 amino acids, during the initial light adaptation, the distribution of arrestin throughout the ROS was not as complete as with the full-length arrestin, showing a strong basal-to-apical gradient (Fig. 8K). When fluorescent intensity was plotted as a function of rod length (Fig. 8N), this basal concentration was clearly evident. This distribution is very similar to the basal-to-apical gradient obtained from treating the tadpoles with the microtubular poison thiabendazole.

Our results indicate that the C terminus of arrestin either contains an element that directly couples arrestin to a light-regulated translocation element, or the C terminus intramolecularly regulates the coupling of arrestin. To determine whether the C terminus is sufficient for translocation, the C-terminal 45 amino acids of arrestin were fused to the N terminus of GFP (C45-GFP), and protein translocation was assessed in vivo in transgenic *Xenopus*. In this fusion construct, C45-GFP was more highly concentrated in the RIS in...
DA tadpoles, similar to Ar-GFP (Figs. 9A, 9D). In response to light adaptation for 1 hour, C45-GFP partially redistributed to the ROS, similar to Ar-GFP, although to a smaller extent (Figs. 9B, 9E). During extended light adaptation (4-hour light), Ar-GFP returned to the RIS, whereas the C45-GFP remained in the ROS (Figs. 9C, 9F). In contrast, in tadpoles expressing GFP alone, there was no change in GFP distribution in response to the various lighting conditions (Figs. 9G–I). These results clearly show that the C-terminal 45 amino acids of arrestin allow GFP to concentrate in the RIS during dark adaptation and that this localization changes in response to light adaptation, allowing GFP to move into the outer segments.

**DISCUSSION**

**Cytoskeletal Elements and Translocation**

The results from the pharmacologic treatment of tadpoles indicate an involvement of the cytoskeleton in the light-driven translocation of arrestin and transducin in rod photoreceptors. In tadpoles that were treated with cytochalasin D, there was a specific slowing of the translocation of transducin during light adaptation from the RIS to the ROS, with no impact on the translocation of arrestin. The fact that arrestin moved normally both to and from the ROS and that transducin moved normally from the ROS, suggest the effect on transducin translocation is not the result of nonspecific clogging of the connecting cilium through which these proteins are moving. Furthermore, treatment of tadpoles with latrunculin B, an agent that inhibits actin polymerization, had essentially identical effects as treatment with cytochalasin D (Figs. 9G–I). These results clearly show that the C-terminal 45 amino acids of arrestin allow GFP to concentrate in the RIS during dark adaptation and that this localization changes in response to light adaptation, allowing GFP to move into the outer segments.
transducin also shows a light-dependent association with phosducin along the length of the rod, and with centrin in the connecting cilium. How all these pieces fit together to regulate transducin translocation remains unclear.

Treatment of tadpoles with thiabendazole, a benzamidole-class reagent with demonstrated effects on acetylated microtubules, also impacted light-driven protein translocation. Most significantly, thiabendazole treatment dramatically slowed the return of arrestin to the RIS from the ROS during dark adaptation (Fig. 5F). A slowing of transducin movement in the same direction in response to light adaptation was also observed. As before, the persistence of normal arrestin and transducin translocation from the RIS to the ROS indicates that the effects on protein translocation of treating the tadpoles with thiabendazole is not a consequence of nonspecific clogging of the connecting cilium. Of note, in thiabendazole-treated tadpoles, arrestin quantitatively translocated to the ROS in response to light, but there was a slower dispersion of arrestin in the ROS (Fig. 5H). This strong basal-to-apical gradient suggests that two separate processes are involved—a thiabendazole-insensitive movement of arrestin from the RIS to the ROS—followed by a thiabendazole-sensitive mechanism that facilitates a more rapid dispersion of arrestin throughout the outer segments.

In rod photoreceptors, microtubules are abundant in both the inner and outer segments. In the RIS, the microtubules are oriented with their plus end toward the endoplasmic reticulum/Golgi complex and their minus ends near the base of the connecting cilium. In the ROS, most of the microtubules are part of the ciliary structure, highly organized as a ring of nine microtubule doublets with their plus ends oriented toward the distal end of the outer segments. Consequently, movement of arrestin from ROS to RIS during dark adaptation, or of transducin from RIS to ROS during light adaptation, presumably involves a minus direction dynein-like motor. In addition to the axonemal microtubules, there are also microtubules running longitudinally that are associated with the incisures along the rim of the outer segments. It is tempting to speculate that these microtubules may have a role in facilitating the spread of arrestin throughout the ROS during light adaptation, since thiabendazole treatment leads to a slower dispersion of arrestin in the light-adapted ROS than occurs in untreated control animals.

Cytoskeletal Involvement in Translocation

Our results fit well with recent findings that indicate the rates of translocation for transducin and arrestin are quite different, both during dark adaptation and light adaptation. In this study, transducin moves quickly to the RIS in response to light (approximately 2 minutes to completely move from ROS to RIS), but then takes nearly 200 minutes to return to the ROS during dark adaptation. In contrast, arrestin returns to the RIS during 25 minutes of dark adaptation. These different rates are consistent with our results showing an association of transducin with microfilaments and an association of arrestin with microtubules during dark adaptation movements, thus predicting different rates of movements for the two proteins.

We cannot exclude the possibility that treating the tadpoles with the cytoskeletal poisons resulted in nonspecific effects on arrestin and transducin translocation. However, the slowing of transit of the connecting cilium.24 How all these pieces fit together to regulate transducin translocation remains unclear.

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We cannot exclude the possibility that treating the tadpoles with the cytoskeletal poisons resulted in nonspecific effects on arrestin and transducin translocation. However, the slowing of translocation from the outer to the inner segments was dramatically slowed (Fig. 8). It is important to note that by removing these 30 amino acids, we have not simply created a nonfunctioning aggregate, since the distribution of this misfolded protein would more likely reflect the available cytoplasmic volume, which is approximately 60% RIS/40% ROS. Instead, the truncated arrestin has 60% of the fusion localized to the outer segments in the dark-adapted photoreceptors. This truncated arrestin retains some light-driven translocation potential, although the initial translocation of Ar(c-30)-GFP to the outer segments in response to light adaptation was not as complete as the full-length arrestin, and was actually much more reminiscent of the thiabendazole-treated tadpoles with a strong basal to apical gradient of localization (Figs. 8K, 8N). This result can be interpreted in two ways. It could implicate the C-terminal 30 amino acids as containing at least a portion of the translocation domain. Alternatively, the removal of the C terminus could induce a conformational change in arrestin that prevents the protein from docking efficiently with the translocation machinery. Regardless of the interpretation, it appears that by removing the C terminus of arrestin we affected the interaction of arrestin with the translocation machinery at a similar point as did treatment with thiabendazole.

Our subsequent study using a fusion of the C-terminal 45 amino acids of arrestin with GFP offers evidence that the translocation domain is at the C terminus of arrestin. In this fusion, the C45-GFP protein was localized almost exclusively to the inner segments in dark-adapted tadpoles, and showed significant translocation to the outer segments during light adaptation. The simplest interpretation of these results is that the C-terminal 45 amino acids of arrestin provide a tether that prevents the protein from docking efficiently with the translocation machinery. Regardless of the interpretation, it appears that by removing the C terminus of arrestin we affected the interaction of arrestin with the translocation machinery at a similar point as did treatment with thiabendazole.
couples to an active motor-driven process. Regardless of the interpretation, the translocation process obviously occurs much more efficiently in the context of the entire arrestin molecule, suggesting that either the conformation of the 45-amino-acid domain may be different in the whole protein, or that there may be additional portions of the arrestin protein that contribute to this “translocation domain.” Further investigations will help refine this domain more precisely. It should be noted that we chose 45 amino acids to use the first ATG codon upstream of the implicated 30-amino-acid C terminus and to provide extra amino acids to allow the peptide to adopt a more native conformation.

The Process of Light-Stimulated Translocation

Is light-stimulated translocation a motor-driven process? The mechanism of arrestin translocation, whether it is diffusion based with different binding partners in the ROS and RIS or a motor-assisted process, remains unresolved. Originally, light-stimulated translocation of arrestin was hypothesized to be a passive process, with arrestin localizing to the outer segments in light-adapted eyes based on its affinity for activated, phosphorylated rhodopsin (R*P). This hypothesis was brought into question by studies using transgenic mice in which the rhodopsin could not be phosphorylated, thus precluding the formation of arrestin's high-affinity binding partner, yet arrestin still translocated normally. However, a recent study notes that arrestin retains a moderate affinity for activated rhodopsin that is not phosphorylated (R*) and presents evidence that this form of rhodopsin is sufficient to provide a binding sink for arrestin in light-adapted outer segments. Further, this study analyzed the rate of diffusion of GFP in rods, showing that equilibrium could be reached between the inner and outer segments in less than 3 minutes, thus concluding that the light-driven translocation of arrestin could be explained by arrestin’s affinity for R* and R*P in the outer segments of light adapted rods, and by its affinity for microtubules in the inner segments of dark-adapted rods.

Our results can be partially fit into this proposed model. We show a significant reduction in the return of arrestin to the inner segments in the presence of thiabendazole which depolymerizes the inner segment microtubules (see Fig. 7). Further, the localization of GFP tagged with the C-terminal 45 amino acids of arrestin to the inner segments of dark-adapted rods could be explained if the C terminus of arrestin, a strongly acidic region, is responsible for conferring an affinity for microtubules. However, the two-sink hypothesis does not completely explain all aspects of our data. For example, our results show that thiabendazole treatment has two effects on arrestin translocation, not only slowing the movement of arrestin to the inner segments during dark adaptation, but also slowing the dispersion of arrestin throughout the outer segments during light adaptation (see Figs. 5E, 5H). Perturbing the microtubules should have no effect on the translocation during light adaptation if arrestin were simply binding to R* or R*P. Similarly, arrestins that are truncated by approximately 30 amino acids also disperse more slowly in light-adapted outer segments (Fig. 8K and Ref. 28). A diffusion-mediated process should not be affected by these truncations. Finally, both our results and

![Figure 8](http://tvst.arvojournals.org/ on 01/21/2018)
The role of protein translocation in photoreceptors is currently undefined. One hypothesis forwarded is a function in phototransduction amplification by the lowering of transducin concentration in the ROS and by shortening the lifetime of activated rhodopsin via an increase in arrestin concentration. Correlative studies support this hypothesis, showing that transducin’s decline in the outer segments parallels the decline in rod signal amplification. Further, disruption of arrestin translocation in Drosophila causes defects in the light adaptation process. Clearly, further work is needed to define conclusively the function of translocation in the photobiology of the retina. Future research designed to identify how arrestin and transducin couple to the translocation machinery will provide a tool enabling us to dissect the role of the proteins’ movements away from their established functions in phototransduction activation and inactivation.

CONCLUSIONS

The role of protein translocation in photoreceptors is currently undefined. One hypothesis forwarded is a function in photoreceptor desensitization. This reduction in rod response during a background illumination would be via a decrease in phototransduction amplification by the lowering of transducin concentration in the ROS and by shortening the lifetime of activated rhodopsin via an increase in arrestin concentration. Correlative studies support this hypothesis, showing that transducin’s decline in the outer segments parallels the decline in rod signal amplification. Further, disruption of arrestin translocation in Drosophila causes defects in the light adaptation process. Clearly, further work is needed to define conclusively the function of translocation in the photobiology of the retina. Future research designed to identify how arrestin and transducin couple to the translocation machinery will provide a tool enabling us to dissect the role of the proteins’ movements away from their established functions in phototransduction activation and inactivation.

REFERENCES


