Molecular Vision 2004; 10:672-81 <http://www.molvis.org/molvis/v10/a81>
Received 24 May 2004 | Accepted 12 September 2004 | Published 15 September 2004

Temporal kinetics of the light/dark translocation and compartmentation of arrestin and α-transducin in mouse photoreceptor cells

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Purpose: To determine the temporal kinetics of the simultaneous translocation of arrestin and rod α-transducin in mice exposed to different lighting environments and to compare the subcellular compartmentation of cone α-transducin with arrestin.
Methods: Double labeling immunofluorescence microscopy and image analysis are used to visualize and quantify the concentrations of rod arrestin and α-transducin in the subcellular compartments of the rod outer segments, the rod inner segments and the synaptic terminals.
Results: The magnitude of the effects of the translocation are clearly contrasted in images of the retinas of animals that have been maximally light adapted verses retinas that have been maximally dark adapted. The onset of light results in a rapid, simultaneous, translocation of arrestin and α-transducin from their respective compartments (α-transducin in the rod outer segment and arrestin in the rod inner segment) to the opposite compartment. Almost all of α-transducin has translocated in less than two min whereas the translocation of the majority of arrestin requires at least five to six min. Translocation in the opposite direction, from light to dark, occurs more slowly for both proteins with arrestin requiring almost 30 min and α-T needing more than 200 min to complete its journey. Under the same lighting conditions, cone arrestin translocation is incomplete. Cone α-transducin does not translocate under any the lighting conditions tested. Unlike the frog, continuous exposure of mice to light does not result in arrestin translocating back to the rod inner segment.
Conclusions: These data suggest that there are four mechanisms involved in the translocation of these two proteins. They also support the conclusion that the more important cellular function of the translocation process is to terminate phototransduction in rod and cone photoreceptors, which could provide protection against light damage. The secondary function of translocation is to maximize rod sensitivity to light during dark adaptation. The restricted localization of cone α-transducin to the cone outer segment is consistent with the function of cones in bright light, just as the concentration of rod α-transducin in dark adapted rod outer segment is consistent with their functioning in dim light.

Mammalian rod and cone photoreceptor cells have evolved unique, exquisite morphological features that are optimized for the detection of light. In general, the cells have a polarized organization in which the light absorbing molecules are concentrated in an "outer segment" at one end of the cell and are separated from the synaptic connections at the opposite end by a nucleus and an "inner segment" which houses the energy producing and protein synthesizing components. All of the protein molecules destined for the rod outer segment (ROS) must pass through a narrow "connecting cilium" between the ROS and the rod inner segment (RIS). It was previously thought that this was a one way conduit that resulted in the polarized distribution of rhodopsin and other proteins into the ROS. However, our lab [1-4] and a handful of others [5-9] demonstrated that the cilium was a connection through which some phototransduction molecules passed in a bidirectional manner. For arrestin and α-transducin (α-T), the direction of this translocation was reversible and dependent on the lighting environment to which the animal was exposed. Because they are localized in opposite compartments (ROS and RIS) under either light or dark conditions, they were always moving in opposite directions when the lighting changed.

Although single-labeled immunocytochemical images from retinas of animals exposed to light or dark have been previously presented to provide a qualitative perspective on the time course of translocation of arrestin alone [1,3,4] or α-T alone [7,10], some recent studies have presented semiquantitative data on the time course of translocation of either α-T in the rat retina [11] or arrestin in the frog retina [12]. In the rat, densitometric analysis of immunoblots of serial sections of the retina were used to investigate the time course of translocation of α-T following the change in the lighting environment whereas densitometry, fluorescence, and immunocytochemistry were used to determine the amount of arrestin and arrestin-green fluorescent protein in frog rods at selected times after the change in the lighting environment.

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The temporal changes in the concentrations of these proteins in these two species were significantly different and included the surprising finding in the frog that prolonged exposure to light resulted in arrestin moving back into the RIS where it resides in the dark. Because the light dependent translocation of these proteins is a significant process for light adaptation in the retina and because the mechanism by which this protein trafficking occurs is important for rods, cones and possibly other neurons, the analysis of the time course of the simultaneous translocation of these proteins in the mouse retina was undertaken. The results demonstrated that the fastest movement of arrestin and α-T occurs within minutes following the onset of light, with α-T relocalizing within 2 min from the ROS to the RIS and arrestin requiring about 4-5 min to relocalize in the opposite compartment (RIS to ROS). The onset of darkness results in the translocation in the opposite direction with arrestin requiring about 20 min and α-T needing more than 200 min. Based on these temporal differences, our data suggest that maximizing the "shut down" of phototransduction is much more important than maximizing the sensitivity of rods for the detection of light. This in turn suggests that the major function of these translocations is to provide protection of rods and cones in bright light rather than increased vision in dim light.

METHODS

Animals used in these studies were cared for and handled according to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Vision and Ophthalmic Research and with IACUC approved animal use protocols that comply with the University of Oklahoma Faculty of Medicine Guidelines for use of Animals in Research. Albino Balb/cJ mice were obtained from Jackson Laboratory and maintained in a 12 h light/dark cycle in the DMEI animal facilities. For dark to light experiments, the mice were dark-adapted for at least 4 h in a dark room and then exposed to 600 Lux of light for selected times, from a variable intensity light source using fiber optic light pipes. For light to dark experiments, the mice were exposed to 2000 Lux of light in a "light box" for 60 min prior to being put into a darkroom. Dim red light, which does not initiate translocation of arrestin or α-T was used to visualize the mice in the dark room. For "dark to light" experiments prior to light exposure, animals were anesthetized by an intramuscular injection of 0.03 ml of a mixture of xylazine and ketamine in the ratio of 1:5. A drop of 1% tropicamide was applied to each eye to dilate the pupils. The mice were killed by cervical dislocation, the eyes removed and immersed in Perfix fixative (Fisher, St. Louis, MO) for 4 h 15 min at room temperature and then kept overnight in 70% ethanol. Subsequently, the cornea and lens were removed and the whole eyecups were embedded in paraffin by routine histological procedures. Sections (5μm) were mounted on glass slides and rehydrated in 0.5 M Tris buffer, pH 7.6 prior to incubation with antibodies. All sections were examined using a Nikon Eclipse 800 microscope. Images were captured with a digital camera and analyzed us-

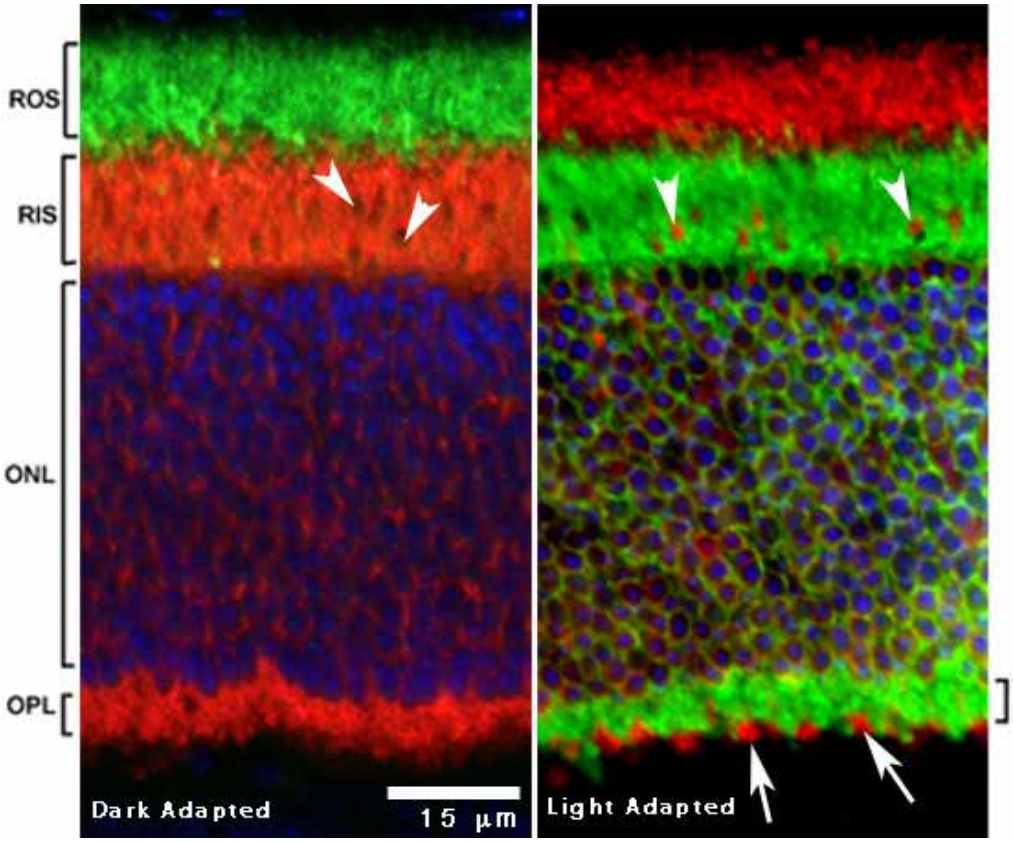

Figure 1. Light-dependent translocation of arrestin and rod α-T. Immunocytochemical localizations of arrestin and rod α-T in dark and light adapted albino mouse retinas were conducted. In the dark, arrestin (red) is localized in the RIS, ONL, and OPL whereas α-T (green) is localized in the ROS. The localizations are reversed in the light adapted retinas with the arrestin localized to the ROS and α-T being in the RIS, ONL, and OPL. Cone inner segments show up as red streaks (arrow heads) in the region of the RIS in the light adapted retina and the cone terminals appear as red spots (arrows) in the OPL. Nuclei are stained with DAPI. The rod outer segment is indicated by ROS, the rod inner segment is indicated by RIS, the outer nuclear layer is indicated by ONL, and the outer plexiform layer is indicated by OPL. The scale bar represents 15 μm.

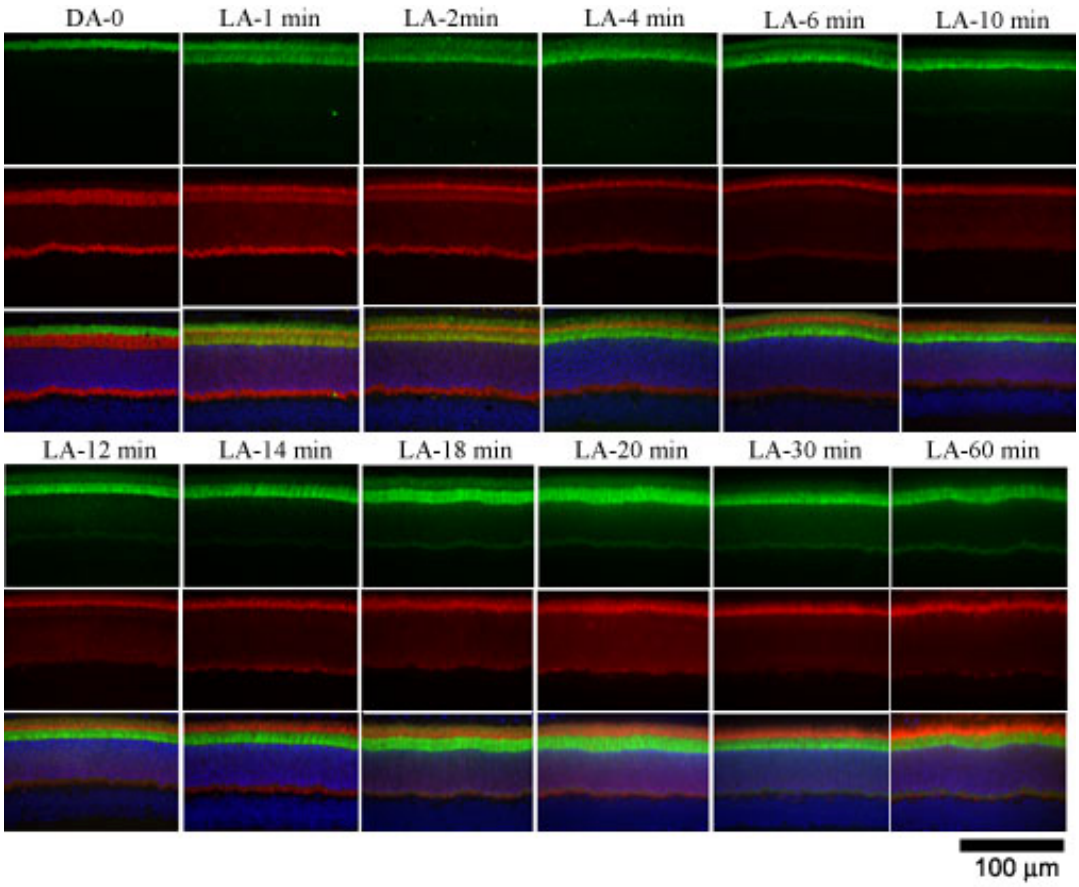

Figure 2. Dark to light temporal translocation of arrestin and rod α-T. Translocation of Arrestin and α-T in albino mouse retinal sections in response to exposure to 600 lux of light for indicated times. The images for each time point are of the same section viewed with a filter to detect Transducin (green, top row), arrestin (red, middle row) or a triple filter for Transducin, arrestin and DAPI stained nuclei (blue, bottom row). The majority of the α-T (green) has translocated from the ROS to the RIS within 2 min of light exposure. Arrestin (red) is seen to initially concentrate as a band at the base of the ROS followed by almost complete translocation from RIS to ROS within 6-8 min of light exposure. The scale bar represents 100 µm.

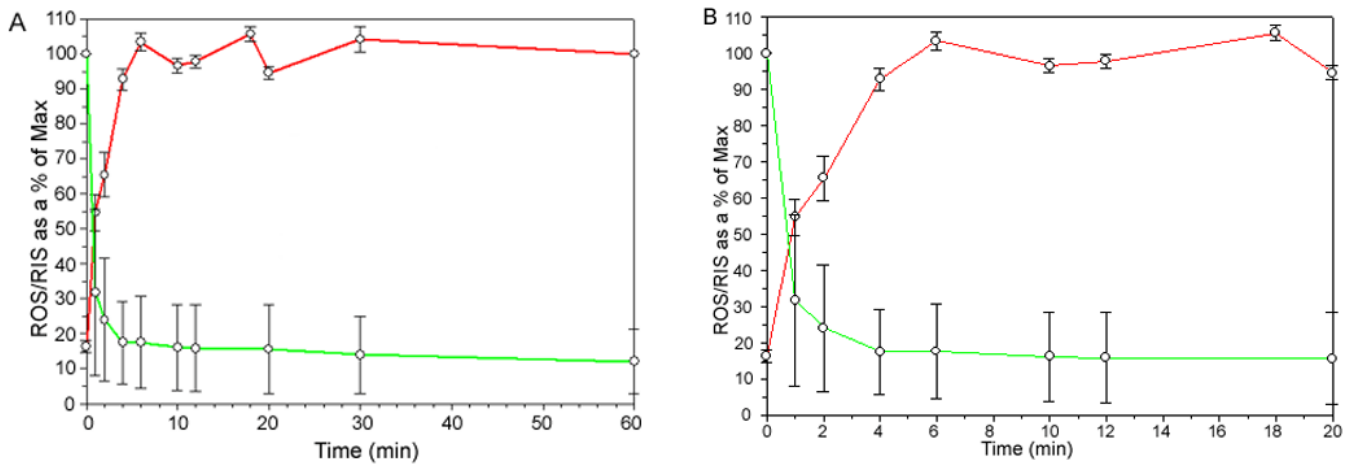

Figure 3. Temporal kinetics of dark to light translocation of arrestin and rod α-T. **A**: Temporal kinetics of dark to light translocation of arrestin and rod α-T. Grayscale immunofluorescent images were quantified by scanning densitometry using Metamorph Image Analysis software and the ROS/RIS ratios determined. The Arrestin graph in red indicates ROS/RIS as a percentage of ROS/RIS at 60 min versus the specified time. The Transducin graph in green indicates ROS/RIS as a percentage of ROS/RIS at 0 min versus the specified time. Each point in the time course experiments is from a single animal and represents the average values (the error bars represent the standard deviation) from three different fields within a section. These data are representative of those obtained from three separate experiments. **B**: The same data are shown on an expanded time scale. The ROS/RIS ratio for α-T reaches its minimum value by 1.5 min whereas arrestin reaches its maximum value in about 4.5 min.

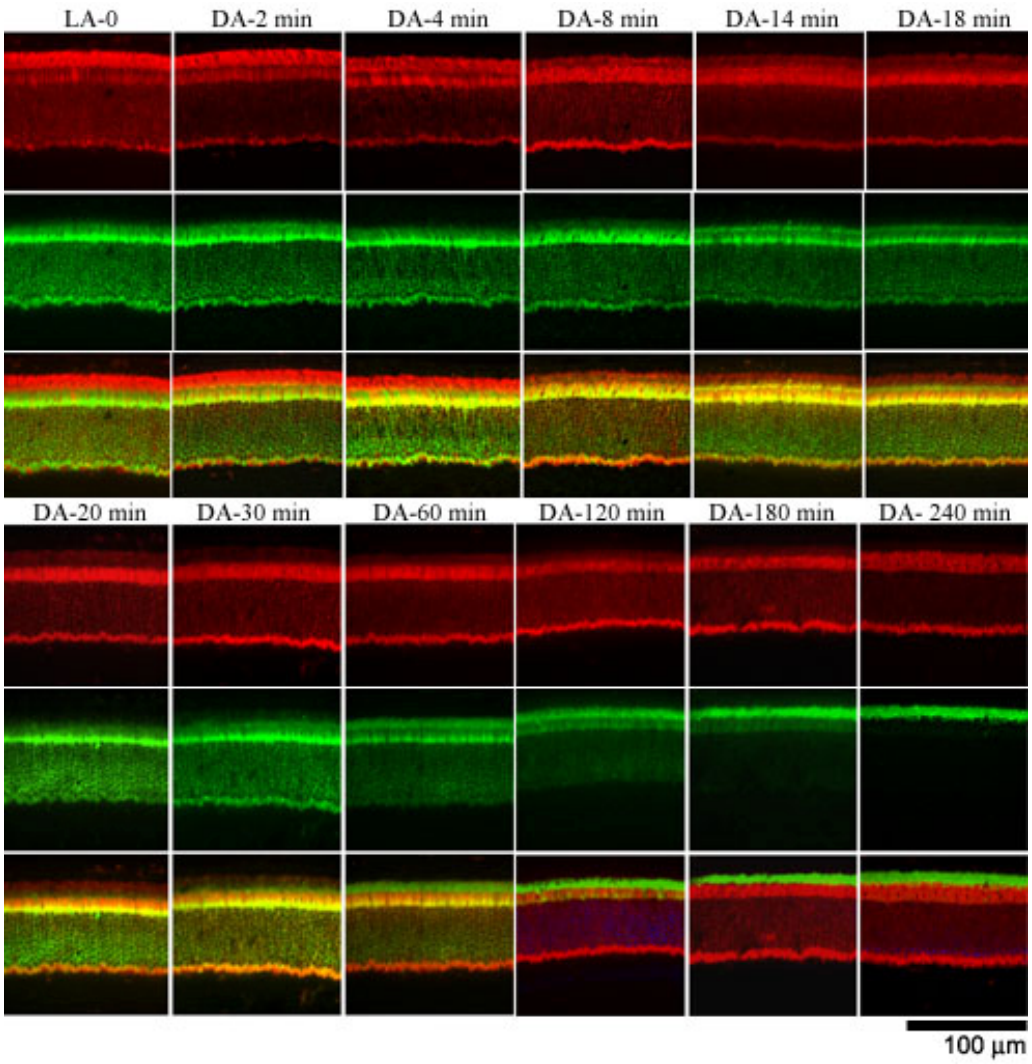

Figure 4. Light to dark temporal translocation of arrestin and rod α-T. Temporal translocation of Arrestin and rod α-T in the albino mouse retina in response to dark adaptation for times of 0, 2, 4, 8, 14, 18, 20, 30, 60, 120, 180, and 240 min was measured. The images for each time point are of the same section viewed with a filter to detect Transducin (green, top row), arrestin (red, middle row) or a triple filter for Transducin, arrestin and DAPI stained nuclei (blue, bottom row). Maximal translocation of arrestin (red) from ROS to RIS occurs within 30 min in the dark whereas α-T (green) takes more than 200 min to translocate from RIS to ROS. The scale bar represents 100 µm.

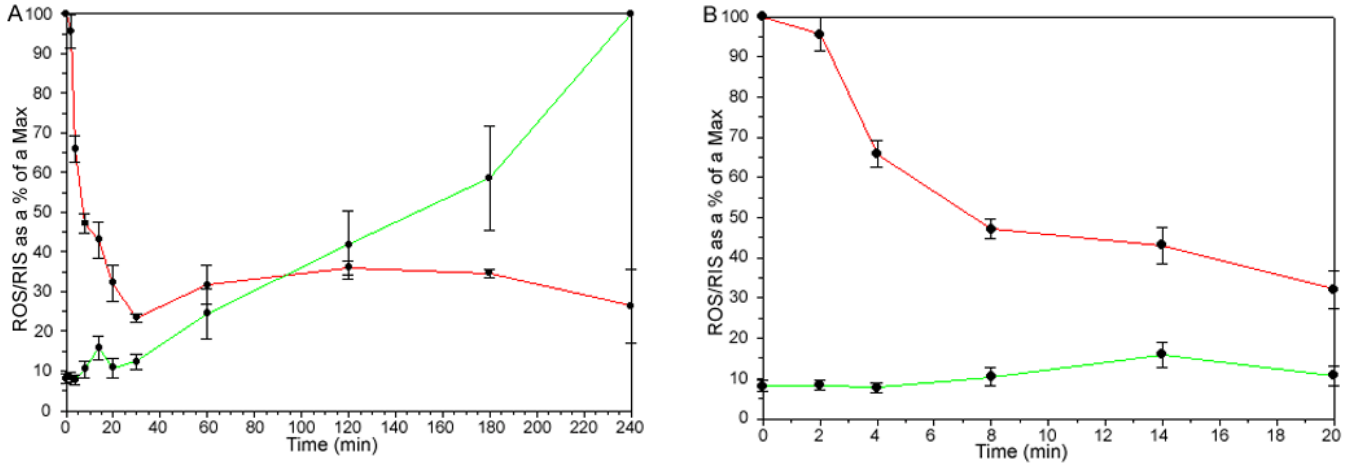

Figure 5. The time course of light to dark translocation of arrestin and α-T. **A**: Temporal kinetics of light to dark translocation of arrestin and rod α-T are shown. The Arrestin graph in red indicates ROS/RIS as a percentage of ROS/RIS at 60 min versus the specified time. The Transducin graph in green indicates ROS/RIS as a percentage of ROS/RIS at 0 min versus the specified time. Each point in the time course experiments is from a single animal and represents the average values (the error bars represent the stardard deviation) from three different fields within a section. These data are representative of those obtained from three separate experiments. **B**: The same data are shown on an expanded time scale. Densitometric scanning of grayscale images and calculation of ROS/RIS ratios confirm that maximal compartmentalization of arrestin occurs within 18 min of dark adaptation whereas α-T requires more than 200 min.

ing Metamorph image analysis software (Version 6.0, Downington, PA).

Immunofluorescent staining: Arrestin was localized using MH785, a mouse monoclonal antibody (kindly provided by Dr. Paul Hargrave, Department of Ophthalmology, University of Florida, Gainsville, FL) raised against bovine arrestin that labels mouse rods and cones whereas the localization of rod α-T was determined using a rabbit antibody (catalog number sc-389; Santa Cruz Biotechnology, Santa Cruz, CA) against rod α-T that does not react with cone α-T. In all cases, arrestin antibody (1: 2000) was visualized using (1/1000) goat anti-mouse antibody conjugated with Alexa Red 568 (Molecular Probes, Eugene, OR) whereas rabbit anti-α-T (1/1000) was amplified using biotinylated horse anti-rabbit IgG (1/1000; Vector Labs, Santa Clara, CA) followed by streptavidin conjugated to Alexa Green 350 (1/1000; Molecular Probes). Previously characterized Rabbit anti cone arrestin antibody [13] was generously provided by Dr. Cheryl Craft, Department of Neurobiology, University of Southern California, Los Angeles, CA. All other primary antibodies gave a single band (data not shown) of the correct molecular weight on western blots against mouse retinal homogenates. Hydrated sections were first incubated for 1 h at room temperature with blocking buffer containing 0.1 M Tris, pH 7.4 and 2% powdered milk. Incubations with primary antibodies were performed at 4 °C for at least 12 h and secondary antibodies and streptavidin were incubated for 1 h at room temperature. All antibodies and streptavidin were diluted in "antibody buffer" containing 2% goat serum, 0.1 M Tris HCl, and 0.01% Triton X-100. The relative concentrations of arrestin and α-T in the RIS and ROS were determined using grayscale digital fluorescent images and Metamorph Image Analysis software. Settings were chosen such that the fluorescent signal was not saturated in the most intense fluorescent region of the image and ratio of intensity for the ROS/RIS determined. The junction between the ROS and RIS was visually determined and the Metamorph program was used to select the areas and determine the intensity as described in the directions accompanying the Metamorph program. The ROS to RIS ratios were calculated as described to provide data comparable to those published for the frog [12]. The average of the percent of the maximum ratio is plotted, where the error bars represent the standard deviation (SD). These settings and shutter speeds were fixed for acquiring each image for quantification. The maximum

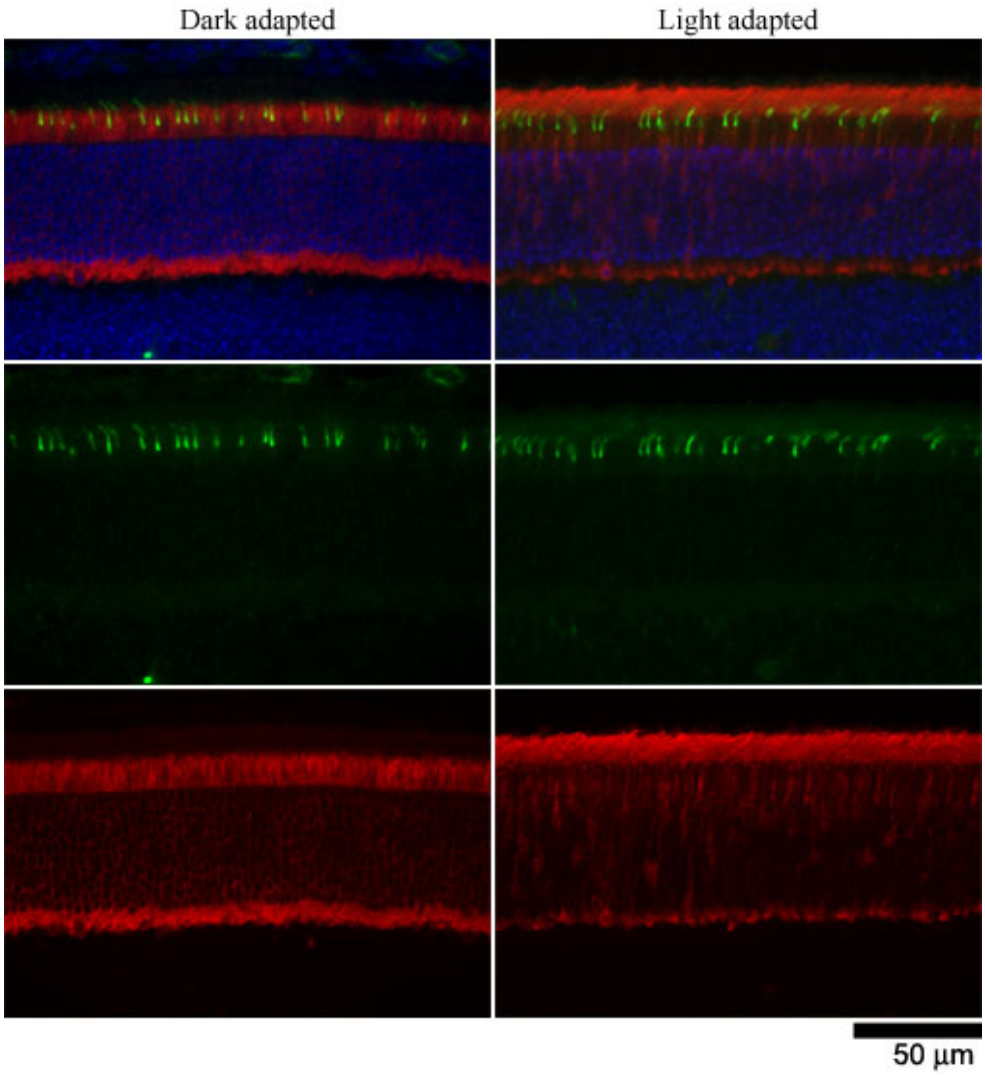

Figure 6. Cone α-T does not translocate. Translocation of Cone α-T does not occur in either light or dark environments. Top row shows double labeling with anti-arrestin (red) and anti-cone α-T (green) antibodies of sections from dark adapted (left) and light-adapted (right) albino mice demonstrating that cone α-T is highly compartmentalized in the COS and does not translocate in light or dark environments. The rod arrestin is seen to be translocated under the same conditions. The same sections viewed with a filter to detect cone α-T alone (green, middle row) and arrestin alone (red, bottom row) are also shown. The scale bar represents 50 µm.

ratio of the staining of arrestin in the ROS/RIS in the completely light-adapted retina was taken as 100% whereas the maximum ratio of staining of α-T in the ROS/RIS in the completely dark-adapted retina was taken as 100%. Each point in the time course experiments is from a single animal and represents the average values (the error bars represent SD) from three different fields within a section. These data are representative of those obtained from three separate experiments.

RESULTS

Light dependent compartmentation: We, and others, had previously examined the transient, light dependent localization of arrestin and α-T in mouse retinas using single labeling techniques with horseradish peroxidase and diamino-benzidine or immunofluorescence. However, this approach required the use of adjacent sections and separate incubations to visualize both proteins. To determine if visualization of arrestin and α-T in the same sections would reveal more information on their distinct compartmentation, sections from the retinas of dark-adapted and from light-adapted albino mice were examined with antibodies detected with two different chromophores, Alexa Red and Alexa Green. Results from such an experiment are seen in Figure 1 and show the exclusive compartmentation of arrestin (Figure 1, red) in the RIS and α-T (Figure 1, green) in the ROS in the dark adapted retina and the equally selective but reversed localizations in the light adapted retinas. Double labeling also reveals the easily visible "red" arrestin positive streaks or spots (Figure 1, arrow heads) in the "green" "α-T-positive RIS region" of the light-adapted retina. It also emphasizes (Figure 1, arrows) the arrestin positive (Figure 1, red) cone synaptic termini that are highlighted by the α-T positive (Figure 1, green) synaptic termini (Figure 1) of the rods. These data support the conclusion that rod α-T moves between the ROS and the RIS but also extend it to demonstrate that rod α-T also translocates into the rod synaptic termini in the light and out of them in the dark. The red streaks or spots within the light adapted RIS (Figure 1) appear to be cone outer segments.

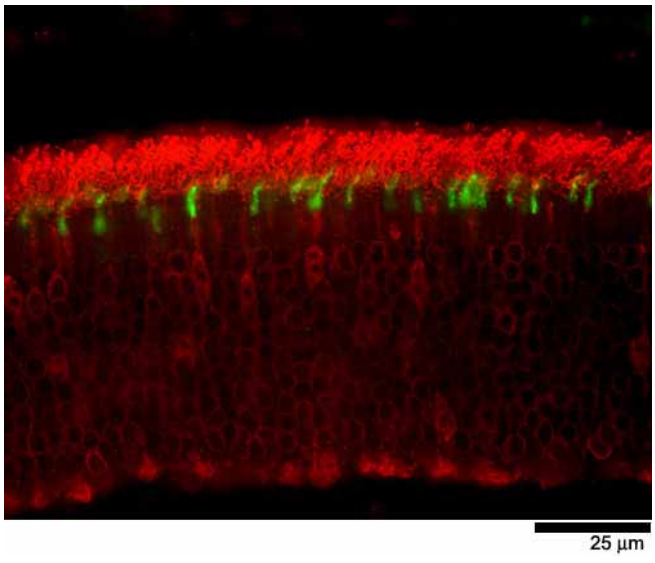

Figure 7. Anti-cone α-T only labels COS. Anti-cone α-T only labels COS. Double labeling of a section of a retina from a light adapted mouse demonstrates the co-labeling of the cones with anti-cone α-T (green) and anti-arrestin (red) whereas rods are only labeled with anti-arrestin. The scale bar represents 25 µm.

Time and light dependent translocation of arrestin and α-transducin in mice: Previous analysis [1,3,4] of arrestin and/or β-Transducin translocations in mouse retina using single labeling had shown that the light dependent translocation of Transducin and arrestin was rapid and occurred over a period of minutes. To simultaneously determine the time course of change in the relative concentrations of arrestin and α-T in the ROS and RIS following the onset of light, dark-adapted albino mice were exposed to 600 Lux of lighting, killed at various times thereafter, and their eyes fixed and processed for immunofluorescence microscopy. Representative sections from such an experiment are shown in Figure 2 and qualitatively demonstrate the simultaneous progressive changes in the compartmentation of arrestin and α-T at various times after the lights were turned on. In the dark adapted retina, almost all of the α-T is in the ROS but even with 1.0 min of light exposure, there is a clear increase in α-T in the RIS and the majority appears to have translocated to the RIS within 2 min. Although arrestin also immediately begins to translocate, it does not move as rapidly as α-T and appears to initially concentrate as a band at the base of the ROS before eventually distributing throughout the ROS. This concentration of arrestin is still seen as a red band when the section is viewed simultaneously with α-T, an observation suggesting either a separate compartment or a very high differential concentration of arrestin versus α-T in this region. To determine if prolonged exposure to light resulted in a reversal of arrestin localization, as had been found in the frog [12], the retinas of mice which had been killed at 1 h intervals from 1 to 12 h after the onset of light were analyzed. At no time during continuous exposure to light (data not shown) did arrestin or α-T reverse their localizations from their normal compartmentation in the light. Prolonged exposure to dark (data not shown) also did not reverse the "dark" localization of either protein.

Densitometric evaluation of the light dependent translocation of arrestin and α-transducin: To evaluate the simultaneous translocation of arrestin and α-T, the grayscale images (from the same section) of immunofluorescence localization of arrestin alone and of α-T alone, were subjected to densitometric analysis using Metamorph Image Analysis software and the ratio of intensity in the ROS/RIS was determined. The results of such an analysis are presented in Figure 3. α-T moves very quickly from the ROS to the RIS and the ratio (ROS/RIS) of its concentration has almost reached the minimum value by 1 to 2 min whereas arrestin translocation, although fast, does not reach it's maximum value until about 4 to 5 min after the onset of light.

Time course of light to dark translocation of arrestin and α-transducin: The reverse change in lighting, from light to dark, results in very different images, Figure 4, which show that arrestin and α-T move much more slowly in the dark and

the images are much more yellow suggesting that both proteins are simultaneously present in the same compartments during their transit to the ROS and the RIS. Arrestin does not appear to be maximally compartmentalized until about 20 min after the mice were put into the dark but α-T requires more than 200 min to maximally compartmentalize. Subjecting the grayscale images of arrestin and α-T localization to the same densitometric analysis (Figure 5) as was done for the dark to light translocation confirms the perception of a slower compartmentation of both arrestin and α-T following the onset of darkness. Arrestin translocates about five times more slowly in the "dark direction" (ROS->RIS) than it does in the light direction (RIS->ROS) whereas α-T compartmentalizes about 100 times more slowly in darkness (RIS->ROS) compared to its less than two min compartmentation in the light direction (ROS->RIS).

Cone α-transducin: To determine if cone α-T also translocates, sections from light-adapted and dark-adapted eyes were double labeled with anti-cone α-T (Figure 6, green) and anti arrestin (Figure 6, red) antibodies. In the dark-adapted retinas (Figure 6), cone transducin is localized to the outer segments of cones, which are seen to occupy the upper half of the thickness of the RIS and extend into the region of the ROS. Double labeling of light-adapted retinas demonstrates the cone transducin positive cone outer segment is continuous with the arrestin positive cone inner segment. When viewed with the red filter only, It can be seen that the cone outer segment actually contains arrestin but the concentration of cone α-T is so high that in the combined images, the cone outer segment look green rather than yellow. To clearly demonstrate the ability of MH785 anti-arrestin to label cones, an enlarged image from a separate light-adapted retina is presented in Figure 7. Therefore, under the same lighting conditions that result in the reciprocal translocation of rod transducin between the RIS in the light and the ROS in the dark, cone α-T does not translocate but remains concentrated in the cone outer segment. Under the lighting conditions used in these experiments, there is always some cone arrestin remaining in the cone inner segment in the light adapted retina.

To independently confirm that the immunostaining of arrestin "streaks" with MH785 in the region of the RIS is actually due to the reaction of the antibody with cone arrestin, a double labeling experiment was performed using MH785 and a rabbit antibody that only reacts with cone arrestin. The results show that the cone structures co-label with both antibodies (Figure 8) and support the identification of the red streaks and synaptic terminals (Figure 1) as cone synapses. These data also demonstrate that cone arrestin does not completely translocate under our lighting conditions.

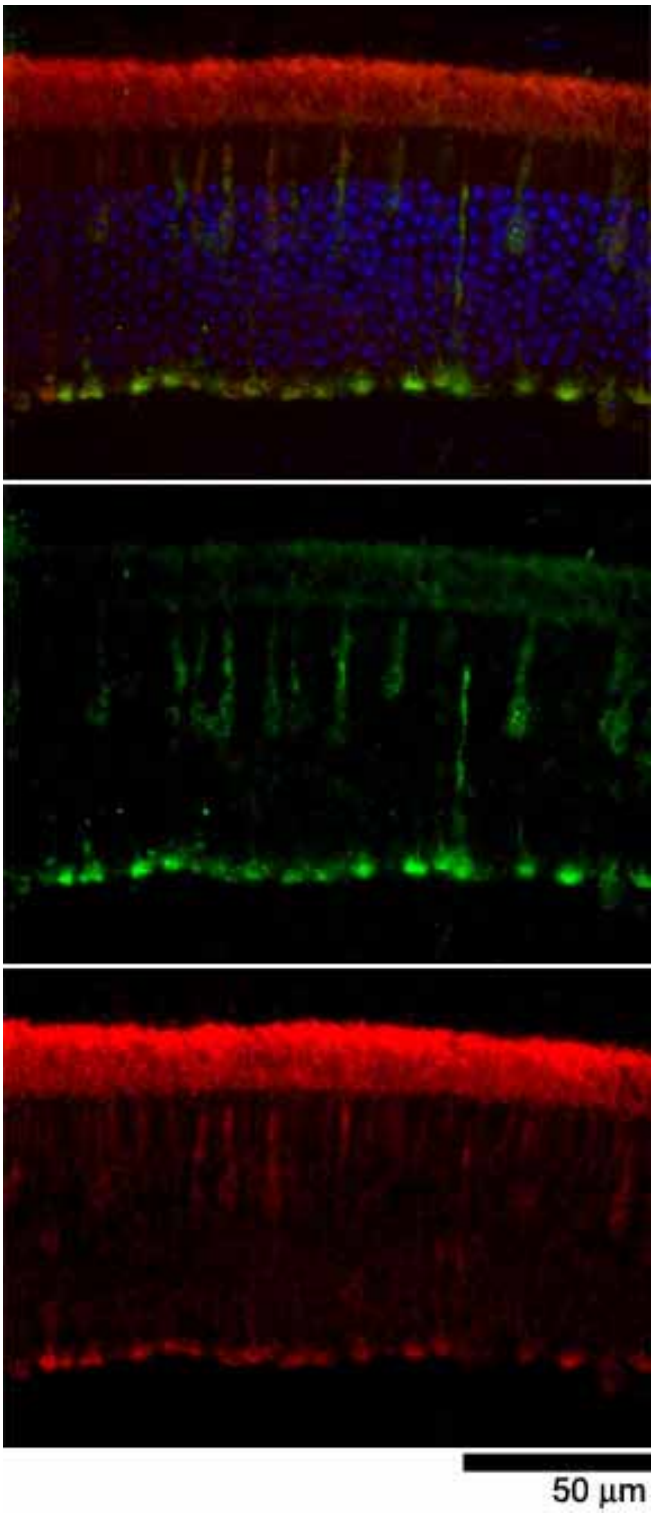

Figure 8. Co-labeling of cones with anti mouse cone arrestin and anti-Arrestin. Co-labeling of cones with anti mouse cone arrestin and anti-Arrestin. Double labeling of a section of a retina from a light-adapted mouse (top panel) demonstrates the co-labeling of cones with rabbit anti mouse cone arrestin (green) and mouse MH785 anti-arrestin (red) and provides independent support of the conclusion that MH785 labels rod and cone arrestin. The same section viewed with a filter to detect mouse cone arrestin alone (middle row) and arrestin alone (bottom row) are also shown. The scale bar represents 50 μm.

DISCUSSION

The mammalian rod photoreceptor cell has a very unique architectural organization that has been optimized for the detection of light. This includes the overall orientation of the rods such that the light absorbing pigment, rhodopsin, is concentrated in separate discs organized in membrane bound stacks (ROS) which point away from the light entering the eye. The

ROS is connected to the RIS by a thin hollow connecting cilium through and along which all proteins destined for the ROS must pass. The vesicular trafficking of proteins has been well studied with rhodopsin being the prototypic example of the polarized subcellular distribution of a membrane protein [14-16]. The demonstration [1-9] that some soluble, phototransduction proteins actually move bidirectionally between the ROS and the RIS, depending on the lighting environment to which the retina is exposed, identified an excellent model system in which to analyze the mechanisms underlying the subcellular translocation of soluble proteins over relatively long distances.

The direct comparison of arrestin and α-transducin localization within the same section of mouse retina produces important correlative data on the unique characteristics of the translocation of these proteins (Figure 1). Densitometric analysis of immunoblots [11] from serial sections of rat retina had indicated that α-T translocated from the ROS to the RIS with a half time of about 5 min. The data from our studies with mice indicate a much shorter transit time with a half time for the light dependent translocation of transducin of about 0.5 min. This difference may simply reflect the speed with which the tissue can be fixed for immunocytochemistry versus dissected, mounted, and frozen for immunoblots, rather than a species or light difference. For rat retina, the half times for the reverse translocation of transducin, light to dark, are similar to our data suggesting that at least 3 h are required. The temporal kinetics for translocation of arrestin have been published only for the frog [12] and were found to be much slower than in the mouse with the half time for dark to light transit being about 25 min and equal or slightly faster from light to dark. For arrestin translocation in the mouse, our data show a half time of about 2 min for dark to light (RIS->ROS) and about 9 min in the light to dark direction (ROS->RIS). Our data also indicate that mouse arrestin does not translocate back into the RIS during prolonged light exposure in contrast to the frog data which show translocation back into the RIS after approximately 90 min of light [12]. These differences in the characteristics of translocation of arrestin most likely reflect the temperature, structural and mechanistic differences between mouse and frog retinas.

Translocation in cones: Cone arrestin has been shown to translocate in a light dependent manner between the cone inner segment and the cone outer segment [13,17] although the extent of translocation is not as complete as that which occurs with rod arrestin. The translocation is in the same direction as rod arrestin such that in the light, cone arrestin is primarily in the outer segments and reverses in the dark. We used anti-cone α-T antibodies to determine the localization of cone α-T in light and in dark-adapted retinas. The immunopositive pattern for cone α-T did not change between light and dark adapted retinas and the synaptic region had barely detectable amounts indicating that cone α-T does not translocate with changes in the lighting environment and that, similar to rhodopsin, it has a restricted, polarized compartmentation within the cone outer segment. That the monoclonal antibody reacts with both rod and cone arrestin is supported by the data seen in Figure 1, Figure 6, Figure 7, and Figure 8. The same lighting conditions which result in translocation of rod arrestin out of the outer plexiform layer (OPL) do not result in the translocation of cone arrestin from the synapses (Figure 8). This may simply reflect a slower mechanism for cone arrestin or a different function for arrestin in cone terminals than in rod terminals.

Hypothetical function of translocation in rods: We had previously postulated that the translocations in rods were functional and consistent with maximizing the ability of rods to detect low levels of light in the dark and shutting down the system in the light. This interpretation is supported by the finding [11] that the amplification of the signaling cascade is directly dependent on the concentration of α-T in the ROS. However, the simultaneous measurement of the amounts of arrestin and α-T in the RIS and the ROS at various times after the onset of light or darkness demonstrates that both arrestin and α-T move faster from one compartment to the other when the lights are turned on than they do when the lights are turned off. Combined with the especially slow translocation of α-T from the RIS to the ROS in darkness, this suggests that an increase in the concentration of arrestin in the ROS, simultaneous with a decrease in the concentration of α-T in the ROS, is more important than changes in the opposite direction. The rapidity of these changes also suggests that the primary function for the translocation is to terminate phototransduction providing protection for rods against light damage whereas the secondary function is to maximize rod sensitivity in darkness. This conclusion is also consistent with the demonstration that an arrestin knockout mouse exhibits greater sensitivity to light damage [18].

Hypothetical function of translocation in cones: Cones are active over six logs of lighting intensity and do not saturate in bright light whereas rods are only active in very dim light and are easily saturated. Consistent with the activity of cones, our data demonstrate that α-T is concentrated in the COS where phototransduction occurs in the light. This localization is the opposite of rod α-T which is concentrated in the RIS in the dark but both localizations are consistent with the functioning of cones in the light and rods in the dark. The apparent anomaly is that cone arrestin has also been shown to be more concentrated in the COS in the light [13,17]. This places both the activating protein (cone α-T) and the inhibiting protein (cone arrestin) in the COS compartment simultaneously when phototransduction is actively occurring. We hypothesize that, like rods, cones have to be protected from light and that this is done with higher concentrations of arrestin in the COS in the light. Because cone opsins do not bind 11-cis retinal as tightly as rod opsin, and because cone arrestin does not bind phosphorylated cone opsin as tightly as rod arrestin binds phosphorylated rod opsin, the presence of cone arrestin does not shut down the system but provides very transient protection against the negative effects of light while the presence of cone α-T enables phototransduction to occur.

Hypothetical mechanisms of translocation: In the mouse, rod α-T translocates faster than arrestin when the retina is exposed to the same amount of light suggesting that some

component(s) of the translocation mechanism is different. Also arrestin moves faster when the lights are turned on than it does with the onset of darkness, which also supports the interpretation that there are two different mechanisms for arrestin itself. Finally, the extreme differences in transit times for α-T under light-dark and dark-light conditions suggests that it also translocates by two distinct mechanisms. These interpretations are consistent with the data and conclusions from experiments [10,19] in which knockout mice lacking either α-T or rod arrestin were capable of translocating the remaining protein. Therefore, four distinct unique mechanisms may be involved; one for each direction of translocation for each protein.

We had previously postulated that the translocations were occurring by microtubule or actin dependent molecular motors [1,3] and this was supported by the demonstration [4,12] that arrestin, in the dark adapted frog rod, is strongly associated with the axoneme and regions between the scalloped edges of the discs which are known to be occupied by microtubules. Such a localized increase in concentration of mouse arrestin was also demonstrated by immunoelectron microscopy [9]. When the subunit, KIF3A, of the kinesin-II microtubule based motor, was inactivated only in the rods using the Cre-loxP system [20], large amounts of opsin and arrestin accumulated in the RIS but there was no effect on the localization of α-transducin. Recent data obtained from knockout mice [10,19] demonstrated that α-T and arrestin translocate independent of each other and in the absence of rhodopsin kinase and therefore independent of phosphorylated rhodopsin. Diffusion through the small connecting cilium cannot account for the fast shift in locations that occur when the lights are turned on. Also, diffusion alone cannot explain the separate compartmentation of these proteins under light or dark conditions. Centrin-1, which itself is restricted to a small region in the axoneme, has been shown to bind to β-transducin [21] but is unlikely, by itself, to account for compartmentation and translocation of α-T. In flies [22], Gq-αand arrestin have been shown to move in response to light and elimination of the phosphoinositide binding site on arrestin was shown to disrupt translocation in both directions [23]. Conditions that increase phosphatidylinositol (3,4,5) triphosphate (PIP_3), inhibited movement out of the rhabdomeres whereas environments with low PIP_3 levels inhibited movement in the opposite direction. The involvement of PIP_3 in migration of the fly proteins, in combination with the demonstration that the tyrosine kinase Src associates with arrestin in a light dependent manner [24] and that α-T is in detergent resistant membranes associated with caveolin-1 in the RIS [25], suggest that translocation of vertebrate arrestin and α-T might also involve phosophoinositide pathways.

ACKNOWLEDGEMENTS

This work was supported by a Jules and Doris Stein Professorship from Research to Prevent Blindness (RPB); National Institutes of Health grants EY06085, EY13050, an NEI core grant (EY12190), P20 RR017703 from the COBRE program of the National Center for Research Resources, general funds from Presbyterian Health Foundation, and by an unrestricted grant from RPB to the Department of Ophthalmology, Dean McGee Eye Institute.

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The print version of this article was created on 15 Sep 2004. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.