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Light-induced stress as a primary evolutionary driver of eye origins

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Abstract

Eyes are quintessential complex traits and our understanding of their evolution guides understanding of trait evolution in general. A long-standing account of eye evolution argues natural selection favors morphological variations that allow increased functionality for sensing light (Darwin 1859; v. Salvini-Plawen and Mayr 1977; Nilsson and Pelger 1994; Nilsson 2013). While certainly true in part, this focus on visual performance does not entirely explain why diffuse photosensitivity persists even after eyes evolve, or why eyes evolved many times, each time using similar building blocks. Here we briefly review a vast literature indicating most genetic components of eyes historically responded to stress caused directly by light, including UV damage of DNA, oxidative stress, and production of aldehydes. We propose light-induced stress had a direct and prominent role in the evolution of eyes by bringing together genes to repair and prevent damage from light-stress, both before and during the evolution of eyes themselves. Stress-repair and stress-prevention genes were perhaps originally deployed as plastic responses to light and/or as beneficial mutations genetically driving expression where light was prominent. These stress-response genes sense, shield, and refract light but only under UV exposure. Once under regulatory-genetic control, they could be expressed before UV stress appeared, evolve as a single unit, and be influenced by natural selection to increase functionality for sensing light, ultimately leading to complex eyes and behaviors. Recognizing the potentially prominent role of stress in eye evolution invites discussions of plasticity and assimilation and provides a hypothesis for why similar genes are repeatedly used in convergent eyes. Broadening the drivers of eye evolution encourages consideration of multi-faceted mechanisms of plasticity/assimilation and mutation/selection for complex novelties and innovations in general.

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I. Introduction

Understanding the evolutionary origins of complex structures and innovative functions are foundational goals of biology. Because we know a lot about structure-function relationships and genetics, eyes serve as models for understanding complex trait evolution (Oakley and Speiser 2015). A commonly accepted explanation for eye evolution is that natural selection acted on variation in morphology, in turn increasing functional capabilities of photoreceptors, simple eyes, and lens-eyes (Nilsson 2009, 2013). Although it explains many aspects of eye evolution, here we discuss how this focus on visual function as the primary driver is incomplete and leaves substantial features of eye evolution unexplained. Furthermore, recent work—mainly on other complex traits—increasingly examines how multiple engines of novelty, including plasticity, contribute to the complexity of life (West-Eberhard 2003; Moczek et al. 2011; Wagner et al. 2018, n.d.). We extend causes of eye evolution beyond visual function to include stress responses as another critical driver. This focus on stress invites discussions about the relative roles of plasticity and mutation in the origins and elaboration of complex traits.

The diversity of animal photoreception can be approximated by four rough, functional categories describing a stepwise increase in complexity of both form and function (Nilsson 2013). First, non-directional photoreceptors use only photosensitive cells, which can only measure the intensity of ambient light for use in multiple behaviors. Second, directional photoreceptors pair light-blocking pigments with photosensitive proteins. Third, low-resolution spatial vision usually uses lens-like material to begin to focus light on the retina. Finally, high-resolution spatial vision uses a lens to focus light finely and precisely onto photoreceptors. This framework hypothesizes that transitions between these morphological categories may be driven by natural selection for increasingly complex behaviors (Darwin 1859; v. Salvini-Plawen and Mayr 1977; Nilsson 2013). In fact, explicit calculations of the physical and optical requirements for the different sensory tasks match well with physical capabilities inferred from the increasingly complex morphologies (Nilsson 2013).

While the gradual elaboration of eyes may be explained by selection on visual function, it is unclear how each of these parts originated before selective pressures to refine visual acuity could shape their evolutionary trajectories (Oakley and Pankey 2008). Besides gradual modifications like deepening of pigment cups and elaboration of lenses, the complexification of eyes required discrete steps, including origins of photoreception, origins of pigmentation adjacent to photoreceptors, and origins of lens-like material in the path of light. While these discrete origins could be explained by purely random mutations that direct expression of components to evolving eyes, the randomness of this mutation-selection model does not account for some salient features of eye evolution. First, despite evolving many times separately, eyes use functionally similar components (v. Salvini-Plawen and Mayr 1977; Picciani et al. 2018). Second, genetic components of eyes often have dual or ancestral roles in responding to stress. Third, for fitness based on visual function to be dramatically higher, morphological novelties should be paired with behavioral capabilities that themselves are innovations, and it may be unlikely that compatible and purely random mutations in both behavior and vision would occur simultaneously. Here we summarize the role of stress in

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evolution of the components of eyes and we hypothesize that response to stress induced by light was an instrumental force in the evolution of eyes, especially for discrete origins of lenses, eye-pigments, and photoreception.

II. Light-Induced Stress and Eye Evolution

A. Responses to light-induced stress were critical for multiple levels of eye evolution

Image forming eyes have evolved incredible complexity optimized for fine scale resolution, object detection and motion tracking. However, we propose canonical visual functions need not be the only driver of eye evolution, instead arising as an emergent property of stress induced evolutionary innovation in response to a pervasive environmental toxin: light. Ultraviolet (UV) light destroys lipids, proteins, and DNA. We hypothesize that components of eyes arose, often repeatedly, from stress response networks that had evolved to predict, preempt, and avoid UV damage — facilitating the multiple, independent origins of eyes. Even before the most rudimentary eyes originated, evolution may have linked independently evolving stress responses to create complex and effective networks to mitigate UV damage. The eventual co-option and genetic-regulatory control of these networks led to the origins of crucial advances in functional complexity: lenses, retinas, and pigment shields. We propose a macroevolutionary history of vision in which stress response pathways are a primary driver of eye origins (Fig 1).

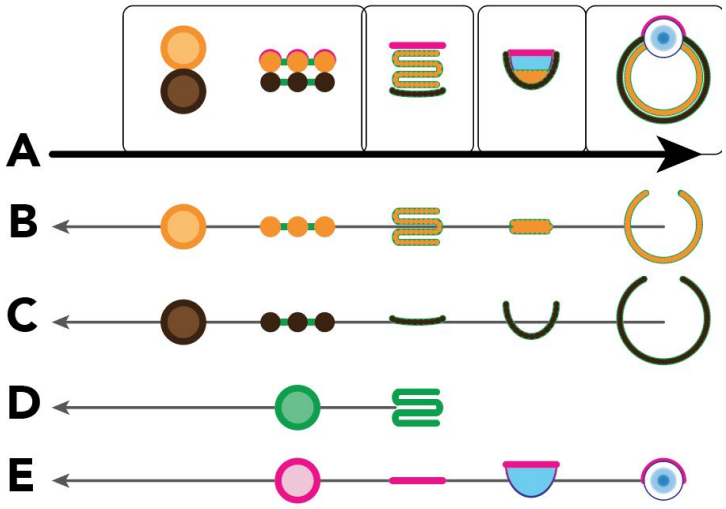

Figure 1. We propose a model of eye evolution with origins rooted in responses to light-induced stress. This builds upon previous ideas (A) that natural selection for increased visual function is the main driver of eye evolution (Nilsson 2013). We agree natural selection acts to elaborate visual complexity. In addition, we propose origins of parts of eyes are rooted in responses to photostress. For example (B) photosensitivity may have originated to repair and predict damage to light-induced stress, eventually leading to opsin-based photoreception. In addition, (C) pigments, such as melanin also originated to respond to light-stress. Third, (D) cilia, which increase surface area of photoreceptive membranes, are driven by UV stress (Chavali and Gergely 2013). Finally, (E) proteins of lenses are varied and have numerous linkages to light-induced stress.

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B. Response to light-stress could involve both plasticity and mutation under selection.

Phenotypic plasticity in response to the environment is one possible engine of innovation, whose contributions to complex trait evolution we are just beginning to address (West-Eberhard 2003; Moczek et al. 2011). Plasticity is quite different from more typical mutation driven explanations of evolution where the primary source of variation is the random mutation of genes that underlie a focal trait. In contrast, phenotypic plasticity may be a faster generator of variation within populations than mutation, creating strikingly labile systems that allow organisms to tolerate a range of environmental regimes. Unlike mutations, which are rare and often detrimental, plastic expression or development may allow immediate and appropriate responses to different environments. Over time, plastic responses may become assimilated into developmental genetic programs, which can be elaborated upon to create complex traits. Therefore, phenotypic plasticity changes the order of events in the origins of novelty compared to mutation-selection. Under mutation-selection, random, undirected mutations create the variation in traits that selection acts upon. With phenotypic plasticity, variation first arises through plastic expression of stress/developmental programs, while mutations may occur later, allowing advantageous plastic expression patterns to become heritable.

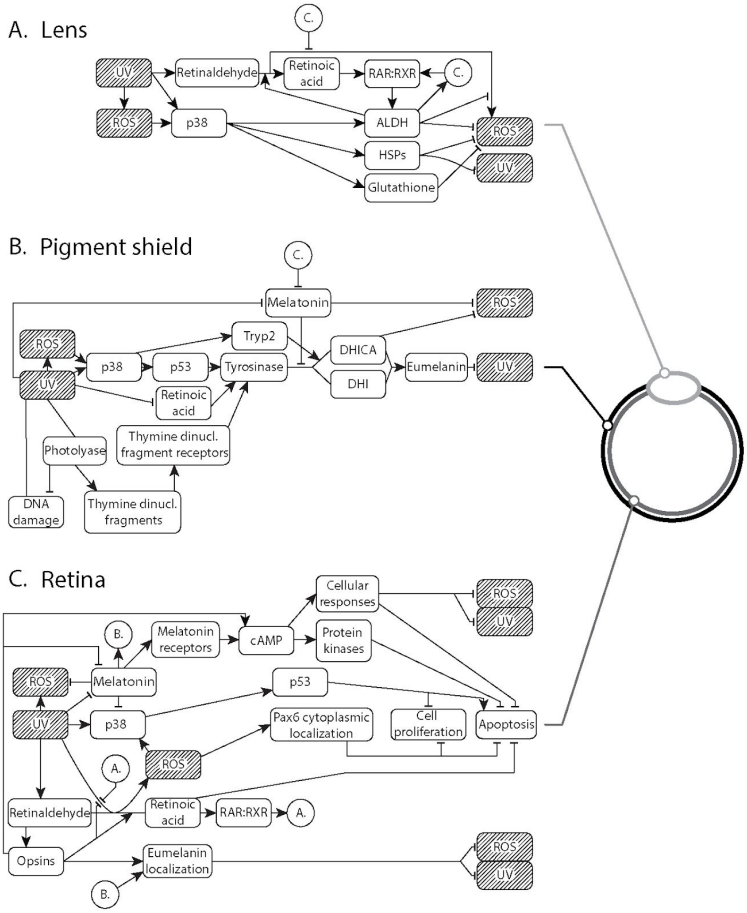

Figure 2. Diagram showing the UV- and ROS-initiated stress response pathways that underlie the origin of each functional part of the vertebrate eye. The lens (A) shows overexpression of UV-blocking, chaperone, and reducing enzymes tied to the expression of light sensitive molecules in the retina (C). The pigment cup (B) is an elaboration of the original pigment shield and is a full-spectrum light blocking membrane formed from specialized portions of stress-related pathways which mitigate ROS and UV stress. The retina (C) shows overexpression of light sensitive proteins and polypeptides evolved to control pathways that rely on pathways (A) to block excess UV light and (B) to produce melanin and metabolize cytotoxic compounds in order to mitigate UV and ROS stressors.

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III. Stress-related innovations underlie crucial components of eye evolution

In the following sections, we discuss in more detail the role stress responses may have played in the evolution of multiple components of eyes that are associated with discrete transitions in functional ability. Beginning with the origin of lenses, we discuss the benefits of light-protection as eyes transitioned from directional light sensors to organs capable of object resolution. We next discuss the evolution of photosensitive proteins that make up retinas, revealing another example of a stress induced evolutionary innovation, tying stress-mitigation pathways to increasingly accurate UV sensors. We then outline a possible evolutionary path behind the molecular roots of pigments and their integration with networks governed by photosensitive proteins. Finally, we describe how these separate components might have come to be regulated together genetically. The following sections lay out a hypothesis that addresses some gaps of acuity-driven eye evolution, showing that stress-responses to light function are a primary driver of eye evolution.

A. Lenses as Emergent Properties of Stress Mitigation

Because lenses allow higher visual acuity, their multiple origins define crucial evolutionary transitions between directional and image-forming vision, while at the same time illustrating connections between stress and eye evolution. The genes recruited to form lenses are quite variable in origin yet very commonly are stress-response proteins. This trend has been noted for some time, but has been explained largely because of proteins' ability to remain transparent and not interfere with the visual function of the eye (Piatigorsky and Piatigorsky 2009; Nilsson 2013). However, by examining these proteins and their roles in UV-stress responses, we see possible origins of lenses by upregulation and concentration of particular proteins in front of photoreceptors to guard against toxins and mitigate UV. Lens proteins absorb UV light, reduce protein-protein interactions, chaperone protein folding, and metabolize cytotoxic compounds that arise as a product of both UV exposure and photoreception (Leiers et al. 2003; Lassen et al. 2008; Ou et al. 2008) (Fig. 2A). In the stepwise framework of eye evolution, the shift between directional photoreception and low resolution, image-forming vision is marked not only by the deepening of a pigment cup, but also the appearance of 'lens-like' material between the retina and the epithelial lining (Nilsson 2013). We hypothesize that the evolution of these specialized lens-like cells stems from the pressure to filter UV light from reaching an increasingly sensitive retina, which would limit external generators of ROS and aldehydes and therefore create a more mild environment for photoreceptors. Thus, the stress-related origins of these crucial novelties suggest that lenses, and by extension image forming vision, originated as a protective mechanism against damage from UV and reactive oxygen species and were later elaborated by natural selection on visual function (Fig. 2A).

Lenses are constructed of stress-response proteins in multiple species (Table 1) (Piatigorsky 1998; True and Carroll 2002; Piatigorsky and Piatigorsky 2009). As one example, the lenses of octopuses recruited several proteins into lenses, the most common are aldehyde dehydrogenases and glutathione S-transferases (Land 2012). The aldehyde dehydrogenases

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are particularly interesting because they belong to the class of proteins upregulated in response to ROS stress in animals. Aldehyde dehydrogenases aggressively find and neutralize dangerous reactive aldehydes such as retinaldehyde (aka. Retinal, the chromophore of opsin-based light sensitivity), 4-hydroxy-2-nonenal, and malondialdehyde, all of which are produced during UV exposure (Lassen et al. 2008). The second protein family in octopus lenses, glutathione S-transferase, detoxifies lipid molecules damaged by free radicals and ROS and acts as a free radical scavenger (Reiter 1993; Lassen et al. 2008). In mammals, lenses are comprised mostly of retinaldehyde dehydrogenase and diverse crystallin proteins. The incorporation and hyperexpression of aldehyde dehydrogenases and crystallins once again shows lenses are elaborations off an original function mitigating damage from UV stress (Piatigorsky and Piatigorsky 2009). A second protein in mammalian lenses, alpha-crystallin, arises from a larger gene family known by a different name: small heat shock proteins. These proteins, similar in function to glutathione S-transferase, work as chaperones to prevent protein-protein interactions and refold damaged proteins in response to ROS, heat, and UV stressors (de Jong et al. 1993). These properties are exceptionally useful in a lens and cornea not only because of their tolerance for environmental stressors, but also because the clarity of the lens suffers when protein-protein interactions occur.

Table 1. Common lens crystallins in vertebrates and invertebrates along with their non-lens functional classification. Table reconstructed from (Piatigorsky 1998) with additional information from (Piatigorsky et al. 2001).

Lens Crystallins		
Vertebrates		
	α	small heat shock proteins/chaperones; all vertebrates
	$\beta\ \gamma$	members of microbial stress protein superfamily; all vertebrates
	ε	lactate dehydrogenase B; ducks, crocodiles
	δ	argininosuccinate lyase; birds, reptiles
	τ	α-enolase; turtles, ducks, other vertebrates
	ξ	novel quinone oxioreductase; guinea pig, camel, degu, llama, rock cavy
	μ	relative of bacterial ornithine cyclodeaminase; Australian marsupials
	η	retinaldehyde dehydrogenase; elephant shrews
	ρ	relative of aldo-keto reductase; frogs
	λ	relative of hydroxyl CoA dehydrogenase; rabbits, hares
	π	glyceraldehyde-3-phosphate dehydrogenase; geckos
Invertebrates		
	S	glutathione S-transferase and its relatives; cephalopods
	Ω/L	relative of aldehyde dehydrogenase; cephalopods, especially octopus; scallops; squid light organ
	J	novel protein, potentially saposin/swaposin homologs; jellyfish
	Drosocrystallin	novel protein; Drosophila

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The repeated evolution of lenses as focusing optics creates opportunities for animals to perform high-resolution spatial vision, but the advantage of improved image resolution may not be what began lens evolution each time. Photoreceptor cells of any organism are exposed to extreme amounts of UV, ROS, and heat stress in order to perform their intended function. In addition, the molecular machinery required for photoreception also generates toxic aldehydes and ROS. As eyes became more sensitive by packing more photosensitive proteins into each cell, the amount of free aldehydes and the rate of ROS production would quickly increase. In order to mitigate damage incurred by this increase in visual acuity, it is likely the expression of stress response proteins such as aldehyde dehydrogenases, heat shock proteins, and homologs of glutathione S-transferase would be useful as a protective layer inside the evolving eye that inhibits protein-protein interactions and maximizes optical clarity of the proteins. Thus, examining the origins of lenses and high-resolution spatial vision reveal that stress may have driven origins in response to lethal UV and ROS stress as eyes became specialized for vision. Elaboration of lenses for fine focusing and a graded refractive index in water (Sweeney et al. 2007) probably evolved later.

B. Protective pigments linked to light exposure

Shielding pigments are a crucial step in the evolution of eyes, allowing for directional photoreception by shading the photoreceptors from one direction (Walne and Arnott 1967; Nilsson 2009). Pigment expression adjacent to photoreceptors could evolve through random mutations and be preserved by natural selection each time eyes originated, alternatively this association could have arisen from the pressure to endure light and oxidative stress while protecting hypersensitized photoreceptors from lethal overexposure (Fig. 2B). In animals, melanin is one of the most well-studied light absorbing pigments recruited into eye spots, eyes, and extraocular photoreceptors. Melanin plays an integral role in protecting cells from UV damage as the end result of highly elaborate and specific protective pathways. Upon exposure to UV, cells undergo immediate pigment darkening through the photooxidation of preexisting melanin, specifically blocking UV light and quenching ROS while balancing the costs of *de novo* melanin synthesis (Fig. 2B). Examining the plastic expression of melanin and how it has been assimilated under improving UV sensors once again reveals that plastic stress-responses could underlie origins of eye evolution.

In vertebrates, melanin synthesis involves damage and stress sensors and is thus linked to light sensing. UV damage to DNA causes neighboring thymines to bond to each other. UV activated proteins, photolyases (PLY), repair these damaged pieces of DNA by excising and discarding damaged segments, creating small fragments of DNA containing thymine dinucleotides. These fragments upregulate tyrosinase, the rate limiting enzyme in melanin synthesis (Eller et al. 1996). Two additional proteins that are involved in every pathway discussed in this paper (Fig. 2A,B,C) can also upregulate tyrosinase activity. The MAPK protein p38 is activated in response to UV light and phosphorylates the inactive form of the p53 tumor suppressor protein. In addition to its many roles repairing DNA damage, controlling cell division, and regulating apoptosis of damaged cells, p53 also upregulates tyrosinase activity to create additional *de novo* melanin

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(Khlgatian et al. 2002; Cui et al. 2007). The circadian rhythm controls p53 activity, making interaction of p38 and p53 much more prevalent during daylight hours although this can be overridden during periods of ROS stress (Lau et al. 2014; Volonte et al. 2015). The photosensitive vitamin-a derivative, retinoic acid, also plays a significant role in regulating tyrosinase activity, controlling *de novo* synthesis in response to retinoic acid light sensing pathways (Orlow et al. 1990; Roméro et al. 1994; Paterson et al. 2013). Lastly, while the previous mechanisms have all encouraged melanin synthesis, melatonin—a ROS and photosensitive molecule—inhibits synthesis post-tyrosinase activity, potentially creating a "pool" of melanin precursors available for quick assembly at low metabolic costs (Fig 2B).

The pre-adaptation this system creates is crucial to enduring moments of high UV stress and suggests that the first link between pigment expression and photoreceptors may have been formed in order to endure moments of extreme UV and ROS stress. Unaided, melanin synthesis has a high oxidative cost and can potentially kill cells already under significant ROS stress. Melanin (eumelanin) is a macromolecule composed of varying ratios of two components, DHI and DHICA. Unless the enzyme Tyrp2 is present, only DHI is produced. Thus, it is likely that eumelanin first appeared as the lattice-like sheets of DHI, and Tyrp2 evolved later to help already stressed cells avoid the ROS associated with DHI synthesis by producing the more specialized and less taxing DHICA (Peles et al. 2010; Galván and Solano 2015; Micillo et al. 2017). DHICA specifically absorbs light in the UV spectrum and acts as a potent ROS scavenger compared to the relatively ineffectual scavenger, DHI (Borovansky and Riley 2011). However, DHI appears necessary to precipitate the more stable DHICA (Aroca et al. 1992; Urabe et al. 1994), This switch is controlled largely through p38, which upregulates Tyrp2 when exposed to UV light as melatonin. Retinoic acid, p53, and thymine dinucleotide fragments also form an elegant series of gates and blocks that switch melanin synthesis from DHI to DHICA and from expensive, *de novo* synthesis to quick, safe assembly from a pool precursors—treading a delicate balance between providing sufficient UV protection and avoiding lethal ROS levels (de Assis et al. 2018). Thus, while pigment shields are needed in order for directional photoreception to evolve, the link between photosensitive proteins and antioxidant pigments was likely forged not for the sake of vision, but in order to protect photosensitive cells from UV and ROS damage.

C. Origins of photoreceptors from sensors of oxidative stress

Retinas can detect minute changes in light by densely packing photosensitive proteins into photoreceptor cells, thereby increasing sensitivity to light and allowing increased visual acuity (Nilsson 2013; Skalicky 2016). In most animal eyes, the photosensitive proteins are opsins (but see Rivera et al. 2012), usually assumed to be present in animal eyes for reasons of visual function, including quick response time and use of a chromophore that can be efficiently regenerated (Nilsson 2013). However, examining opsins' evolutionary history, we suggest the driving process behind its repeated co-option into retinas is instead rooted in its notable predisposition to endure and mitigate photostress. In addition to the stressors discussed earlier in this paper, UV light produces the toxic compound retinaldehyde, a necessary ligand for opsin

photosensitivity (Tolleson et al. 2005). Extant opsin proteins tightly bind this free aldehyde and upregulate secondary messengers that perform a myriad of regulatory tasks in the cell (Provencio and Foster 1995; Peirson and Foster 2006; Shichida and Matsuyama 2009; Wicks et al. 2011). Thus, we posit that the light sensitivity of opsins and their subsequent use in retinas is a byproduct of their original function tracking retinaldehyde levels to coordinate stress-initiated mitigation pathways (Fig 2C).

Nuclear retinoic acid receptors, melatonin receptors, and opsins all play roles in directing stress responses to UV exposure, but opsins have evolved as both the most accurate photodetector and a potent activator of UV-protective pathways. However, without the co-expression of each of these sensors into ROS and UV quenching stress pathways, now found in retinas, reaching the concentration of photoreceptors needed for visual tasks would quickly kill cells through photooxidative stress and cytotoxic products of photodegradation. Melatonin acts as a powerful scavenger of ROS but it is quickly broken down by both ROS and UV light, leading to intracellular melatonin concentrations that closely track oxidative and UV stress levels (Xu et al. 2009). Melatonin receptors and their homologs evolved as a subfamily of GPCRs early in the history of eukaryotes (Schippers and Nichols 2014) that track these levels and can be used as a proxy for external light levels. Melatonin receptors regulate intracellular adenylyl cyclase in response to local melatonin concentrations, and are integral in many ROS and UV damage mitigating pathways like melanin production. (Fischer et al. 2008; Galano et al. 2011; Yan et al. 2018). Retinoic Acid (RA) can also be used as a sensor for UV stress, but unlike melatonin, retinoic acid fluctuations are more specifically tied to UV exposure, dropping more than fifty percent in the presence of UV light (Tolleson et al. 2005). Nuclear retinoic acid receptors use the low intracellular concentrations of RA that accompany light to stall cell division and inhibit apoptosis (Mangelsdorf et al. 1990; Konta et al. 2001; McNamara et al. 2001). Ancestral opsins likely also evolved as a retinoid receptor, initially regulating intracellular cyclic nucleotide levels (cAMP or cGMP) in response to retinaldehyde, the metabolic precursor to RA (Koyanagi et al. 2008; Shichida and Matsuyama 2009). In addition, their evolutionary history primed their downstream signalling pathways to intertwine with existing GPCR initiated stress response pathways (Peirson et al. 2009; Semo et al. 2010; Iyengar 2013; Bertolesi et al. 2015).

The close ties between opsins and other stress response initiators allows them to withstand the stresses of dedicated, high density photoreceptors, and could explain their co-option into each independent evolution of a retina. As eyes transition along Nilsson's (2013) stepwise evolutionary framework, the amount of photosensitive proteins in the retina is constantly increasing, forcing additional stress on photoreceptive cells. Light-sensing of this caliber is a double-edged sword, where the cells that are hypersensitized to light, and therefore the quickest to succumb to high levels of exposure are also the cells that must be exposed to the brightest light levels in order to fulfill their function. To survive these conditions, mitigation responses to light stress must be linked to immediate detection of UV light. Opsins perform as part of a specific UV stress response network initiated by light exposure: Nuclear retinoic acid receptors detect a drop in RA associated with UV exposure, melatonin receptors respond to the increase in ROS, and opsins detect the increase in retinaldehyde that accompanies vitamin-a

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metabolism/photodegradation (Tolleson et al. 2005). Whether opsins photosensitivity is an inherent or derived characteristic, their ability to immediately detect photons striking a cell was likely co-opted as a regulator for other UV stress responses, creating a lynchpin for immediate and comprehensive UV protection. Because of this, opsins represent one of the only photosensitive proteins that could be expressed in high enough quantities to perform visual tasks yet also mitigate photo-stress to such an extent that these photoreceptors weren't immediately killed by cytotoxic byproducts of UV exposure. Thus, we find it possible that opsin's repeated co-option into retinas is initially due to its role as a cytosolic retinaldehyde and UV light receptor, while its use as a visible light receptor is a secondary elaboration on these ancestral functions.

D. Genomic regulation transforms stress response networks into a single evolutionary unit

The concept that selection for improved visual function drives eye evolution rests on the assumption that the eye morphology is heritably expressed as a single, cohesive unit. This evolutionary step is typically achieved by movement away from transient, plastic expression to a more coordinated developmental process divorced from stressors and driven by transcription factors (Wagner et al. n.d.). One of the largest gaps in our knowledge of eye evolution is how these regulatory relationships evolved between transcription factors and the stress response pathways outlined above (Nishina et al. 1999). In animals, these UV/ROS response pathways are largely controlled by the PAX family of transcription factors, which contribute to development and patterning of lenses, pigment shields, and retinas in all currently known eyes (Ashery-Padan and Gruss 2001). By examining literature surrounding a vertebrate copy of PAX, PAX6, we once again see links to ROS and UV stress responses that may have driven the disambugation of light-mediated stress responses from the stimulus of UV exposure (Mikkola et al. 1999; Ou et al. 2008; Laggner et al. 2017). The sequence similarity between PAX6 promoters and the promoters driving crucial portions of the UV-response network facilitated PAX6 to become adopted as an 'unified' activator (Cvekl et al. 2017). With UV light still sufficient, but no longer necessary, developmental processes could express the response pathways above in cohesive, photosensitive 'eyespots' permanently presensitized to track levels of UV light from a distance, setting in motion the evolution of more complex eyes. In addition, PAX6's role in ROS quenching (Ou et al. 2008; Laggner et al. 2017) shows that stress induced evolution may have played a significant part not only in the origins of each piece of an eye, but also in the origin of each 'eye' as a cohesive, heritable unit upon which selection for visual function could begin to innovate (Fig. 1).

This pattern of stress-induced phenotypes being assimilated then elaborated into new functions via mutation-selection extends all the way to the unification of each piece of an eye under PAX6. It is even a piece of UV-specific protective pathways and likely co-occurred alongside the other pathways it regulates. The binding sites for PAX6 as a transcription factor occupy an incredible position of similarity to sites used to regulate a number of stress response networks. Heat shock elements (found in lenses), antioxidant response elements (found throughout the eye), and p53 binding sites (cell cycle arrest and pigment expression) are all exceedingly similar

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to the 'optimal' PAX6 binding site (Cvekl et al. 2017). Very few mutations are needed to change any one of these promoter regions into one that would recognize PAX6 as an activator. Through these mutations, each independently evolved stress response previously discussed became subsumed into a cohesive regulatory network with heritable expression patterns subject to selection instead of transient external stimuli. It is through this amalgam of both plasticity and mutation under selection that we see specific response and protective pathways emerge from general repair pathways to connect plastic, stress induced pathways as a single evolutionary unit each time eyes have evolved.

III. Summary and Conclusions

A. Stress-induced origins complete a framework of eye evolution

Common hypotheses for the evolution of eyes rely on selection for increased visual function to explain the origins and elaboration of structures and functions of eyes. While this framework provides a reasonable explanation for the elaboration of existing traits (e.g. increasing precision of lens focusing after its origin) — it remains incomplete in its explanation of the origins of these traits. Attempting to justify the origin of eyes based solely on selection for improved visual acuity creates a logical fallacy of circular reasoning, leaving no obvious evolutionary starting point for eyes or parts of eyes to independently evolve. By focusing on eye evolution through the lens of mitigating stress from light, we suggest that the repeated co-option of genes from particular functional categories into each origin of lenses, retinas, and pigment shields could be driven by selection to mitigate photostress. Therefore, stress, not vision, may have often created *initial* selection to maintain co-expression and evolve co-regulation of genetic mechanisms bringing together parts of eyes before the behavioral connections to light sensitivity could select for improved visual acuity.

B. Future directions & Acknowledging limitations

Thinking about stress-induced origins as an engine of novelty inspires significant hypotheses that related to processes that potentially drive complex trait evolution as a whole. Yet we acknowledge much work needs to be done to test these ideas presented. In constructing this framework, we have faced a number of restrictions that represent excellent avenues for future research. Next, we discuss several limiting factors and potential investigations that could address them, including explicit comparative work to infer the history of function and the potential confirmation bias of studies that review vast amounts of literature.

First, our hypotheses need to be tested with explicit comparative methods. At present, the hypotheses rely mainly on findings from basic biology and clinical research that describe protein functions in the present day. This presents two unique problems which future comparative studies would address: the timeline of stress network evolution and ancestral protein function. Without specific comparative studies, including those that take advantage of time-calibrated molecular phylogenies, it is impossible to place an order to the evolutionary

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events that occurred as stress networks co-opted new actors and were in turn co-opted into innovations and novelties. At the same time, with the incredible volumes of untapped sequence data, reconstructing well supported histories of genes is fast becoming a matter of streamlining computational pipelines. Combined, investigating the history of gene function using comparative techniques will allow testing explicit hypotheses, such as responses to stress being more ancient functions than visual functions.

A particularly compelling application of comparative techniques would be to examine the ancestral function of opsins. A critical point in the evolutionary history of animals was the ability for opsins to tightly bind retinaldehyde, forming the first bistable opsins that could be used for reliable, quick, light detection. Through experimental mutation, comparative studies, and ancestral sequence reconstruction (Chang and Donoghue 2000), we may be able to unravel how this specific function evolved. Perhaps the drive to detect smaller concentrations of retinaldehyde created a selective pressure to increase binding efficiency without the need for any form of visual system in place. Assuming the ancestral opsin was a retinaldehyde receptor, the longer each molecule of retinaldehyde remained bound, the more sensitive the cell would be to smaller and smaller concentrations of this cytotoxic compound. This pressure may have culminated in an opsin with the ability to permanently bind retinaldehyde, no longer tracking intracellular concentrations, but instead detecting the isomer of retinaldehyde trapped in the binding pocket, which changes in response to UV light. This scenario makes particular predictions, (1) that the ancestor of all opsins may lack the ability to permanently bind retinal, but will function as a retinoid receptor, and (2) that close relatives of the opsin family may have preserved their ancestral function as retinoid receptors.

A second concern that inspires future research is our synthesis of literature from a wide array of unrelated fields risks confirmation bias. The breadth and depth of study required to unravel the molecular mechanisms of cell physiology at the level required for this paper exists mainly in a few model organisms. Because of this, our conclusions are drawn from a small subset of the tree of life, relying on examples from vertebrates and a select few invertebrates. This critique highlights the need for future work and opens the door for future comparative studies to reinforce the support we have found in a handful of well researched organisms. The issue of confirmation bias presents a unique point of interest in this study. In such broad, reaching reviews, there is always the risk that in searching for stress related functions, we have overlooked alternative explanations. Interestingly, although we summarized a limited selection of pieces from the developmental network tied to eye evolution, we find that origins from light-induced stress responses likely extend far beyond our restricted exploration (Sancar 2000; Galibert et al. 2001; Weber 2005; Piatigorsky and Piatigorsky 2009; Rivera et al. 2012; Engelen et al. 2013; Tamaru et al. 2013).

The advent of reliable single cell sequencing and advances in theory surrounding cell type evolution (Wagner et al. n.d.; Arendt et al. 2016) provides another way to address issues surrounding both confirmation bias and restrictive sampling. By sequencing genes expressed in individual cells, we can examine relative enrichment of particular stress networks in particular organs. We predict cells that make up eyes will have greater representation of stress-response genes than many other cell types. Perhaps counter to this prediction, we acknowledge how

shockingly common it is to find stress-related genes involved in almost any innovation or novelty (Feder and Hofmann 1999; Kültz 2005; Chavali and Gergely 2013; Wagner et al. 2018). Pleiotropic function of developmental genes and novelties tracing back to stress-responses have been superficially noted during countless studies in nearly every field, a pattern that, from an evolutionary perspective, becomes more interesting the more evidence is gathered (West-Eberhard 2003; Palmer 2004; Chevin et al. 2010; Ramaswami 2014).

In conclusion, broadening our interpretations of the processes that drive eye evolution reveals an expanded framework that explains the origin and evolution of eyes, vision, and photoreception. Examining stress as a potential engine of plasticity and variation elucidates, once again, a surprisingly common occurrence of stress responses underlying many complex traits and developmental modules. We believe this near-ubiquitous co-option of stress genes in novelties may highlight an underappreciated macroevolutionary pattern that will illuminate, in part, the relative contributions of *de novo* evolution and co-option to evolution as a whole. We are excited to see future research on cell motility, sensory systems, and cell type evolution test these hypotheses and begin to delve into larger evolutionary questions about the roles of *de novo* evolution and co-option.

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