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Role of crystallins in ocular neuroprotection and axonal regeneration

Solon Thanos [a, b, *, 1], Michael R.R. Böhm [a, b, 1], Melissa Meyer zu Hörste [c, d, 1], Verena Prokosch-Willing [a, 1], Maren Hennig [e, 1], Dirk Bauer [e, 1], Arndt Heiligenhaus [a, e, 1]

[a] Institute of Experimental Ophthalmology, Germany
[b] Center of Excellence "Cells in Motion" (CiM), School of Medicine, University of Münster, Albert-Schweitzer-Campus 1, D15, 48149 Münster, Germany
[c] Massachusetts Eye and Ear Infirmary (MEEI), Boston, MA, USA
[d] Eye Clinics, University Essen, Germany
[e] Departement of Ophthalmology at Franziskus Hospital Münster, Germany

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ABSTRACT

Neuroprotection is an emerging challenge in ophthalmology due to the particularly exposed location of retinal neurons and to the steadily increasing rate of intraocular surgical and pharmacological treatments applied to various eye diseases. Within few decades neuroprotection has developed from strongly contested approaches to being recognized and introduced as a potentially clinical application. One of the groups of putative substances for neuroprotection comprises αA- and αB-crystallins, which are types of heat-shock proteins and are considered to be molecular chaperones. The β/γ-crystallins form their own superfamily and are characterized as proteins with a distinct structure containing four Greek key motifs. Besides being abundant in the ocular lens, crystallins are also expressed in both the developing and mature retina. Crystallins are dramatically up-regulated in numerous retinal pathologies, including mechanical injury, ischemic insults, age-related macular degeneration, uveoretinitis, and diabetic retinopathy. Crystallins of the α family are thought to play a crucial role in retinal neuron survival and inflammation. Crystallins of the β/γ superfamily are also small proteins with a possible emerging role in retinal tissue remodeling and repair. One of the typical retinal diseases associated with crystallins is the experimental glaucomatous neuropathy that is characterized by their expression. Another typical retinal disease is the atrophy that occurs after mechanical injury to the optic nerve, which is associated with the need to regrow retinal axons. We have shown in regenerative models *in vivo* and *in vitro* that βB2-crystallin actively supports the regenerative growth of cut retinal axons, thereby offering targets for neuroprotective and regenerative treatments. In this review we discuss the discovery that βB2-crystallin is clearly up-regulated in the regenerating retina *in vitro*. βB2-Crystallin is produced and secreted during axon elongation, while β/γ-crystallins promote axon growth both *in vivo* and *in vitro* by acting either directly by uptake into cells, or indirectly by enhancing the production of ciliary neurotrophic factor from astrocytes to synergistically promote axon regrowth. We also discuss methods to induce the continuous production of crystallins at the site of injury and repair based on the use of transfected neural progenitor cells. This review ultimately leads to the conclusion that the postinjury fate of neurons cannot be seen merely as inevitable, but instead should be regarded as a challenge to shaping the neuroprotective and regenerative conditions that promote cell survival and axon repair.

* Corresponding author. Institute of Experimental Ophthalmology, School of Medicine, University of Münster, Albert-Schweitzer-Campus 1, D15, 48149 Münster, Germany. Tel.: +49 251 8356915; fax: +49 251 8356916.
E-mail address: solon@uni-muenster.de (S. Thanos).

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Contents

1. Introduction

Several previous reviews have discussed the form and classification of lens crystallins. The present review therefore only provides a short summary of these proteins in the lens, and then focuses on the potential role of crystallins within the retina.

2. Retinal crystallins

2.1. Crystallin localization within ocular tissues

Crystallins represent a heterogeneous group of highly abundant proteins in the ocular lens that augment its refractive properties (Wistow and Piatigorsky, 1988; Piatigorsky, 1992; Bloemendal and de Jong, 1991; Graw, 2009; Parthasarathy et al., 2011). The three major families of crystallins (α, β, and γ) are organized in the lens in a spatially and developmentally regulated manner that determines the refractive power of the lens tissue (Lubsen et al., 1988; Wistow, 1990; Wistow and Piatigorsky, 1988). The first class of α-crystallins belong to the small heat-shock proteins (HSPs) that act as molecular chaperones during embryonic development (Sax and Piatigorsky, 1994; Horwitz, 1992, 2003). They are classified into αA- and αB-crystallins (human gene symbols: *CRYAA* and *CRYAB*) and are encoded by separate and single copy genes (reviewed by Andley, 2007; Graw, 2009). While αA-crystallin (mouse gene symbol: *cryaa*) is generally restricted to the lens, αB-crystallin (mouse gene symbol: *cryab*) is also expressed outside the lens, suggesting the presence of functional differences between αA- and αB-crystallins. In the mouse, α-crystallins are expressed during embryonic development and then are silenced after birth. Within the lens epithelium, mutations in *cryaa* diminish the protective features of αA-crystallin (Andley et al., 2002), while the protective abilities of αA- and αB-crystallins differ, as could be expected from their different distributions within organs and tissues (Andley et al., 2000). Mutations in *cryaa* (Mackay et al., 2003) and *crygd* account for recessive or dominant forms of cataract (Mackay et al., 2004; Graw, 2009).

Lens development is regulated by several genetic and epigenetic mechanisms, while lens differentiation is characterized by the expression and accumulation of crystallins (Cvekl and Duncan, 2007; Kataoka, 2007; Whiston et al., 2008). Crystallins are therefore regulated at different molecular levels, including the posttranslational level. For example, stress-induced phosphorylation of serine residues 19, 45, and 59 negatively regulates the assembly of αB-crystallin into higher order complexes (Ito et al., 1997, 2001), while hypoxia-induced overexpression of αB-crystallin in cultured astrocytes is induced by $TGF_{\beta}1$ and $TGF_{\beta}2$ (Yu et al., 2007). In terms of intracellular signaling, αB-crystallin modulates the transcriptional factor NF-kappaB activity and protects cells from tumor-necrosis-factor (TNF)-α cytotoxicity *in vitro* (Adhikari et al., 2011). The hormonal regulation of crystallins has also been demonstrated, with αA-crystallin being characterized as a sex-steroid-regulated protein (D'Anna et al., 2011). Modifications to crystallins may change their function, as shown by the chaperone activity of αB-crystallin, which is altered by phosphorylation and the loss of the dimeric crystallin substructure (Aquilina et al., 2004; Arrigo and Simon, 2010). Although a considerable body of data related to crystallin expression and function has been obtained using cultured cells, and hence is not directly transferable to the *in vivo* organism, it points to potentially similar molecular events taking place *in vivo*.

The second class of the β/γ superfamily consists of βA1/A3-, βA2-, βA4-, βB1-, βB2-, and βB3-crystallins as well as γA-F- and γS-crystallins (Jaenicke and Slingsby, 2001). In rodents and humans, the γ-crystallin cluster is encoded by six genes, each of which is responsible for a single functional peptide. In humans, but not in mice, γ-crystallins are encoded by pseudogenes (ψ8E and ψ8EF) (Meakin et al., 1987), while *cryga*/*CRYGA*, *crygb*/*CRYGB*, *crygc*/*CRYGC*, and *crygd*/*CRYGD* are expressed in the ocular lens of both humans and mice. Mutations in the human CRYM gene result in non-syndromic deafness (Graw, 2009). In contrast to the αA-crystallins, βB2-crystallin expression begins in the lens after birth, and *crybb2* knockout in the mouse results in progressive cataracts (Zhang et al., 2008). Besides a functional βB2-crystallin locus in humans, there is a second βB2-crystallin-derived pseudogene. Conversion of the βB2 locus to the pseudogene results in human lens opacification and cataract formation (Vanita et al., 2001). Lens cataractogenesis can be influenced by other ocular tissues such as the vitreoretinal compartment (Beebe et al., 2011). Crystallins may also influence multiple events during development. For example, trans-differentiation of ocular structures into each other, such as from the cornea to the lens in larval *Xenopus laevis* development, involves crystallin-synthesizing optic vesicles (Cannata et al., 2008; Day and Beck, 2011). Lens regeneration in the rat depends on various