



Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

From *eyeless* to neurological diseases

Jochen Graw

Helmholtz Zentrum München, Institute of Developmental Genetics, Ingolstaedter Landstr, 1, D-85764 Neuherberg, Germany

ARTICLE INFO

Article history:

Received 23 September 2015

Accepted in revised form 6 November 2015

Available online xxx

Keywords:

Pax6

Pitx3

Beta-crystallins

Eye disease

Neurogenesis

Parkinson's disease

Neurodegeneration

Schizophrenia

ABSTRACT

Age-related cataracts are frequently associated with degenerative changes in the ocular lens including the aggregation of proteins – mainly crystallins, but also other proteins including amyloids (A β) leading to the hypothesis that cataracts could be used as “biomarkers” for Alzheimer disease. Even if this hypothesis was rejected by David Beebe's last paper (Bei et al., Exp. Eye Res., in press), it is a fascinating aspect to look for commonalities between eye diseases and neurological disorders.

In this review, I discuss such commonalities between eye and brain mainly from a developmental point of view. The finding of the functional homology of the *Drosophila eyeless* gene with the mammalian *Pax6* gene marks a first highlight in the developmental genetics of the eye – this result destroyed the “dogma” of the different evolutionary routes of eye development in flies and mammals. The second highlight was the finding that *Pax6* is also involved in the development of the forebrain supporting the pleiotropic role of many genes. These findings opened a new avenue for research showing that a broad variety of transcription factors, but also structural proteins are involved both, in eye and brain development as well as into the maintenance of the functional integrity of the corresponding tissue(s). In this review recent findings are summarized demonstrating that genes whose mutations have been identified first to be causative for congenital or juvenile eye disorders are also involved in regenerative processes and neurogenesis (*Pax6*), but also in neurodegenerative diseases like Parkinson (e.g. *Pitx3*) or in neurological disorders like Schizophrenia (e.g. *Crybb1*, *Crybb2*).

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1. Introduction

At the beginning of his career, David Beebe published some papers on δ -crystallins together with Joram Piatigorsky (Beebe and Piatigorsky, 1977). At that time, at the beginning of the 1980s, the idea was launched that ancient proteins can sometimes have more than one function – and the δ -crystallin was one of the striking examples for the recruitment of enzymes as lens structural proteins (Wistow and Piatigorsky, 1987). Today, one generation and 30 years later, this evolutionary concept has been widely accepted thanks to the revolutionary progress in gene sequencing. This progress, however, made us also aware that a gene is commonly expressed not only in one single tissue – and this is true not only for the crystallins (Graw, 2009), but also for some other genes being fundamental for eye development and its functional integrity.

In one of his last papers, David Beebe wrote in this journal as summary of a small case control study with Alzheimer patients: “We conclude that cataract grade or lens opacity is unlikely to

provide a non-invasive measure of the risk of developing Alzheimer dementia” (Bei et al., 2015). The underlying hypothesis based upon the observation that amyloid- β aggregates were found in cataracts of postmortem Alzheimer Disease patients (Bei et al., 2015; Frederikse et al., 1996). David Beebe's paper showed that it might be very unlikely to associate cataracts with Alzheimer's disease. Nevertheless, this biochemical aspect of protein aggregation might be a fascinating hypothesis as a common theme underlying a broad variety of neurodegenerative disorders, and the prion-hypothesis of protein-induced misfolding in the Creutzfeldt-Jakob-Disease was recently expanded to other disorders like Alzheimer (accumulation of β -amyloid), Parkinson (α -synuclein) or frontotemporal dementia (τ -protein) (Jucker and Walker, 2013; Halliday et al., 2014), and it might be discussed, whether it holds true also for crystallin aggregation in the formation of age-related cataracts.

Since these biochemical aspects still remain in a rather hypothetical stage, developmental genetics might give a rather verified hint for common pathways of eye diseases and neurological disorders, since a broad variety of genes are expressed both in the developing eye and the developing brain. The actual search (July 27, 2015) in the Mouse Genome Informatics database (mgi; <http://>

E-mail address: graw@helmholtz-muenchen.de.<http://dx.doi.org/10.1016/j.exer.2015.11.006>

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www.informatics.jax.org/) for the phenotype selection in the categories “behavior/neurological” and “embryogenesis” and “nervous system” and “vision/eye” leads to a list consisting of 136 genes; omitting the restriction to “embryogenesis”, the number of common genes increased up to 453. This list contains mainly genes coding for transcription factors [like *Eya1* (eyes absent 1 homolog/*Drosophila*), *Mitf* (microphthalmia associated transcription factor), *Pax6* (paired box 6), *Pitx3* (paired-like homeodomain transcription factor 3), *Rax* (retina and anterior fold homeobox) or *Six1* (sine-oculis related homeobox 1)], but also some genes encoding structural proteins [like *Col*(1a1, 2a1, 4a1) or *Lam*(a1, c1)]. Here, I would like to highlight just a few of them – *Pax6*, *Pitx3* and *Crybb2* – indicating my personal view on the way from “eye to brain”.

2. From eye to brain

2.1. PAX6: master control gene in eye development and (master regulator) in neurogenesis

Small-eye mutants in the mouse and eyeless mutations in *Drosophila* have been identified since the beginning of experimental genetics, just because of their obvious phenotypes. One of the mouse mutants (gene symbol *Sey*) was the first being characterized as mutation in the *Pax6* gene (paired box 6; Hill et al., 1991). Quiring et al. (1994) showed that the eyeless gene of *Drosophila* (gene symbol: *ey*), the *Sey* gene in mice, and the gene causing aniridia in humans are homologous genes. Since the pioneering experiment demonstrating that the mouse *Pax6* gene can ectopically induce eye development in *Drosophila*, *Pax6* is considered as the “master control gene of eye development” (Halder et al., 1995). And shortly later, Stoykova et al. (1996) observed in the *Sey* mutant mice also forebrain patterning defects. Today, *Pax6* is considered also as one of the master regulator genes of neurogenesis in the brain (Paridaen and Huttner, 2014).

One of the key features for deciphering the role of *Pax6* in the brain was the finding of its expression in the radial glia cells which were considered previously as cables for migrating neurons and were thought to transform into glial cells at later stages of development; however, it turned out today that they are neuronal stem and progenitor cells in the ventricular zone (Malatesta and Götz, 2013). For the detailed dissection of the role of the various domains of the PAX6 transcription factor – the paired domain (PD), the homeodomain (HD) or the transactivating Proline-Serine-Threonine (PST) domain (Fig. 1), several *Pax6* alleles have been used, which have all been identified in mutation screens for eye diseases: *Leca2* (R128C) and *Leca4* (N50K) as lens-corneal adhesions (Thaung et al., 2002), *Pax6*^{4Neu} (S273P, Favor et al., 2001), *Sey* as small eye (G194X; Hill et al., 1991) or *Aey18* as anomaly of the eye (the mutation is in intron 5 leading to a splice defect, skipping exons 5a and 6; Graw et al., 2005).

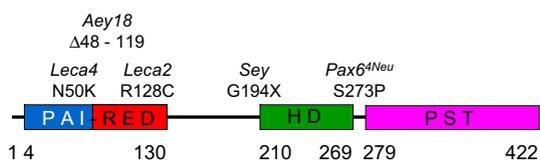


Fig. 1. Schematic drawing of the functional domains of PAX6: The canonical transcription factor PAX6 of the mouse (UniProt P63015, 422 amino acids) consists of 3 functional domains: the paired domain (aa 4–130), the homeodomain (green; aa 210–269) and the Pro-Ser-Thr-rich domain (PST domain, magenta; aa 279–422). The paired domain can be divided into the sub-domains PAI (blue) and RED (red). The mouse alleles mentioned in the text and leading to various *small-eye* phenotypes are given above, and the consequences for the amino-acid sequence are indicated. Scale is not to draw.

As examples, Haubst et al. (2004) compared the *Pax6*^{4Neu} mutants (mutation inactivates the HD domain) with the *Aey18* (mutation leads to a functional loss of the PD domain): the *Pax6*^{4Neu} mutant has just subtle defects of forebrain development, whereas in *Aey18* mutants the regulation of neurogenesis, cell proliferation and patterning are severely affected in the developing forebrain. Based upon structural data, the paired domain was subdivided (“hyphenated”) into two halves (pai-red) and have been therefore named as PAI (N-terminal) and RED (C-terminal) subdomains (Jun and Desplan, 1996). Thanks to the eye mutants *Leca4* (N50K) and *Leca2* (R128C), the functional difference of these two subdomains could be elaborated: the PAI subdomain mutation affects neurogenesis, while the PAI and RED subdomain mutations reduce and increase, respectively, the number of mitoses (Walcher et al., 2013). In continuation of this approach, Huettl et al. (2015) analyzed divergent functions of the PAI and RED sub-domains at distinct levels within the neural tube: within the hindbrain, the mutation of the PAI sub-domain (*Leca4*) severely affects patterning of the domain of the progenitor motor neurons and their establishment later on. Interestingly, the mutation in the RED sub-domain (*Leca2*), while not playing a role for neuronal generation at either level within the neural tube, affects axonal pathfinding of hypoglossal axons within the hindbrain. Since all these experiments have been performed in homozygous mutant embryos, it might be noteworthy that among the *Pax6* mutations discussed here, only the homozygous *Leca2* allele can survive after birth to adulthood (Walcher et al., 2013) – all other homozygous *Pax6* alleles die at birth (there is one other exception reported, *Pax6*^{132–14Neu}; Favor et al., 2008). Therefore, it remains highly speculative, how these effects during embryonic development might influence the behavior during adulthood. Besides the mouse mutant, also a spontaneous *Pax6* mutation in the rat was reported, rat small eye (*rSey*²; Matsuo et al., 1993). The mutation in the rat leads to a “break” downstream of the homeodomain including loss of the transactivating PST domain. Heterozygous rat mutants showed impaired prepulse inhibition (PPI), and they also exhibited changes in their social interaction (more aggression and withdrawal) in addition to impairment in rearing activity and in fear-conditioned memory as compared to wild-type rats (Umeda et al., 2010). These authors summarize their findings that the heterozygous *rSey*² mutants have some phenotypic components of autism. If also similar experiments will be done for the various mouse mutants, we may expect some more clues for human mental health or illness – most likely depending on the site of the mutation.

2.2. Eye and Parkinson's disease: Pitx3

Mutations in the human *PITX3* gene encoding the paired-like homeodomain transcription factor 3 were reported first to be causative for autosomal dominant anterior segment mesenchymal dysgenesis (ASD) and cataract (Semina et al., 1998). These original findings have been confirmed in several additional studies (Berry et al., 2004; Addison et al., 2005; Finzi et al., 2005; Bidinost et al., 2006; Burdon et al., 2006; Summers et al., 2008; Verdin et al., 2014). Among the various mutations, a 17bp duplication (c.640_656dup) is the most recurrent mutation being causative for ASD in 12 of the 18 families tested so far (Verdin et al., 2014). This mutation leads to a frameshift and, therefore, to a replacement of the 82 C-terminal amino acids of the wild type with 94 new residues. This replacement deletes the OAR box (named according to the first 3 members of this protein family, OTP, ARISTALESS and RAX).

Surprisingly, no eye defect has been found in a 17-year-old male patient with an ~317 kb hemizygous deletion on chromosome 10q24.32 involving also *PITX3* (Derwińska et al., 2012). His Smith-Magenis syndrome-like phenotype includes mild intellectual

impairment, sleep disturbance, hyperactivity, and self-destructive behavior. Since L-DOPA is absent from his cerebrospinal fluid, treatment with L-DOPA led to mild mitigation of some of the symptoms (Derwińska et al., 2012).

In the mouse, mutations affecting the *Pitx3* gene are responsible for the aphakia (gene symbol *ak*) (Varnum and Stevens, 1968; Semina et al., 2000; Rieger et al., 2001), eyeless (gene symbol *eyl*) (Rosemann et al., 2010) and microphthalmia/aphakia (gene symbol *miak*) phenotypes (Wada et al., 2014); all three mutations are of spontaneous origin and demonstrate a recessive mode of inheritance. The *ak*-mouse was one of the most famous eye mutants in the pre-genomic area; the molecular analysis demonstrated 2 deletions in the promotor area including the untranslated 1st exon, which leads to a loss of *Pitx3* expression in the developing eye (Semina et al., 2000; Rieger et al., 2001). In the *eyl*-mutants, a point mutation (416insG) leads to a frame shift and therefore, to an inactive hybrid protein (Rosemann et al., 2010). Similarly, the *miak* mouse is characterized by a point mutation (444C -> A) leading to a stop codon after position 147 (Y148X). This mutation lets the homeodomain intact, but more than the half of the C-terminal part (including the OAR region) is lost (Wada et al., 2014). In the developing lens, *Foxe3* is a direct target gene of PITX3 (Ahmad et al., 2013).

After the first characterization of the role of *Pitx3* during eye development, the group of Marten Smidt and Peter Burbach in Leiden (NL) discovered molecular and cellular alterations in the *Pitx3*-deficient midbrain dopaminergic system of the *ak*-mice (Smidt et al., 2004; Smits et al., 2005). A more detailed anatomical investigation revealed that the *Pitx3*-expressing region in the wild-type mice can be associated with the substantia nigra pars compacta – this is exactly the region, where dopaminergic neurons are missing (or get lost) during Parkinsonism. However, the loss of dopaminergic neurons in the *ak* mice did not lead to an altered walking pattern or tremor in the *ak* mice suggesting that motor control itself is not affected in the *ak* mice (Smidt et al., 2004).

In the substantia nigra, PITX3 was shown to be a direct activator of the gene encoding tyrosine hydroxylase (gene symbol *Th*), which is considered to catalyze the rate-limiting step in dopamine synthesis (Maxwell et al., 2005). Also, epidemiological studies demonstrated associations between polymorphic sites in the (human) *PITX3* gene and Parkinson's disease (Fuchs et al., 2009; Haubenberger et al., 2009; Bergman et al., 2010).

2.3. Eye and schizophrenia: *Crybb1* and *Crybb2*

Crystallins have been believed for decades as the only organ-

specific proteins (Bloemendal, 1981a). Mutations in the crystallin-encoding genes are causative for various forms of congenital or juvenile forms of cataracts (for a review see Churchill and Graw, 2011), and posttranslational modifications of the crystallins are discussed to be major risk-factors for age-related cataracts (Truscott and Friedrich, 2015). However, by technical progress it became more and more evident that at least some crystallin genes are expressed also in tissues outside the lens. As one example, *Cryab* gene (encoding α B-crystallin) should be mentioned, which is expressed in the heart and muscles; corresponding mutations in the human gene (*CRYAB*) lead, therefore, to cataracts and cardiomyopathies (for review see Graw, 2009).

Moreover, the *Crybb2* gene is coming now into the focus, too. It encodes the β B2-crystallin, which was previously referred to as the “basic principle crystallin” (Bloemendal, 1981b) because of its strong expression in the lens. However, later it turned out that it is expressed also in testes (DuPrey et al., 2007) and in several regions of the brain (hippocampus, olfactory bulb, cerebellum, cerebral cortex) (Ganguly et al., 2008). Behavioral analysis of a corresponding homozygous mouse mutant (*Crybb2*⁰³⁷⁷; the point mutation in intron 5 affects splicing and leads to additional 19 amino acids in front of exon 6) showed a decreased acoustic startle reflex in combination with an increased prepulse inhibition. More detailed examination of these mutant mice revealed a decrease of parvalbumin-positive interneurons in the hippocampus and an increased translation of input-to-output neuronal activity in the dentate gyrus of the hippocampus (Sun et al., 2013).

More surprisingly, a meta-analysis of gene expression quantitative trait loci in the human cortex with five psychiatric disorders (attention-deficit hyperactivity disorder, autism, bipolar disorder, major depressive disorder and schizophrenia) demonstrated the *CRYBB2* gene as the most significant association ($q = 1.75 \times 10^{-38}$; Kim et al., 2014). The corresponding SNP (single nucleotide polymorphism) rs997872 is described as intronic of *CRYBB2* with a global minor allele frequency of 25.8% (dbSNP at <http://www.ncbi.nlm.nih.gov/snp/?term=rs997872>; Sept. 15, 2015); however, a more detailed search for this SNP in the ENSEMBL database revealed that it is located in intron 3 of the *CRYBB2* pseudogene, ψ *CRYBB2*, which is located ~200 kb downstream of the functional *CRYBB2* gene (http://www.ensembl.org/Homo_sapiens/Transcript/Exons?db=core;g=ENSG00000100058;r=22:25230000-25484000;t=ENST00000354451; Sept. 15, 2015; the genomic organisation of the human *CRYB*-cluster is given in Fig. 2).

In line with the above-mentioned data, Spadaro et al., 2015 reported in 2015 interesting data on the “schizophrenia-related gene”

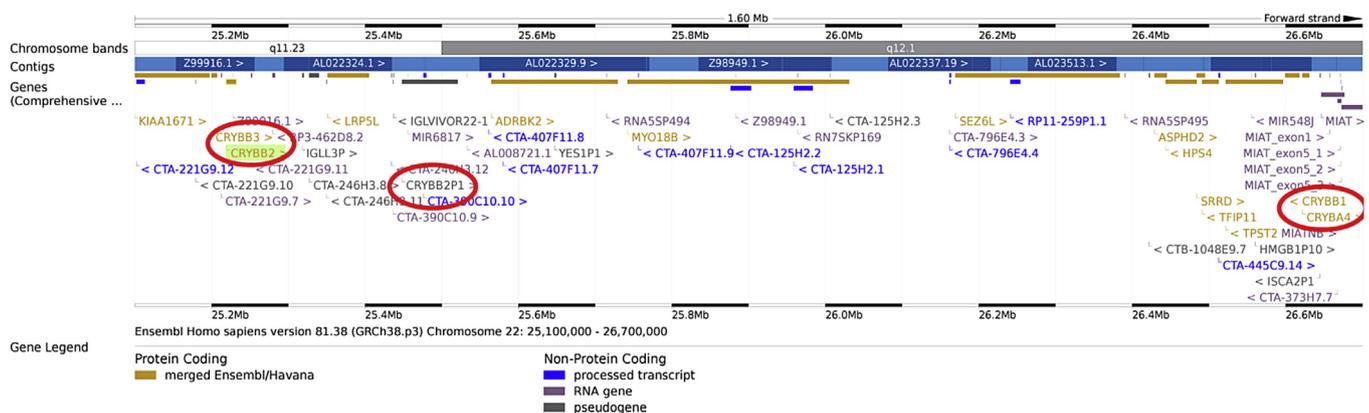


Fig. 2. Genomic organization of the *Cryb* cluster on mouse chromosome 5: The figure shows the 4 *CRYB* genes on human chromosome 22 including the additional pseudogene of *CRYBB2*, all circled in red. The two groups of *CRYBB2*/*CRYBB3* and *CRYBB1*/*CRYBA4* are separated by 1.4 Mb including several protein-coding and non-protein coding genes. The pseudogene of *CRYBB2* (*CRYBB2P1*) is ~2 kb downstream of the regular *CRYBB2* gene; the orientation of the *CRYBB1* and *CRYBA4* gene is in a head-to-head orientation indicating that they are in an opposite orientation with common regulatory regions. (source: Ensembl release 81 - July 2015).

Crybb1 (encoding β 1-crystallin). First, in a set of fear conditioning experiments, the authors observed a significant increase in the level of *Crybb1* transcripts (but not in the closely related *Cryba4* gene (encoding β A4-crystallin)). And in contrast, when the authors knocked down the *Crybb1* expression level in the brain using short hairpin RNAs and a lentiviral system for gene transfer in a stereotaxic surgery, they observed a decrease in anxiety-related behavior of the knockdown mice (i.e. more time spent in the center of their test arena, while travelling significantly shorter distances). Similarly, *Crybb1* knockdown mice also spent significantly more time within the open arms of the elevated plus maze. The authors summarize their finding as “a novel role for *Crybb1* in mediating fear and stress-associated responses with its repression appearing to reduce anxiety-like behavior” (Spadaro et al., 2015). In humans, mutations in the *CRYBB1* gene leads either to dominant (Mackay et al., 2002) or to recessive cataracts (Cohen et al., 2007).

3. Conclusion and outlook

In conclusion, we have demonstrated at a few examples that there is a close relationship between genetically defined disorders affecting both the eyes and the brain. In the beginning of the paper, the question was asked whether lens opacification can be used as a biomarker for Alzheimer's disease. At the end, I would like to ask the question in the other way around: can we identify molecules being important for the development of Alzheimer also in the eye? Indeed, there are some hints that the amyloid β has been detected also in the retina – and there is some hope that by *in-vivo* imaging of the retina a more effective diagnosis of Alzheimer's disease might be possible – independent from patient's cognitive input (Chang et al., 2015).

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