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Distinct Embryonic Expression and Localization of CBP and p300 Histone Acetyltransferases at the Mouse αA-Crystallin Locus in Lens

Ying Yang, Louise V. Wolf and Ales Cvekl*

Department of Ophthalmology and Visual Sciences Albert Einstein College of Medicine, Bronx, NY10461 USA

Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx NY10461, USA

Mouse αA-crystallin gene encodes the most abundant protein of the mammalian lens. Expression of αA-crystallin is regulated temporally and spatially during lens development with initial expression in the lens vesicle followed by strong upregulation in the differentiating primary lens fibers. Lens-specific expression of αA-crystallin is mediated by DNA-binding transcription factors Pax6, c-Maf and CREB bound to its promoter region. Its 5′-distal enhancer, DCR1, mediates regulation of αA-crystallin *via* FGF signaling, while its 3′-distal enhancer, DCR3, functions only in elongated primary lens fibers *via* other lens differentiation pathways. DCR1 and DCR3 establish outside borders of a lens-specific chromatin region marked by histone H3 K9 acetylation. Here, we identified CREB-binding protein (CBP) and p300 as major histone acetyltransferases (HATs) associated *in vivo* with the mouse αA-crystallin locus. Both HATs are expressed in embryonic lens. Expression of CBP in primary lens fiber cells coincides with αA-crystallin. In the chromatin of lens epithelial cells, chromatin immunoprecipitations (ChIPs) show that the αA-crystallin promoter is notably devoid of any significant presence of CBP and p300, though DCR1 and a few other regions show the presence of these HATs. In the chromatin obtained from newborn lens, CBP was localized specifically at the promoter region with about ten times higher abundance compared to the entire αA-crystallin locus. In contrast, p300 is distributed more evenly across the entire locus. Analysis of total histone H3 and H3 K9 acetylation revealed potential lower density of nucleosomes 2 kb upstream from the promoter region. Collectively, our data suggest that moderate level of αA-crystallin gene expression in lens epithelial cells does not require the presence of CBP and p300 in the promoter. However, the lens-specific chromatin domain contains both promoter localized CBP on the "background" of locus-spread presence of CBP and p300.

*Corresponding author

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Introduction

The eukaryotic genome is packed into a highly organized nucleoprotein structure, the chromatin fiber.[1] The regulation of gene expression through chromatin requires coordinated actions of multiple enzymes and proteins to modify chromatin structure to either promote or repress transcription. These "chromatin remodeling" activities include local and global modifications of core histone proteins, change of positions of individual nucleosomes and removal of nucleosomes from promoter regions.[2,3] It is hypothesized that a specific combination of these local/global activities are required for transcriptional regulation of any particular gene.[4]

Post-translationally modified core histones, with acetylated and methylated lysine and methylated arginine residues, serve as distinct marks to recruit

Abbreviations used: CBP, CREB-binding protein; HATs, histone acetyltransferases; ChIP, chromatin immunoprecipitation; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

E-mail address of the corresponding author: cvekl@aecom.yu.edu

proteins to the chromatin, and are thought to operate *via* the "histone code" of epigenetic gene regulation.[5,6] Acetylation of lysine residues in histone tails is catalyzed by a family of histone acetyltransferases (HATs), including ATF2, CREB-binding protein (CBP), MOZ, p300, P/CAF, pCIP, SRC-1, TAF250 and Tip60.[7] These HATs exhibit different substrate specificities, affinities for interacting proteins and can acetylate non-histone substrates.[7] Acetylated histones are recognized by bromodomain-containing proteins such as ATP-dependent chromatin remodeling enzymes Brg1 and Snf2h, which are catalytic subunits of a variety of multiprotein chromatin remodeling complexes SWI/SNF and ISWI, respectively.[8,9] These chromatin remodelers can move nucleosomes along the DNA or remove them completely to generate nucleosome-free regions in chromatin.[10]

During development, transcription of batteries of genes is controlled precisely in space and time. A large number of studies have shown that specific DNA-binding transcription factors bound to DNA in chromatin serve as platforms to recruit chromatin remodeling enzymes.[4,11] In parallel, modifications of local chromatin structure facilitate binding of additional DNA-binding transcription factors and this process culminates with active transcription.[4,10,12]

The key structural proteins of mammalian lens are 15 genes encoding the α- and β/γ-crystallins required for lens transparency and light refraction.[13–15] The αA-crystallin is the most abundant crystallin in mammalian lens.[13–15] Structurally and functionally, αA-crystallin belongs to a family of small heat shock proteins acting as molecular chaperones.[13–15] Expression of αA-crystallin is regulated tightly both temporally and spatially at the level of transcription with the onset of expression in the lens vesicle around E10.5 of mouse embryonic development.[16] αA-crystallin expression is boosted dramatically during lens fiber cell differentiation starting at E12.5.[16] Using H3 K9 acetylated histone as a marker of transcriptionally active chromatin, we recently identified at least a 16 kb domain of lens-specific chromatin harboring the entire αA-crystallin (*Cryaa*) locus. Two enhancers, DCR1 and DCR3, are near its 5′/3′ borders.[17] Responsive to FGF signaling in cultured lens explants, the DCR1 in combination with a 1.9 kb αA-crystallin promoter is able to recapitulate αA-crystallin endogenous expression pattern in the transgenic mouse model.[17] In contrast, the 1.9 kb promoter/DCR3 transgene was activated only in more elongated primary lens fibers. Array of Pax6-binding, Maf-binding and CRE-binding sites have been identified within both DCR1 and the promoter.[17–21] Local presence of chromatin remodeling enzymes Brg1 and Snf2h was linked to the presence of Pax6 and c-Maf in the αA-crystallin locus.[17] Nevertheless, there remained the question of the possible roles of HATs, CBP and p300 suggested by earlier gene reporter and transgenic mouse overexpression studies.[22,23]

CBP and p300 are highly structurally homologous HATs involved in cell proliferation, differentiation and apoptosis.[24,25] CBP/p300 have been shown to function as scaffolds or bridges between two DNA-binding transcription factors,[26] and to acetylated histones *via* their internal bromodomains or other non-histone proteins.[27–30] Mutations in human CBP cause Rubinstein-Taybi syndrome, characterized by a wide array of ocular defects, including cataracts and glaucoma together with mental retardation and malformed thumbs and toes.[31,32]

Although CBP and p300 are highly homologous and co-expressed in a variety of tissues, gene targeting studies suggest that their functions are not redundant.[33–37] Here, we show that both CBP and p300 are expressed in lens with different expression patterns. Distinct physical recruitment patterns of CBP and p300 through the αA-crystallin locus *in vivo* by chromatin immunoprecipitation (ChIP) suggest that they play unique roles in regulating the αA-crystallin gene expression, in agreement with studies of other genes during neural and cardiac development.[37]

Results

CBP and p300 have distinct expression patterns during mouse lens development

Previously, CBP/p300 expression was reported in the epithelial cells of E15.5 lens.[23] However, the antibody originally used recognized both CBP and p300 proteins. To identify precisely the expression patterns of CBP and p300 during mouse lens development, we analyzed CBP and p300 expression in earlier lens developmental stages using specific antibodies against CBP or p300. During the invagination stages of lens development from E10.5 to E11.5 (Figure 1(a) and (b), and (d) and (e)), both CBP and p300 are detected in the lens pit as well as lens vesicle. As expected, expression of both proteins is detected also in the non-lens surface ectoderm, developing optic cup and in the periocular mesenchyme. As primary lens fiber cells differentiate, they express high levels of αA-crystallin,[16] and fill up the lens vesicle by E14.5. At this stage, stronger signals of CBP were detected predominantly in the primary lens fiber cells and transitional zone, while only a few cells were highlighted in the lens epithelium (Figure 1(c) and (g)). However, p300 appears to be expressed strongly in both the lens epithelium and the primary lens fiber cells (Figure 1(f) and (h)). Interestingly, cytoplasmic rather than nuclear localization of p300 was observed in E10.5 mouse lens pit (Figure 1(d)). This transient cytoplasm localization was observed only at this stage and was reproducible using a variety of modifications of the immunostaining procedures (data not shown).

Next, we analyzed the mRNA levels of CBP and p300 by quantitative RT-PCR using microdissected two days old rat lenses. We found approximately