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LAP2ζ binds BAF and suppresses LAP2β-mediated transcriptional repression

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Abstract

Proteins of the nuclear envelope have been implicated as participating in gene silencing. BAF, a DNA- and LEM domain-binding protein, has been suggested to link chromatin to the nuclear envelope. We have previously shown that LAP2β, a LEM-domain inner nuclear membrane protein, represses transcription through binding to HDAC3 and induction of histone H4 deacetylation. We now show that LAP2ζ, the smallest LAP2 family member, is also involved in regulation of transcription. We show that similar to other LEM-domain proteins LAP2ζ interacts with BAF. LAP2ζ-YFP and BAF co-localize in the cytoplasm, and overexpression of LAP2ζ leads to reduction of nucleoplasmic BAF. Mutations in the LAP2ζ-YFP LEM domain decrease its interaction with BAF retaining the nucleo-cytoplasmic distribution of BAF. Co-expression of LAP2β and LAP2ζ results in inhibition of LAP2β-induced gene silencing while overexpression of LAP2ζ alone leads to a small increase in transcriptional activity of various transcription factors. Our results suggest that LAP2ζ is a transcriptional regulator acting predominantly to inhibit LAP2β-mediated repression. LAP2ζ may function by decreasing availability of BAF. These findings could have implications in the study of nuclear lamina-associated diseases and BAF-dependent retroviral integration.

Keywords: LAP2; BAF; LEM; Nuclear envelope; Nuclear lamina; Transcription; Histone modification; Epigenetics; Chromatin; PIC

Introduction

The nuclear lamina, outlining the nuclear envelope, is composed of lamins A/C and B and their binding partners at the inner nuclear membrane (INM). The nuclear lamina forms a scaffold which stabilizes nuclear structure and serves as a platform for the organization of protein complexes involved in chromatin organization, DNA replication, transcription and signal transduction (Gruenbaum et al., 2005). Mutations in nuclear lamina proteins have been shown to cause a wide array of genetic diseases such as Hutchinson-Gilford progeria, dilated cardiomyopathy, Emery-Dreifuss muscular dystrophy and others collectively termed laminopathies or

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nuclear envelopathies (Somech et al., 2005a; Worman and Bonne, 2007). Although ubiquitously expressed, mutations in these proteins lead to diverse, tissue-specific, clinical manifestations. One hypothesis regarding the role of the nuclear lamina in disease pathogenesis is based on cumulative evidence showing involvement of nuclear lamina proteins in gene repression (Shaklai et al., 2007). Several observations support such involvement: silent heterochromatin clusters at the nuclear periphery, gene-poor chromosomes are more peripherally configured than gene-rich chromosomes (Croft et al., 1999), and transcriptionaly silent genes are located at, or translocated to the nuclear periphery (Brown et al., 1997; Dietzel et al., 2004; Kosak et al., 2002; Malhas et al., 2007). Transcriptional repressors have also been found to associate with nuclear lamina proteins. The lamin B receptor (LBR) binds heterochromatin protein 1 (HP1) and histones H3/H4 under deacetylating conditions (Kourmouli et al., 2000; Polioudaki et al., 2001). We have shown that the INM protein lamina-associated polypeptide 2β (LAP2β) recruits histone deacetylase 3 (HDAC3) and the transcriptional repressor germ-cell-less (GCL) to the nuclear envelope (Nili et al., 2001; Somech et al., 2005b), and both lamin A and LAP2α bind the hypophosphorylated form of the retinoblastoma protein (Mancini et al., 1994; Markiewicz et al., 2002). Although data regarding the components of these lamina-bound complexes has begun to accumulate, the dynamics of their formation are yet unknown.

The LAP2 family of INM proteins consists of six alternatively spliced isoforms; LAP2α, β, δ, ε, γ and ζ (Berger et al., 1996; Foisner and Gerace, 1993; Furukawa et al., 1995; Harris et al., 1994). All members share a common, 186 amino acids long N-terminal region which contains two functional domains; the LEM (LAP2, emerin and Man1) domain which binds to barrier-to-autointegration factor (BAF), a non-specific DNA-binding protein (Shumaker et al., 2001) and the ''LEM-like'' domain which binds DNA directly (Cai et al., 2001; Foisner, 2001). Four family members (LAP2β, δ, ε and γ) are type II integral proteins of the INM (hence termed ''membrane isoforms'') and contain a lamin B-binding domain (Berger et al., 1996; Furukawa et al., 1995). The membrane isoforms differ in the size of the ''LAP2β-specific region'' they express due to alternative splicing. LAP2β contains the complete ''LAP2β-specific region'' previously shown by us to bind GCL and HDAC3 (Nili et al., 2001; Somech et al., 2005b). LAP2ε, δ and γ, each contain a smaller fraction of the ''LAP2β-specific region'' in descending order (Berger et al., 1996). LAP2α contains a unique C-terminal region with a lamin A-binding domain, and is predominantly intranuclear (Dechat et al., 2000). LAP2α was shown to be part of the Moloney murine leukemia virus (MMLV) pre-integration complex (PIC) where it interacts with BAF stimulating intermolecular integration and suppressing viral autointegration (Suzuki et al., 2004). Mutations in LAP2α have recently been implicated as a rare cause of familial dilated cardiomyopathy due to decreased prelamin A binding (Taylor et al., 2005). The sixth LAP2 family member, LAP2ζ, is the smallest isoform and has been cloned thus far only in mouse (Berger et al., 1996). LAP2ζ is a short version of the LAP2 membrane isoforms sharing their common LEM domain containing N-terminal but lacking the lamin-binding and nuclear localization signals. Mouse LAP2ζ terminates in a unique five amino acids long exon at its C-terminus (Berger et al., 1996).

BAF is a 10-kDa, conserved, essential chromatin protein (Segura-Totten and Wilson, 2004). BAF binds non-specifically to double-stranded DNA (Umland et al., 2000; Zheng et al., 2000), histone H3 and H1.1 (Montes de Oca et al., 2005), lamin A (Lee et al., 2001), BAF-like (Tifft et al., 2006) and LEM-domain proteins (Cai et al., 2001; Furukawa, 1999; Lee et al., 2001; Shumaker et al., 2001). It is located in the nucleoplasm, cytoplasm and nuclear membrane (Lee and Craigie, 1988, 1994; Segura-Totten and Wilson, 2004). BAF was first isolated from retroviral PICs as a cellular component exploited by the virus in the integration process (Chen and Engelman, 1998; Engelman, 2003; Lee and Craigie, 1988, 1994; Lin and Engelman, 2003; Suzuki and Craigie, 2002). Its intracellular distribution has recently been shown to vary according to the cell cycle with nuclear localization correlating to progression of S phase (Haraguchi et al., 2007). The physiological functions of BAF are unknown. In accordance with its DNA-binding ability BAF has been shown to influence chromatin structure (Mansharamani et al., 2003; Margalit et al., 2007; Segura-Totten et al., 2002). BAF represses cone-rod homeobox (Crx)-dependent transcription (Wang et al., 2002) and has recently been shown to bind histones (Montes de Oca et al., 2005). Regulation of BAF depends on its modification state (Bengtsson and Wilson, 2006) and binding partners identified to date in the nucleus, nuclear membrane and PIC (Montes de Oca et al., 2005; Segura-Totten and Wilson, 2004; Tifft et al., 2006).

Here we show the cloning and characterization of human LAP2ζ. We show that LAP2ζ binds BAF through the LEM domain and that both proteins co-localize in the cytoplasm. Overexpression of LAP2ζ leads to depletion of BAF from the nucleus and enhances transcription. We also show that co-expression of LAP2ζ with either LAP2β or HDAC3 results in suppression of the inhibitory activity of the latter two proteins. We therefore suggest that LAP2ζ is a transcriptional regulator which may exert its effect through manipulation of BAF localization possibly interfering with its capacity to bridge between chromatin and the nuclear envelope.