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The multiple activities of CtBP/BARS proteins: the Golgi view

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The C terminal-binding protein (CtBP) family functions in the nucleus as co-repressors of transcription and has a crucial role in differentiation, apoptosis, oncogenesis and development. Recently, the products of the *CtBP1* gene have been implicated in important cytoplasmic functions, including membrane fission in intracellular trafficking, the partitioning of the Golgi complex during mitosis and the organization of ribbon synapses. This has led to a redefinition of the CtBPs as multifunctional proteins. Shuttling of CtBPs between the nucleus and the cytoplasm can be finely regulated by post-translational modifications. In addition, the structural homology with the dehydrogenase family of proteins and the ability of CtBPs to bind NAD^+ and acyl-CoAs have offered clues to the molecular mechanisms that enable these proteins to have different functions. Here, we discuss the cytoplasmic roles of the CtBPs and the possible mechanisms that enable them to switch between cell compartments and multiple functions.

The CtBPs, a protein family with multiple cellular functions

The CtBP (C terminal-binding protein) protein family has attracted interest over the past decade because of its many important functions, both in the nucleus as transcriptional co-repressors, and in the cytosol in the control of membrane trafficking. In mammals, the CtBP family is encoded by two genes: *CtBP1* and *CtBP2*. CtBP1 has two splice variants, CtBP1-L (long; previously known as CtBP1) and CtBP1-S/BARS (short; previously known as CtBP3/BARS) (Figure 1) [1]. CtBP2 and RIBEYE are splice variants of the *CtBP2* gene (Figure 1). CtBPs are structurally similar to the ancient protein family of the D-hydroxyacid dehydrogenases [2,3]. Among this dehydrogenase family, the probable ancestor of the CtBPs is phosphoglycerate dehydrogenase, which functions as a rate-limiting enzyme in serine synthesis and is the closest functional CtBP homologue (Figure 2) [3].

The first members of the CtBP family to be cloned were CtBP1-L and CtBP2, which were characterized as important transcriptional repressors (Box 1) [4]. CtBP1-S/BARS (here referred as CtBP/BARS for simplicity) and RIBEYE were identified independently and cloned on the basis of their roles in membrane trafficking (CtBP/BARS) and the localization in synaptic ribbons (RIBEYE) [5,6]. CtBP/BARS functions in membrane fission at different intracellular transport steps [7] and RIBEYE is a component of the synaptic ribbon in sensory neurons [8]. Recently, CtBP1-L has also been shown to be present in the synaptic ribbon and in the active zone of the mammalian synapse, where its function remains to be determined [8]. A single form of CtBP is present in invertebrates, where it has a role in the regulation of transcription and development [9,10], and in plants, where it has a different C terminus, and where its function has been mainly related to the organization of microtubules; a role in transcription has been suggested but not yet directly proven [11,12].

In mammals, the high degree of homology (97%) between CtBP1-L and CtBP/BARS suggests functional redundancy. Initially, however, CtBP1-L was characterized as a transcription factor, and CtBP/BARS as a fission protein, suggesting that these splice variants had separate functions. However, the observation that CtBP1-L behaves similarly to CtBP/BARS in transport assays (A.L. *et al.*, unpublished data) indicates that CtBP1-L can be considered a dual-function protein. Whether CtBP/BARS also functions as a transcriptional co-repressor remains unknown. Here, we focus on the cytoplasmic roles of the CtBPs as controllers of intracellular trafficking, specifically in the process of membrane fission. We discuss the mechanisms by which the CtBPs might switch their locations and roles in different cell compartments, and the possible physiological significance of this multiple functionality. For details of the transcriptional activity (Box 1), see reviews [4,13,14].

CtBP/BARS membrane fissioning activity

CtBP/BARS was originally identified as a 'brefeldin A (BFA)-dependent ADP-ribosylation substrate' during a search for factors that control membrane tubulation [15,16]. BFA is a fungal toxin that promotes the disassembly of the Golgi complex into tubules. Although BFA was known to block the GTP exchange factor for the small GTPase Arf, this did not fully explain the mechanism of the tubular disassembly of the Golgi complex. In a series of studies, it was shown that CtBP/BARS induces the formation of narrow constrictions in Golgi tubules as well as the complete fission of the Golgi tubules *in vitro* (Box 2) [17,18]. In addition, BFA promotes the ADP ribosylation of CtBP/BARS with high selectivity and potency, and this reaction inhibits the effects of BARS

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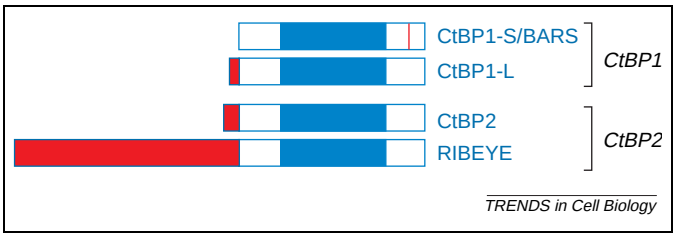

Figure 1. The CtBP protein family. The sequences of the CtBP family members show a conserved region in all CtBP proteins (white), which includes a dehydrogenase homology region (blue) [3]. Regions with no recognizable homology are shown in red. Full-length CtBP1-S/BARS consists of 430 amino acids and has a predicted molecular mass of 47 kDa. CtBP1-S/BARS has 97% identity to human and mouse CtBP1-L, and 79% identity to human and mouse CtBP2 [3]. The divergent region between CtBP1-L and CtBP1-S/BARS is at the N-terminus, where CtBP1-L has 11 additional amino acids [3,5,19].

on Golgi tubule fission [15,17]. ADP ribosylation might therefore facilitate the formation and elongation of Golgi tubules and synergize with the effects of BFA on the Arf GTPase, contributing to the disassembly of the Golgi complex [5,17,18].

CtBP/BARS-dependent fission requires long-chain acyl-CoAs as essential cofactors [17]. These acyl-CoAs were originally proposed to be acyl donors for a lipid-specific acyl-transferase activity of CtBP/BARS, which leads to the conversion of lysophosphatidic acid into phosphatidic acid [17] (a lipid that facilitates the fissioning process [16]; Box 2). However, the acyl-transferase activity [17,19] could result from an activity associated with, rather than intrinsic to, CtBP/BARS [20]. Moreover, this activity facilitates, but is not essential for,

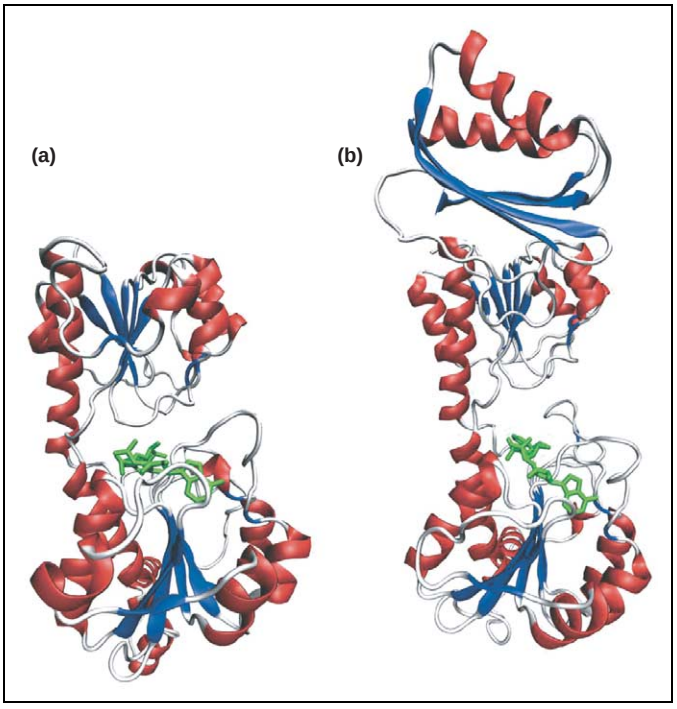

Figure 2. Ribbon diagram of the NAD(H)-bound, three-dimensional structures of CtBP1-S/BARS (residues 1–350) **(a)** and D-3-phosphoglycerate dehydrogenase (3-PGDH) **(b)**. CtBP1-S/BARS consists of two compact domains separated by a deep cleft; the two domains are structural variants of the Rossmann dinucleotide-binding domain [3]. Note the structural similarities between CtBP1-S/BARS and 3-PGDH. Secondary structural elements are indicated: β-sheet elements in blue; α helices in red. The bound NADH (green) is shown in a ball-and-stick representation. The structural rendering was generated using the Visual Molecular Dynamics program (VMD) [72].

several fission-requiring activities of BARS (the mitotic fragmentation of the Golgi complex, the formation of post-Golgi transport intermediates and the fission of COPI-coated vesicles [7,21,22]). More work is required to clarify the significance of this acyl-transferase activity in membrane fission. The alternative explanation as to why acyl-CoAs are required for CtBP/BARS-induced membrane fission is because they bind to the dinucleotide-binding domain (known as the Rossmann fold) of CtBP/BARS, and thereby induce a change in protein conformation that might promote fission [3,22].

CtBP/BARS induces membrane fission *in vivo*

CtBP/BARS-dependent membrane trafficking steps

Intracellular transport involves the formation of vesicular or tubular carriers, which bud and segregate from donor compartments (Box 2, Figure I) [16,23] and then fuse with an acceptor compartment. Membrane fission is a fundamental event in membrane trafficking.

Increasing evidence suggests that there are several fission machineries *in vivo*. Many fission events are driven by the proteins of the dynamin family, a versatile and structurally diverse group of large GTPases that can be subdivided into 'classic' dynamins and dynamin-related proteins [24–30]. Other fission events are controlled by coat protein complex II (COPII) [31,32] and by CtBP/BARS. CtBP/BARS is involved in membrane fission at several transport steps, including transport from the Golgi complex to the basolateral membrane in epithelial cells, fluid-phase endocytosis and retrograde transport of the KDEL receptor to the endoplasmic reticulum by COPI-coated vesicles [7,22]. COPI is composed of the coatomer, which is a seven-subunit protein complex that participates in the formation of Golgi-derived coated vesicles [34]. Dynamin is not involved in any of these steps (and, conversely, several dynamin-dependent steps do not involve CtBP/BARS), suggesting that CtBP/BARS and dynamin drive non-overlapping fission machineries [7].

The evidence showing that CtBP/BARS activates membrane fission *in vivo* is based on the analysis of the dynamics and structure of transport carriers. During trafficking from the Golgi complex to the basolateral plasma membrane in epithelial cells, the large pleiomorphic tubular carriers that operate between these two trafficking stations elongate out of the Golgi complex along microtubules before detaching to move to the plasma membrane [33]. When CtBP/BARS is inhibited (e.g. by dominant-negative mutants, specific antibodies or RNA interference), these tubules are unable to detach from the Golgi complex but continue to elongate out from and retract to the Golgi, blocking transport. This indicates that the fission of the tubular carriers is inhibited. Conversely, the microinjection of active recombinant CtBP/BARS results in a rapid formation of transport intermediates, possibly through super-stimulation of their fission machinery [7].

The retrograde traffic of the KDEL receptor to the endoplasmic reticulum requires CtBP/BARS, and COPI-coated vesicles are thought to be essential carriers at this stage [35,36]. When CtBP/BARS was inhibited, COPI-coated vesicles bud normally, but do not detach from Golgi