CHAPTER 10

CtBP3/BARS and Membrane Fission

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Abstract

CtBP3/BARS was the third protein of the CtBP (C-terminal binding protein) family to be identified. It was initially isolated as a 50-kDa cytosolic protein during the characterisation of the molecular targets of the toxin brefeldin A (BFA). As this protein is a substrate of BFA-dependent ADP-ribosylation, it was initially named BARS-50 (BFA-dependent ADP-ribosylation substrate), or BARS. After its purification and cloning, the protein was shown to be the third member (hence CtBP3/BARS) of the CtBP transcription corepressor family of proteins, sharing a high degree of aminoacid identity with CtBP1 (97%). CtBP3/BARS induces membrane fission in isolated Golgi membranes and is necessary for the fragmentation of the Golgi complex that occurs at the beginning of mitosis; its direct role in transcription regulation has not yet been specifically investigated. The CtBPs are thus a multifunctional protein family that can modulate both nuclear and cytosolic functions.

CtBP3/BARS As a Substrate of BFA-Dependent ADP-Ribosylation

As indicated above, CtBP3/BARS was initially identified as the 50-kDa substrate of BFA-dependent ADP-ribosylation.[1,2] BFA is a fungal toxin[3] that induces a very rapid block of secretion.[4,5] As with other toxins, BFA has been widely used over the last twenty years as a tool to elucidate the molecular mechanisms of transport. In addition to blocking secretory traffic, BFA induces a dramatic morphological reorganisation of the Golgi complex and the redistribution of both resident and cargo proteins from the Golgi complex to the endoplasmic reticulum (ER).[5-8] Moreover, BFA affects the morphology and function of the endosomal/lysosomal compartments by inducing the tubulation and fusion of the endosomal membranes.[9,10]

The first molecular target of BFA was identified as the exchange factor for the small GTPase ARF.[11,12] In 1994, we showed that BFA was also able to induce the ADP-ribosylation of two cytosolic substrates of 38 kDa and 50 kDa.[2] The 38-kDa substrate was identified as an isoform of glyceraldehyde 3-phosphate dehydrogenase (GAPDH),[2] a glycolytic enzyme with multiple cellular functions;[13] however, only a small percentage of the total cellular GAPDH is modified by BFA.[2] The 50-kDa substrate (CtBP3/BARS) was shown to contribute to the ability of BFA to disassemble the Golgi complex, indicating a possible role for this protein in the control of the structure of this organelle.[14] In order to obtain the protein sequence, CtBP3/BARS was purified from rat brain cytosol by following its ADP-ribosylation in the presence of [^{32}P]-NAD through four chromatographic steps.[15] After an 800-fold enrichment of cytosolic CtBP3/BARS, it was separated by two-dimensional gel electrophoresis, trypsin-digested, and subjected to protein microsequencing. The peptide sequences obtained from microsequencing were used to generate two probes to screen a rat brain cDNA library. One clone (GenBank Accession Number AF067795) contained a full-length open reading frame (ORF) that coded for a

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CtBP Family Proteins, edited by G. Chinnadurai.

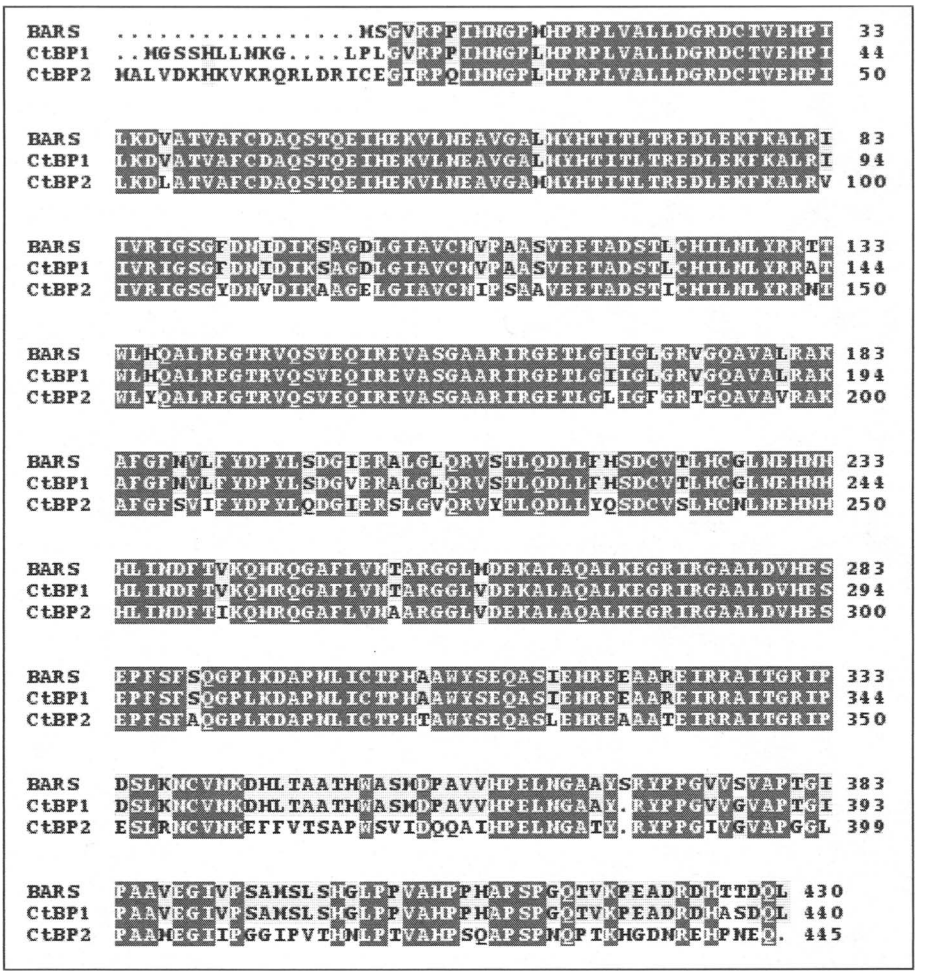

Figure 1. CtBP3/BARS belongs to the CtBP family. CtBP3/BARS is aligned with mouse CtBP1 (accession number AJ010483) and mouse CtBP2 (accession number AF059735). Identical residues are in white on a dark grey background; conserved residues in the sequences are in black on a light grey background. CtBP1 and CtBP3/BARS are also now referred to as CtBP1-L and CtBP1-S, respectively, as they represent the long and short splice variants deriving from the CtBP1 gene.

430-aminoacid protein with a predicted mass of 47 kDa. When transfected into COS7 cells, the cloned cDNA expressed a 50-kDa cytosolic protein that proved to be a substrate of ADP-ribosylation induced by BFA. The use of antibodies raised against CtBP3/BARS peptides or against a GST-CtBP3/BARS fusion protein in immunoprecipitation experiments also confirmed that the cDNA isolated in the screening actually coded for CtBP3/BARS.[15]

Rat CtBP3/BARS is highly similar to CtBP1 and CtBP2. These latter two proteins have been cloned in human and mouse. At the aminoacid level, CtBP3/BARS shares a 97% identity with human and mouse CtBP1 (accession numbers: U37408 and AJ010483), and a 79% identity with human and mouse CtBP2 (accession numbers: AF016507 and AF059735) (Fig. 1). A significant difference between CtBP1 and CtBP3/BARS resides the N-terminal portion, where the two proteins differ in sequence and length. Another sequence feature particular to