

# CtBP3/BARS and Membrane Fission

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### Abstract

**C**tBP3/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 multi-functional 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.<sup>1,2</sup> BFA is a fungal toxin<sup>3</sup> that induces a very rapid block of secretion.<sup>4,5</sup> 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).<sup>5-8</sup> Moreover, BFA affects the morphology and function of the endosomal/lysosomal compartments by inducing the tubulation and fusion of the endosomal membranes.<sup>9,10</sup>

The first molecular target of BFA was identified as the exchange factor for the small GTPase ARE.<sup>11,12</sup> In 1994, we showed that BFA was also able to induce the ADP-ribosylation of two cytosolic substrates of 38 kDa and 50 kDa.<sup>2</sup> The 38-kDa substrate was identified as an isoform of glyceraldehyde 3-phosphate dehydrogenase (GAPDH),<sup>2</sup> a glycolytic enzyme with multiple cellular functions;<sup>13</sup> however, only a small percentage of the total cellular GAPDH is modified by BFA.<sup>2</sup> 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.<sup>14</sup> In order to obtain the protein sequence, CtBP3/BARS was purified from rat brain cytosol by following its ADP-ribosylation in the presence of [<sup>32</sup>P]-NAD through four chromatographic steps.<sup>15</sup> 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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BARS	.....MSGVRRPTDNGPLHPRPLVALLDGRDCTVEHPI	33
CtBP1	..MGSSHLNKG...LPLGVRRPTDNGPLHPRPLVALLDGRDCTVEHPI	44
CtBP2	HALVDKHKVKRQRLDRICEGIRPQTDNGPLHPRPLVALLDGRDCTVEHPI	50
BARS	LKDVAIVAFCDQAQSTQEIHEKVLNEAVGALHYHTITLTREDLEKFKALRI	83
CtBP1	LKDVAIVAFCDQAQSTQEIHEKVLNEAVGALHYHTITLTREDLEKFKALRI	94
CtBP2	LKDLAIVAFCDQAQSTQEIHEKVLNEAVGALHYHTITLTREDLEKFKALRV	100
BARS	IVRIGSGFDNIIDINSAGDLGIAVCHVPAASVEETADSTLCHILNLYRRIT	133
CtBP1	IVRIGSGFDNIIDIKSAGDLGIAVCHVPAASVEETADSTLCHILNLYRRAT	144
CtBP2	IVRIGSGYDNIIDIKAGELGIAVCHVPSAAVEETADSTLCHILNLYRRNT	150
BARS	WLHQAALREGTRVQSVEQIREVASGAARIRGETLGLIGLGRVGOAVALRAK	183
CtBP1	WLHQAALREGTRVQSVEQIREVASGAARIRGETLGLIGLGRVGOAVALRAK	194
CtBP2	WLHQAALREGTRVQSVEQIREVASGAARIRGETLGLIGFGRTGQAVAVRAK	200
BARS	AFGFNVLFYDPYLSDGIERALGLORVSTLQDLLEHSDCVTLHCGLEMEHH	233
CtBP1	AFGFNVLFYDPYLSDGVERALGLORVSTLQDLLEHSDCVTLHCGLEMEHH	244
CtBP2	AFGFSVIFYDPYLDGIERSLGVORVYTLQDLLEHSDCVSLHCHLEMEHH	250
BARS	NLINDFTVKQHRQGAFLVMTARGGLVDEKALAQAALKEGRIRGAALDVHES	283
CtBP1	NLINDFTVKQHRQGAFLVMTARGGLVDEKALAQAALKEGRIRGAALDVHES	294
CtBP2	NLINDFTIKQHRQGAFLVMAARGGLVDEKALAQAALKEGRIRGAALDVHES	300
BARS	EPFSFSOGPLKDAPMLICTPHAAWYSEQASTEHREAAAREIRRAITGRIP	333
CtBP1	EPFSFSOGPLKDAPMLICTPHAAWYSEQASTEHREAAAREIRRAITGRIP	344
CtBP2	EPFSFAOGPLKDAPMLICTPHAAWYSEQASTEHREAAATEIRRAITGRIP	350
BARS	DSLKNCVNRDHLTAATHWASIDPAVVHPELNGAAYSRYPGVVSVAPTGI	383
CtBP1	DSLKNCVNRDHLTAATHWASIDPAVVHPELNGAAYRYPGVVSVAPTGI	393
CtBP2	ESLRNCVNRKFFVTSAPNSVIDQQAHPPELNGATYRYPGVVSVAPGGL	399
BARS	FAAVEGIVPSAMSLSHGLPPVAHPPHAPSPGQTVNPEADRDHSDOL	430
CtBP1	FAAVEGIVPSAMSLSHGLPPVAHPPHAPSPGQTVNPEADRDHSDOL	440
CtBP2	FAAVEGIIPGGIPVTHNLPVAHPPHAPSPNQPQKHGDNREHPNEQ	445

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.<sup>15</sup>

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