Gene Expression Patterns 7 (2007) 266–273

www.elsevier.com/locate/modgep

BCS1L is expressed in critical regions for neural development during ontogenesis in mice

Heike Kotarsky [a,*], Imran Tabasum [a], Susanna Mannisto [b,c], Markku Heikinheimo [b,c,d], Stefan Hansson [e], Vineta Fellman [a,b]

[a] *Department of Pediatrics, Clinical Sciences, Lund, Lund University, Lund, Sweden*
[b] *Department of Pediatrics, University of Helsinki, Helsinki, Finland*
[c] *Program for Developmental and Reproductive Biology, and Children's Hospital, University of Helsinki, P.O. Box 63, 00014 Helsinki, Finland*
[d] *Department of Pediatrics, Washington University, School of Medicine, St. Louis, MO 63310, USA*
[e] *Department of Obstetrics and Gynecology, Clinical Sciences, Lund, Lund University, Lund, Sweden*

Received 13 April 2006; received in revised form 7 September 2006; accepted 8 September 2006
Available online 16 September 2006

Abstract

BCS1L is a chaperone necessary for the incorporation of Rieske FeS and Qcr10p into complex III (CIII) of the respiratory chain. Mutations in the BCS1L gene cause early fetal growth restriction and a lethal neonatal disease in humans, however, the pathogenesis remains unclear. Here, we analysed the expression of BCS1L during mouse embryonic development and compared its expression with that of the mitochondrial markers Porin, GRIM19, Core I, and Rieske FeS. BCS1L was strongly expressed in embryonic tissues already at embryonic days 7 (E7) and 9 whereas the expression of Porin and Rieske FeS was not as evident at this time point. At E11, BCS1L, Porin, and Rieske FeS had overlapping expression patterns in organs known to contain high numbers of mitochondria such as heart, liver and somites. In contrast, BCS1L was differently distributed compared to the mitochondrial proteins Porin, Rieske FeS, Core I and Grim 19 in the floor plate of the E11, E12 and E13 neural tube. These results show that the expression pattern of BCS1L only partially overlaps with the expression of Porin and Rieske FeS. Thus, BCS1L alone or in cooperation with Rieske FES may during development have previously unknown functions beside its role in assembly of complex III. The floor plate of the neural tube is essential for dorsal ventral patterning and the guidance of the developing neurons to their targets. The predominant expression of BCS1L in this region, together with its presence in peripheral ganglia from E13 onwards, indicates a role for BCS1L in the development of neural structures.

Keywords: BCS1L; Complex III; Mitochondria; Chaperone; Floor plate; Ganglia; Mouse ontogenesis

1. Results and discussion

The mitochondrial respiratory chain is composed of five large protein complexes, encoded by either mitochondrial or nuclear genes and assembled in the mitochondrial membrane. The assembly of complex III (CIII) is facilitated by the chaperone bcs1 in yeast, and BCS1L in higher eukaryotes. By analysing respiratory deficient mutants of *Saccharomyces cerevisae* a gene necessary for the expression of functional bc1 complex was identified and named bcs1 (Nobrega et al., 1992). Yeast bcs1 is a mitochondrial membrane protein and belongs to the family of AAA ATPases (ATPases associated with a variety of activities) (Confalonieri and Duguet, 1995). Proteins belonging to this diverse family are mainly involved in substrate remodelling influencing protein folding and unfolding or the assembly of protein complexes (Hanson and Whiteheart, 2005; Ogura and Wilkinson, 2001). The mitochondrial AAA proteins, mAAA- and i-AAA protease are, for example, involved in quality control of mitochondrial proteins (Arnold and Langer, 2002).

* Corresponding author. Tel.: +46 46 222 07 46; fax: +46 46 222 07 48.
E-mail address: Heike.Kotarsky@med.lu.se (H. Kotarsky).

1567-133X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2006.09.005

The bcs1 protein is anchored in the inner mitochondrial membrane by a single transmembrane domain; it also contains an internal mitochondrial targeting signal and an import auxiliary region facilitating the import of bcs1 protein into the inner mitochondrial membrane (Fölsch et al., 1996; Stan et al., 2003). Once inside the inner membrane, bcs1 acts as a molecular chaperone assisting the incorporation of Rieske FeS protein into the cytochrome bc_1 complex in an ATP dependent manner (Cruciat et al., 1999).

Until now, most studies on the function of bcs1 have been performed in yeast. However, bcs1-like proteins are expressed in various eukaryotic organisms including animals, fungi and plants and comprise a group of their own within the AAA protein family (Frickey and Lupas, 2004). A human gene ortholog with 50% amino acid identity to yeast bcs1 has also been identified (Petruzzella et al., 1998) and termed BCS1-like protein or BCS1L. Mutations in this gene, located on human chromosome 2, cause a severe neonatal disease presenting with various phenotypes, however always including lactic acidosis (de Lonlay et al., 2001; De Meirleir et al., 2003; Visapaa et al., 2002). In Finnish patients a homozygous missense mutation, S78G (Visapaa et al., 2002), causes a specific phenotype, GRACILE syndrome MIM (603358) characterized by Growth Retardation starting early during fetal life, aminoaciduria of Fanconi type, cholestasis, iron overload, lactic acidosis and early death during the first days or weeks of life (Fellman et al., 1998). In contrast to the lethal GRACILE syndrome, mutations in CIII subunits cause CIII deficiency with mainly subtle symptoms such as exercise intolerance (Rotig et al., 2004). Furthermore, molecular analyzation of respiratory chain deficiencies indicates that mutations in assembly genes are as important as mutations in structural genes for the development of disease (Rotig and Munnich, 2003). For example, the targeted deletion of chaperones involved in the assembly of complex I, GRIM19 and AIF, generated a lethal phenotype in the mouse (Huang et al., 2004; Vahsen et al., 2004). Recently, a new complex I chaperone, has been identified, a mutation in the corresponding gene termed B17.2L causes a progressive mitochondrial disease in humans (Ogilvie et al., 2005).

In order to clarify *in vivo* functions of the chaperone BCS1L, we assessed the embryonic expression pattern of BCS1L using immunohistochemistry and *in situ* hybridisation on mouse tissues. Its distribution was compared with the expression of certain mitochondrial proteins: the commonly used mitochondrial marker Porin, the two complex III subunits Rieske FeS protein and Core I and the complex I chaperone Grim 19. Our results indicate elevated expression of BCS1L in certain neuronal structures compared to these proteins thus suggesting a possible unknown function of this protein.

1.1. Characterization of the BCS1L antibody

A rabbit anti-BCS1L antibody was generated based on a peptide located in the C-terminal part of the mouse BCS1L protein (Fig. 1A). This anti-BCS1L antibody specifically recognized the C-terminal peptide in an ELISA assay (data not shown) and reacted with two proteins (approximately

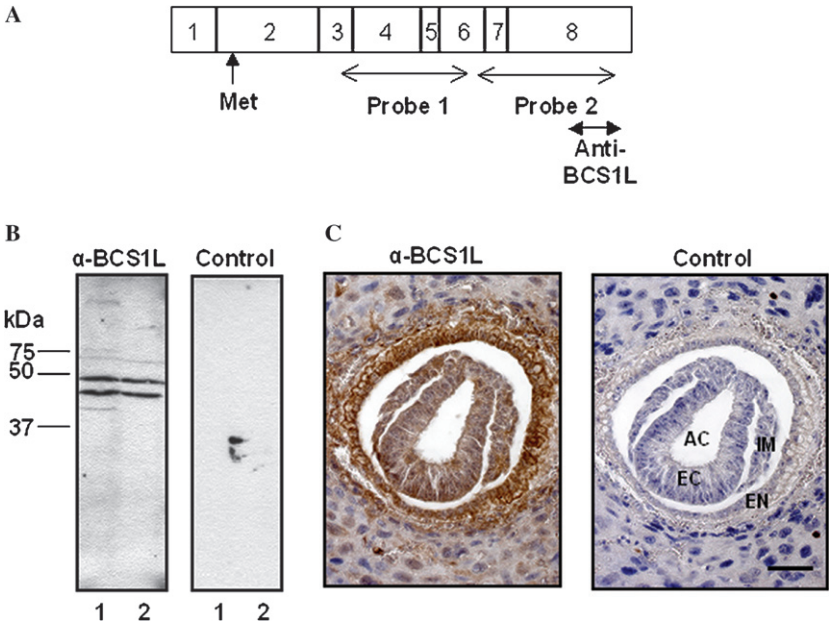

Fig. 1. (A) Schematic drawing of BCS1L mRNA and protein structure. Exons coding for BCS1L are labelled with numbers and the transcription start for BCS1L is marked with Met. Probe 1 and probe 2 indicate the location of the two *in situ* hybridisation probes. α-BCS1L defines the location of the peptide used to generate the anti-BCS1L antibody. (B) Western blot detecting BCS1L in mouse tissue extract from small intestine (lane 1), colon (lane 2) is shown in the left panel. The binding of the antibody was blocked by pre-incubation with its corresponding peptide as shown in the right panel. (C) Immunohistochemical detection of BCS1L in E7 embryo (left panel) and blocking of the antibody binding by pre-incubation with peptide (right panel). Abbreviations: AC, amniotic cavity; EC, extraembryonic ectoderm; IM, intraembryonic mesoderm; EN, extraembryonic endoderm. Scale bar, 50 μm.