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Biochimica et Biophysica Acta 1711 (2005) 183–196

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

Sensitivity of the brain transcriptome to connexin ablation

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Received 29 July 2004; received in revised form 24 November 2004; accepted 2 December 2004
Available online 22 December 2004

Abstract

Extensive studies on mice with total or partial disruption of either connexin43 (Cx43) or connexin32 (Cx32) have detected only subtle changes in central nervous system structure, growth, development, or function. We have used high density cDNA arrays to analyze the regulation, control, and coordination of the abundances of 7446 distinct transcripts in four brains, each of Cx43 null (K43), Cx43 heterozygous (H43), and Cx32 null (K32) mice as compared to the brains of wildtype (W) mice. The use of multiple samples allowed the determination of the statistical significance of gene regulation. Significantly regulated genes encoded proteins of all functional categories, extending beyond those that might be expected to depend on junctional communication. Moreover, we found a high degree of similarity between genes regulated in the K43 and H43 brains and a remarkable overlap between gene regulation in brains of K43 and K32. The regulated genes in both K43 and H43 brains showed an outstanding inverse coordination with the levels of expression of Cx43 in W brain, indicating that the regulated genes are largely predictable from their co-variance with Cx43 in the wildtype samples. These findings lead to the hypothesis that connexin expression may represent a central node in the regulation of gene expression patterns in brain.

Keywords: Connexin43; Connexin32; Connexin43 null mouse; Connexin32 null mouse; Connexin43 heterozygous mouse; Gene expression

Contents

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doi:10.1016/j.bbamem.2004.12.002

1. Introduction

More than half of the approximately twenty known mammalian connexins [1] are expressed in the nervous system either during development or in the mature brain [2–5]. The importance of the expression of these connexins has been assumed to be in the formation of gap junctions, thereby providing direct cytoplasmic continuity from one cell to another; such coupling mediates rapid impulse transmission between certain neurons and less rapid transmission of second messengers between the glia (see [6,7] for reviews). However, cDNA array studies of astrocytes cultured from connexin43 (Cx43) deficient mice have detected a large number of genes with altered expression, including those related to apoptosis and growth, as well as transcription factors expected to impact broadly on gene expression patterns [8,9]. In order to explore the impact of connexin expression on the expression of other genes, we have undertaken gene expression studies described below comparing brains of newborn wildtype mice with those lacking Cx32 or Cx43. These connexins were chosen for comparison because they are normally expressed in distinct glial elements of the brain.

2. Expression of Connexin32 and Connexin43 in the nervous system

Connexin32 (Cx32 or gap junction beta-1) is the primary component of hepatocyte gap junctions [10,11]. Moreover, Cx32 is a major gap junction component in the myelinating cells of the CNS (oligodendrocytes: [4,12,13]) and in the PNS (Schwann cells: [13–15]), where it is hypothesized to play the unusual role of providing a nutritional shunt from the outermost Schwann cell cytoplasm to the innermost, adaxonal regions [13,16,17]. Cx32 mutations are responsible for the X-linked form of Charcot-Marie-Tooth disease (CMTX), a progressively developing demyelinating peripheral neuropathy [14,18]. Cx32 knockout mice are viable through adulthood, and older animals are prone to slowed peripheral nerve conduction, analogous to the late onset of human CMTX disease [19,20].

Connexin43 (Cx43 or gap junction alpha-1) is the most widespread gap junction protein in mammals, where it occurs in almost every tissue [21,22]. Cx43 is a primary component of intercellular gap junction channels in cardiac tissue [23] and in astrocytes, the most abundant glial cell type in the brain [24–27]. In astrocytes, Cx43 gap junctions mediate ionic and metabolite exchange that contributes to potassium siphoning from around the active neurons and to long-range signaling (so-called “calcium waves”; see [7] for a review). Because Cx43 is distributed within the domains of single astrocytes, as well as between processes of neighboring astrocytes [28,29], it is likely that it forms autaptic contacts onto single astrocytes, somewhat analogous to the arrangement of Cx32 in Schwann cells. Cx43 also occurs between neural progenitors in early development [30–32]. Cx43 null mice die at birth due to a developmental cardiac abnormality, where hyperplasia blocks blood flow exiting from the right ventricular outflow tract to the lungs [33]; heterozygous Cx43(+/−) mice do not display this defect and are viable. Brains of Cx43 null mice are grossly normal [34], although the migration of neural progenitors is demonstrably altered compared to wildtype littermates when certain time points are critically examined [35]. Previous microarray studies have indicated that Cx43 expression is altered in both acute and chronic stages of multiple sclerosis [36] in Alzheimer disease [37], Huntington’s disease [38], and in other neural disorders [39,40].

3. Application of cDNA arrays to the analysis of gene expression in connexin null brains

3.1. cDNA microarrays

For the studies described here, we have used cDNA arrays produced at Albert Einstein College of Medicine. These arrays were co-hybridized with DNAs obtained by reverse transcription in the presence of fluorescent dUTPs (labeled with 532 nm emitting Cy3 or 635 nm emitting Cy5) of total RNA extracted from the tissues to be compared and a sample reference. Fluorescence signals of the co-hybridized arrays are then compared. As illustrated in Fig. 1A, a spot in the pseudo-color 8-bit image of the merged two 16-bit tiffs obtained by dual scanning of the hybridized array at 635 nm and 532 nm will appear yellow if that mRNA is similarly abundant in the two extracts or will be biased toward green or red if the abundance of mRNA is higher in one source or the other.

The arrays contain 27,571 spotted sequences: 15,693 spots probe 7455 distinct mouse genes encoding known protein products, 11,686 spots correspond to mouse ESTs whose annotation was incomplete at the date of the study (and thus eliminated from the expression analysis), and 192 spots contain bacterial sequences for quality control of the arrays.