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ScienceDirect
European Journal of Cell Biology 86 (2007) 683–700
European Journal of Cell Biology
www.elsevier.de/ejcb

Characterization of connexin30.3-deficient mice suggests a possible role of connexin30.3 in olfaction

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Received 31 October 2006; received in revised form 5 January 2007; accepted 8 January 2007

Abstract

We have generated connexin30.3-deficient mice in which the coding region of the connexin30.3 gene was replaced by the *lacZ* reporter gene. The expression pattern of this connexin was characterized using β-galactosidase staining and immunoblot analyses. In skin, β-galactosidase/connexin30.3 protein was expressed in the spinous and granulous layers of the epidermis. Specific β-galactosidase/connexin30.3 expression was also detected in the thin ascending limb of Henle's loop in the kidney. In addition, we found β-galactosidase/connexin30.3 in progenitor cells of the olfactory epithelium and in a subpopulation of cells in the apical layer of the vomeronasal organ. Connexin30.3-deficient mice were fertile and displayed no abnormalities in the skin or in the chemosensory systems. Furthermore, they showed normal auditory thresholds as measured by brain stem evoked potentials. These mice did, however, exhibit reduced behavioural responses to a vanilla scent.

Keywords: Gap junctions; Cx30.3; Olfactory epithelium; Vomeronasal organ

Introduction

Gap junctions are intercellular conduits that permit diffusional exchange of ions, secondary messenger molecules and metabolites up to 1000 Da and can serve for transmission of electrical signals between two contacting cells. Each gap junction channel consists of two hemichannels, the connexons, which are contributed by two neighbouring cells. Each connexon is composed of six connexin (Cx) protein subunits (Nicholson, 2003). In the mouse genome, 20 connexin genes (*Cx*) have been identified (Söhl and Willecke, 2003). They show cell type- and differentiation-dependent expression patterns, whereby most cell types express more than one connexin isoform. Deletion of several connexin genes has proven diverse functions of gap junctions in different organs (White and Paul, 1999; Willecke et al., 2002; Wei et al., 2004).

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doi:10.1016/j.ejcb.2007.01.005

In adult murine epidermis, eight connexin isoforms are known to be expressed (Richard, 2000), with changing patterns during embryogenesis and after birth (Risek et al., 1992; Choudhry et al., 1997). In the adult mouse epidermis, Cx43 and Cx40 are expressed in the basal layer, and Cx43 and Cx31 are expressed in the spinous layer. In the differentiating granulous layer, Cx43 disappears gradually during development, but Cx31 is present continuously along with Cx26 (Kamibayashi et al., 1993; Butterwerk et al., 1994). In rat epidermis, Cx37 was localized in all epidermal layers except the stratum corneum (Goliger and Paul, 1994). Transcripts of *Cx30.3* and *Cx31.1* were found in mouse epidermis, but the corresponding protein expression pattern has not been analysed due to lack of antibodies against these connexins (Hennemann et al., 1992; Dahl et al., 1996).

Several human skin disorders are known to be caused by mutations in connexin genes. Mutations in the gene for *Cx30.3* or *Cx31* were demonstrated to be causally involved in erythrokeratodermia variabilis (EKV) (Richard et al., 1998; Macari et al., 2000). However, in humans the loss of functional Cx30.3 did not produce an epidermal phenotype (van Geel et al., 2002). In order to determine the general expression pattern of Cx30.3 and to investigate the function of Cx30.3, we generated Cx30.3-deficient mice in which the coding region of *Cx30.3* was replaced by a *lacZ* reporter gene under control of the endogenous *Cx30.3* promoter. Cx30.3 was detected in the epidermis, in the descending limb of Henle's loop in the kidney, in the olfactory epithelium, and in the vomeronasal organ. We noticed that deletion of Cx30.3 in the epidermis did not lead to obvious phenotypic alteration of the epidermis. Instead, we found that Cx30.3 might be involved in olfactory perception. Compared to their wild-type litter mates, the Cx30.3-deficient mice exhibited significant behavioural differences towards vanilla scent.

Material and methods

Construction of the targeting vector pCx30.3ko

A fragment of genomic 129/Sv mouse DNA spanning about 12 kb of the *cx30.3* locus was isolated from a recombinant lambda phage library (Stratagene, La Jolla, CA). The Cx30.3 knockout construct was embedded in a pBluescript II KS(±) vector backbone (Stratagene). A 1.3-kb KpnI-XbaI fragment and a 5.7-kb AccI-EagI fragment were used as 5′ and 3′ homologous regions, respectively. The 432-bp fragment between the XbaI site and the start codon of *Cx30.3* was amplified by PCR and inserted between the 5′ homologous region and the *lacZ* coding DNA with a nuclear localization signal (NLS-*lacZ*) (cf. Theis et al., 2000). A selection marker cassette, carrying the mouse phosphoglycerate kinase (PGK) promoter-neomycin selection cassette, was placed downstream of the NLS-*lacZ* coding DNA (Meyers et al., 1998). The selection cassette was flanked by frt sites and inserted in reverse orientation with respect to the NLS-*lacZ* coding region (Fig. 1A). The final targeting vector pCx30.3ko was analysed by restriction mapping and partial sequencing. The function of the frt sites was verified by transformation of Flp recombinase-expressing *Escherichia coli* (Buchholz et al., 1996).

Screening of embryonic stem cell clones

HM-1 embryonic stem cells were transfected with 250 μg DNA of the targeting vector pCx30.3ko (linearized downstream of the 3′ homologous region by NotI digestion) and selected with 350 μg G418 (Sigma, St. Louis, MO) per ml of medium as described previously (Magin et al., 1992; Degen et al., 2004). Resistant clones were analysed for homologous recombination by PCR and Southern blot hybridization. Recombination at the 5′ homologous site was examined using a 5′ upstream primer external to the targeting vector (QZ12; 5′-AGGCTG-GGGTACACAAACATGGCC-3′) and a *lacZ*-specific 3′ downstream primer (QZ10; 5′-ACAGAGGACAGAG-GACCAGAGGAG-3′) under the following conditions: 20 min of DNA denaturation at 94 °C, addition of Taq DNA polymerase (Promega, Madison, WI), followed by 38 cycles of 94 °C for 30 s each, 64 °C for 45 s, and 72 °C for 2 min, and final elongation at 72 °C for 10 min. The resulting amplicon had a size of 1.6 kb. Recombination at the 5′ homologous region was examined by Southern blot analysis. DNA from PCR-positive clones was digested with SpeI and fractionated on a 0.75% agarose gel. After blotting of DNA onto Hybond N+ membranes (Amersham Biosciences, UK), the digested DNA was fixed by UV-cross-linking. A 620-bp AccI-AccI fragment outside the 3′ homologous region served as external probe and was radioactively labelled using Megaprime DNA Labelling System (Amersham Biosciences). Hybridization was performed using Quick Hyb hybridization solution (Stratagene) according to instructions provided by the manufacturer. The *Cx30.3* wild-type allele yielded a 18-kb fragment compared to a 13-kb fragment of the mutated allele. In addition, a 563-bp BamHI- PvuII fragment from the neo selection cassette served as internal probe. After digestion of genomic DNA with SpeI, the internal probe detected a 10.5-kb fragment of the mutated allele.

Generation of Cx30.3-deficient mice ($Cx30.3^{lacZ/lacZ}$)

Three homologously recombined embryonic stem cell clones were injected into C57BL/6 mouse blastocysts to