Characterization of isoform-specific expression and function of Bcl11a in the dorsal telencephalon

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Abstract

The zinc-finger transcription factor Bcl11a plays important roles in neural development and it is well established that different isoforms are generated from the *Bcl11a* gene locus. However, the biological significance of the different isoforms remains unclear. Studies in humans suggest that *BCL11A* mutations differentially affecting isoform expression lead to varying clinical symptoms. To systematically explore expression and putative functions, we developed tools that allow isoform-specific analysis in mice, including isoform-specific antibodies, FLAG- and Myc-tagged expression constructs to characterize Bcl11a isoform expression in cell culture and different brain regions of developing mice. To test *in vivo* functions in cortical neurons, we overexpressed Bcl11a isoforms using *in utero* electroporation. Our findings show that Bcl11a isoforms exhibit spatiotemporal expression differences at the tissue and subcellular levels. *In vivo* overexpression of Bcl11a isoforms in wildtype brains differentially affects neuronal polarization and positioning, suggesting functional differences of Bcl11a isoforms.

I. Bcl11a isoforms

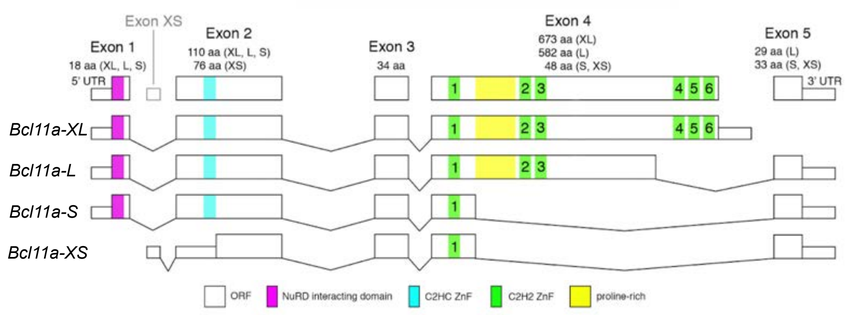

Figure 1: Alternative splicing of *Bcl11a* leads to four isoforms in mice.

(Simon et al., 2020)

II. Isoform-specific Bcl11a antibodies

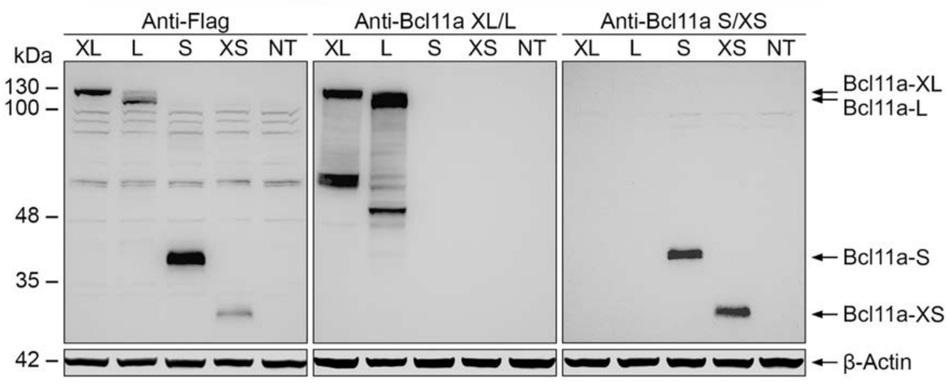

Figure 2: Lysates of N2a cells transfected with Flag-tagged *Bcl11a*-isoforms were analyzed by Western blotting. Flag-antibody detects all four isoforms at their corresponding molecular weight (left). Antibodies against Bcl11a XL/L (middle) and Bcl11a S/XS (right) show isoform-specificity. NT, lysate of not transfected cells; XL/L/S/XS, lysate of cells transfected with *Bcl11a-XL/L/S/XS*.

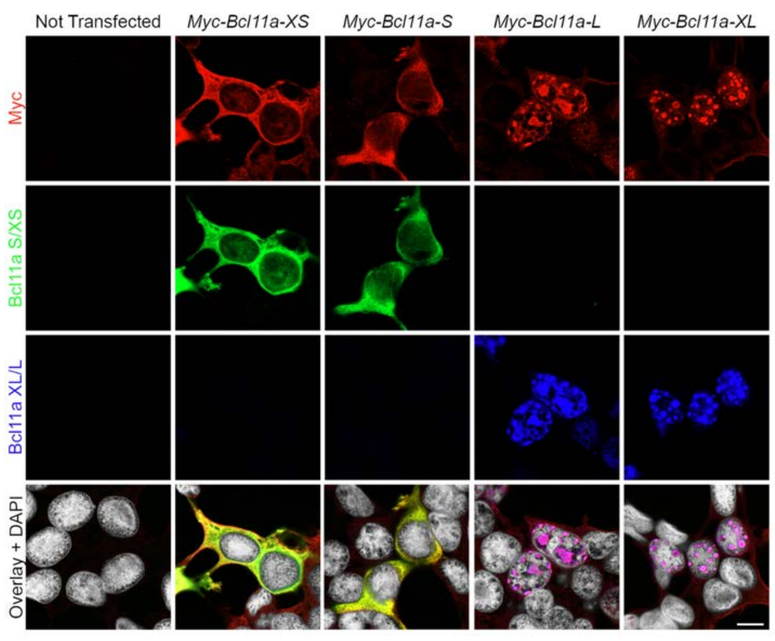

Figure 3: HEK293 cells were transfected with Myc-tagged *Bcl11a*-isoforms as indicated. Staining with an antibody against Myc (red) shows that Bcl11a-XS and -S are expressed in the cytoplasm, whereas Bcl11a-L and -XL are expressed in paraspeckles in the nuclei. The Bcl11a S/XS antibody (green) specifically detects Bcl11a-S and -XS, and the Bcl11a XL/L antibody (blue) is specific for Bcl11a-XL and -L. Nuclei were stained with DAPI (white). Scale bar, 10 µm.

(Brode, R., Wiegreffe, C., Britsch, S., unpublished)

III. Co-Transfection

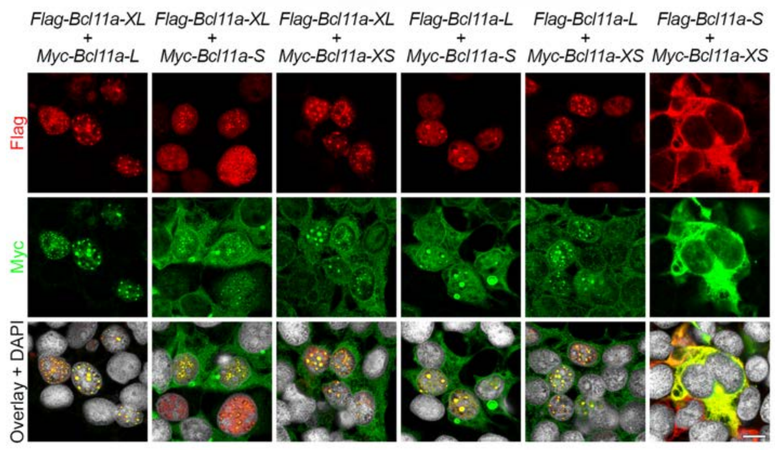

Figure 4: Representative images of HEK293 cells transfected with combinations of Flag-tagged (red) and Myc-tagged (green) *Bcl11a*-isoforms as indicated. Co-transfection has no apparent effect on the subcellular localization of Bcl11a-XL and -L. By contrast, both Bcl11a-S and Bcl11a-XS are partly translocated to the nucleus when co-transfected with Bcl11a-L or Bcl11a-XL. Nuclei were stained with DAPI (white). Scale bar, 10 µm.

(Brode, R., Wiegreffe, C., Britsch, S., unpublished)

IV. Axonal expression of Bcl11a S/XS in primary cortical neurons

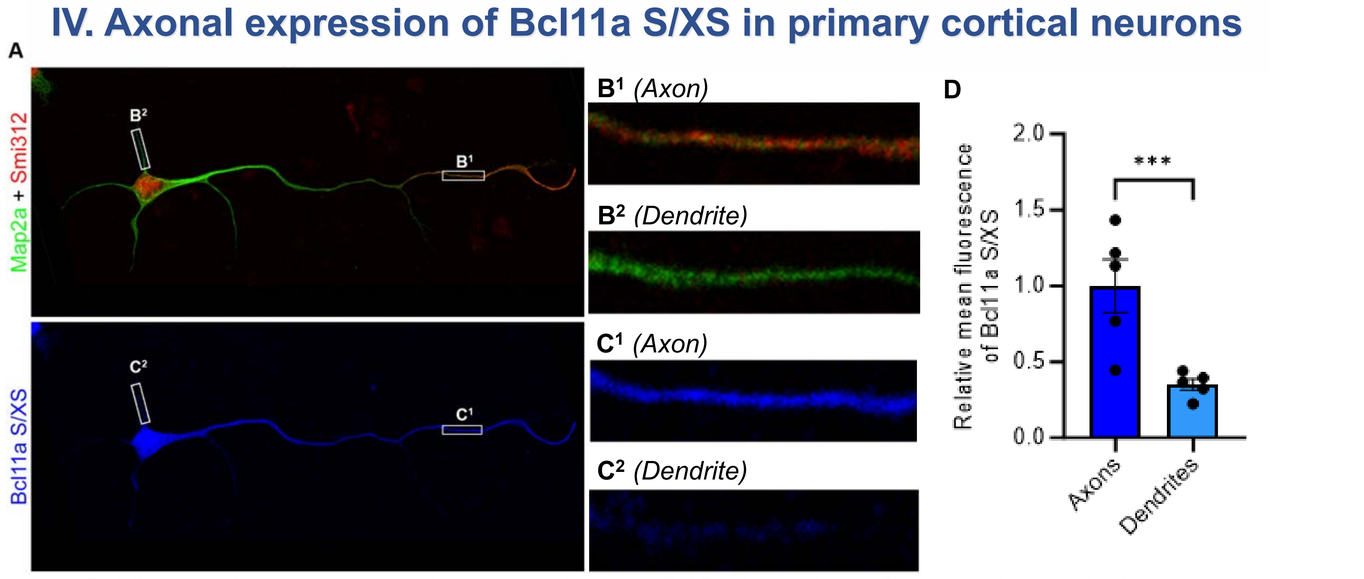

Figure 7: (A-C) Immunocytochemistry of a representative primary cortical neuron (E14.5, cultured for 3 days). Dendrites are stained with Map2a (green) and the axon additionally with Smi312 (red). Bcl11a S/XS (blue) is expressed in the entire perikaryon and appears to be preferentially expressed in the axon.
(D) Quantification of the mean fluorescence level of Bcl11a S/XS in axons and dendrites reveals that Bcl11a S/XS expression is higher in the axons compared to the dendrites. n = 5 technical replicates. Mean ± SEM. Student's t test; ***p<0.001.

(Brode, R., Wiegreffe, C., Britsch, S., unpublished)

V. Isoform-specific expression in the corpus callosum

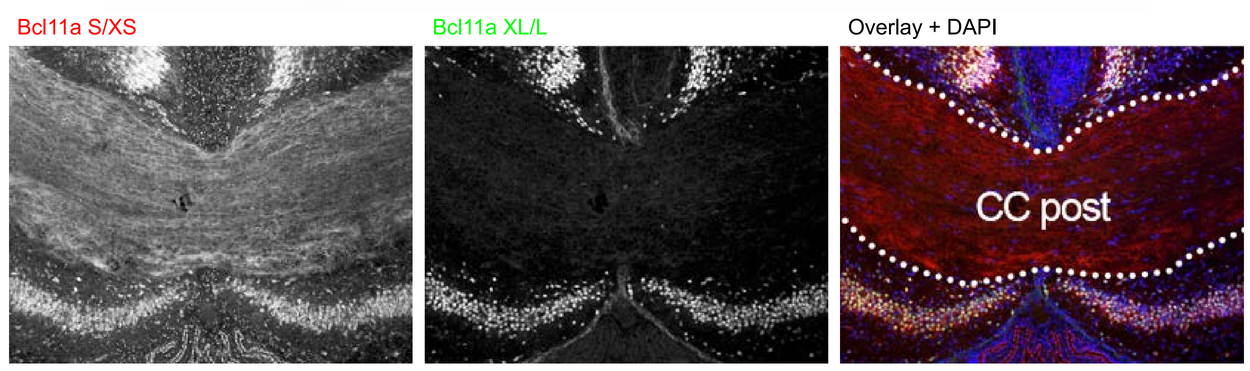

Figure 8: Immunohistochemistry on coronal frozen sections at development stage P2 of the posterior corpus callosum. CC post, posterior corpus callosum. Nuclei were stained with DAPI (blue).

(Brode, R., Wiegreffe, C., Britsch, S., unpublished)

VI . Isoform-specific *in utero* over expression

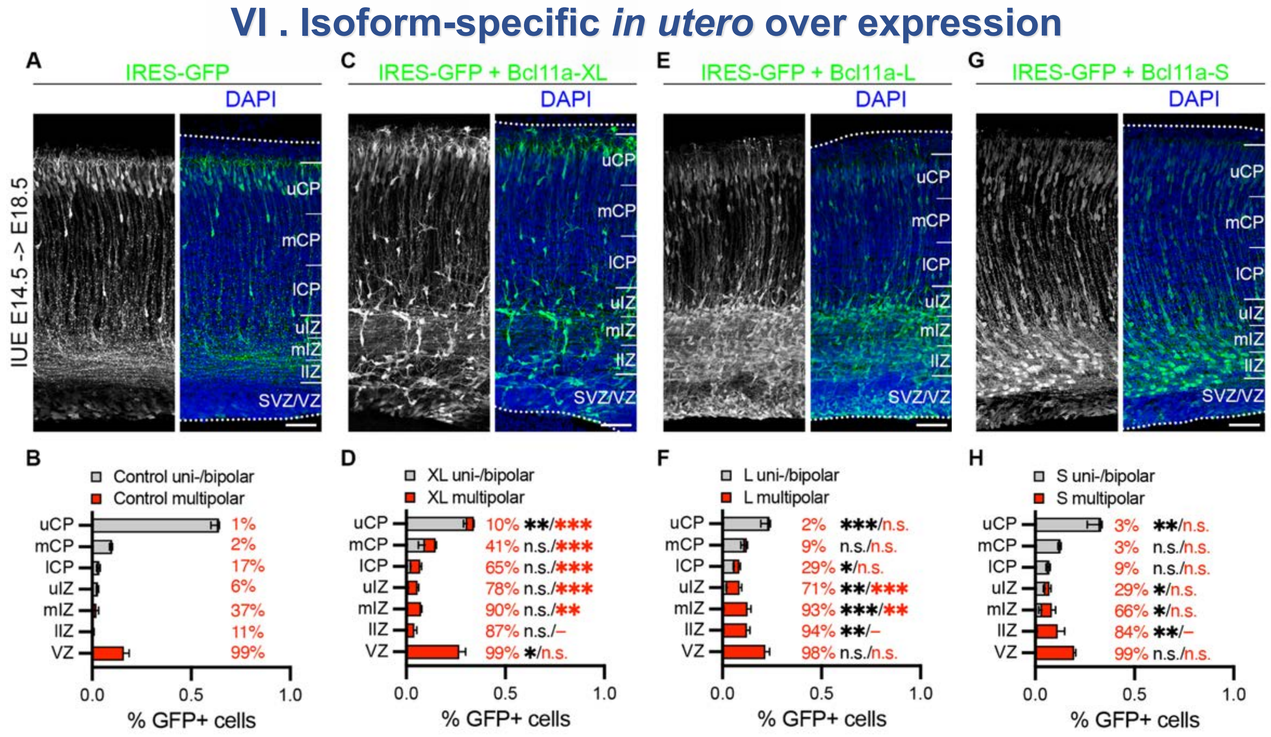

Figure 7: (A/C/E/G) *In utero* electroporation (IUE) of an *IRES-GFP*-vector containing no insert (control) or one of the *Bcl11a*-isoforms as indicated. uCP/mCP/ICP, upper/middle/lower cortical plate; uIZ/mIZ/ lIZ, upper/middle/lower intermediate zone; SVZ/VZ, (sub-)ventricular zone. Scale bar, 100 µm.
(B/D/F/H) Quantification of the respective relative distribution of uni-/bipolar and multipolar neurons (n = 4). Mean ± SEM. Percentages show the proportion of multipolar cells in each zone. Significance levels refer to the distribution of all neurons (black) or polarity (red) in the respective zone compared to control. One-way ANOVA followed by Dunnett's post hoc test. n.s., not significant; *p<0.05; **p<0.01; ***p<0.001; –, cell number too low for statistical analysis.

(Brode, R., Wiegreffe, C., Britsch, S., unpublished)

VII. Conclusions and perspective

- Our developed tools include isoform-specific antibodies and will be useful to further improve our understanding of Bcl11a function and its associated disorders.
- Bcl11a isoforms have overlapping but also differential spatiotemporal expression and function in forebrain development.
- We could detect the short Bcl11a isoforms at the protein level in axons, supporting speculations about functions during axonogenesis

VIII. References

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