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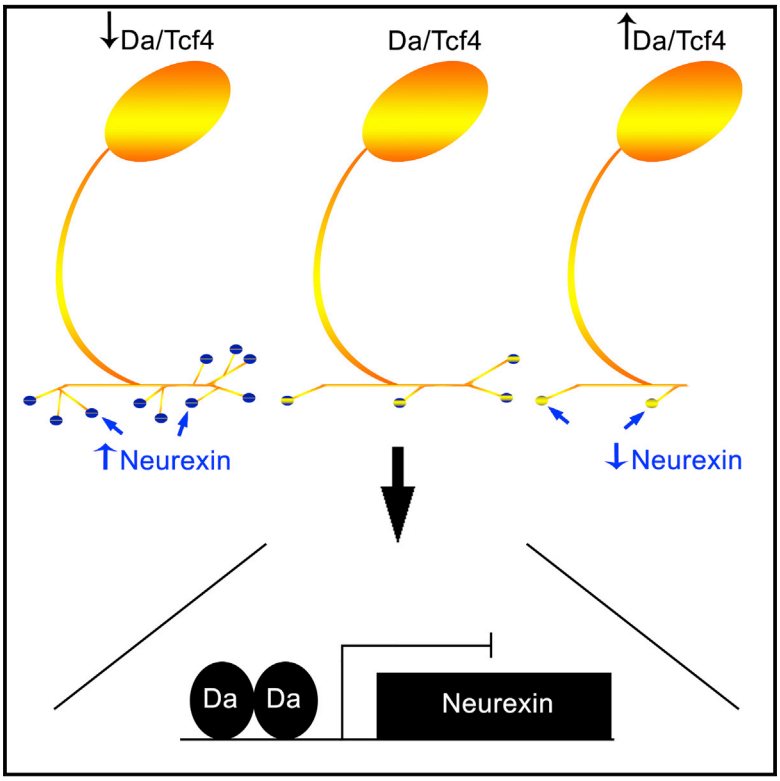
Type I bHLH Proteins Daughterless and Tcf4 Restrict Neurite Branching and Synapse Formation by Repressing Neurexin in Postmitotic Neurons

Graphical Abstract



Authors

Mitchell D'Rozario, Ting Zhang, Edward A. Waddell, ..., Faith Liebl, Wenhui Hu, Daniel R. Marenda

Correspondence

whu@temple.edu (W.H.), daniel.marenda@drexel.edu (D.R.M.)

In Brief

Class I bHLH proneural proteins are highly conserved transcription factors generally recognized as critical for neurogenesis. D'Rozario et al. show that the *Drosophila* and mouse class I bHLH proteins Daughterless and Tcf4 are present in postmitotic, differentiated neurons and function to restrict neurite branch and synapse number.

Highlights

- bHLH proteins Daughterless and Tcf4 are present in postmitotic neurons
- bHLH proteins Daughterless and Tcf4 restrict branching in postmitotic neurons
- Daughterless and Tcf4 repress Neurexin expression in postmitotic neurons
- Daughterless homodimers restrict branching in postmitotic neurons

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Type I bHLH Proteins Daughterless and Tcf4 Restrict Neurite Branching and Synapse Formation by Repressing Neurexin in Postmitotic Neurons

Mitchell D'Rozario,[1] Ting Zhang,[2] Edward A. Waddell,[1] Yonggang Zhang,[2] Cem Sahin,[3] Michal Sharoni,[1] Tina Hu,[1] Mohammad Nayal,[1] Kaveesh Kutty,[1] Faith Liebl,[4] Wenhui Hu,[2,*] and Daniel R. Marenda[1,5,*]
[1]Department of Biology, Drexel University, Philadelphia, PA 19104, USA
[2]Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA 19140, USA
[3]Department of Electrical and Computer Engineering, Drexel University, Philadelphia, PA 19104, USA
[4]Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL 62026, USA
[5]Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19104, USA
*Correspondence: whu@temple.edu (W.H.), daniel.marenda@drexel.edu (D.R.M.)
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SUMMARY

Proneural proteins of the class I/II basic-helix-loop-helix (bHLH) family are highly conserved transcription factors. Class I bHLH proteins are expressed in a broad number of tissues during development, whereas class II bHLH protein expression is more tissue restricted. Our understanding of the function of class I/II bHLH transcription factors in both invertebrate and vertebrate neurobiology is largely focused on their function as regulators of neurogenesis. Here, we show that the class I bHLH proteins Daughterless and Tcf4 are expressed in postmitotic neurons in *Drosophila melanogaster* and mice, respectively, where they function to restrict neurite branching and synapse formation. Our data indicate that Daughterless performs this function in part by restricting the expression of the cell adhesion molecule Neurexin. This suggests a role for these proteins outside of their established roles in neurogenesis.

INTRODUCTION

Proneural proteins of the class I/II basic-helix-loop-helix (bHLH) family are transcription factors that are highly conserved from invertebrates to humans (reviewed in Guillemot, 2007; Powell and Jarman, 2008). Class I bHLH proteins are expressed in a broad number of tissues during development, while class II bHLH protein expression is more tissue restricted. Generally, class I bHLH proteins heterodimerize with class II bHLH proteins to activate gene expression (Powell and Jarman, 2008). However, class I bHLH proteins have also been reported to homodimerize (Cabrera and Alonso, 1991) and restrict (Lim et al., 2008) or activate (Tanaka-Matakatsu et al., 2014) gene expression. Class I bHLH proteins also form heterodimers with class V bHLH proteins of the inhibitor of differentiation (ID) family. This I/V interaction inhibits class I bHLH DNA binding and functions as a dominant-negative interaction to compete against binding of type I factors with type II factors, thus preventing gene expression (Massari and Murre, 2000; Quednow et al., 2014).

Our current understanding of the function of class I/II bHLH transcription factors in both invertebrate and vertebrate neurobiology is largely focused on their function as master regulators of embryonic neurogenesis (Powell and Jarman, 2008). A wealth of literature shows that the expression of class I/II bHLH proteins is both necessary and sufficient to initiate programs that lead to the differentiation of neural stem/progenitor cells (NSCs/NPCs) (Guillemot, 2007). However, we are also beginning to appreciate the roles that class II bHLH proteins play in terminally differentiated cells. For example, in *Drosophila*, the class II bHLH protein Atonal functions to promote postmitotic neuron migration, axon guidance, and arborization in the dorsal cluster neurons in the brain (Hassan et al., 2000). In mammals, the Atonal homolog Atoh1/Math1 is essential for migration of postmitotic retrotrapezoid nucleus neurons required for proper respiration (Huang et al., 2012). However, to date, there is no published finding on the function of any class I bHLH protein in postmitotic neurons in either vertebrates or invertebrates.

Mutations in *Transcription Factor 4* (*TCF4*; a human class I bHLH protein) have been reliably identified in genome-wide association studies as a susceptibility risk factor for schizophrenia (Schizophrenia Psychiatric GWAS, 2011; Stefansson et al., 2009; Steinberg et al., 2011) and have also been associated with Pitt-Hopkins syndrome (Amiel et al., 2007; Brockschmidt et al., 2007; Sepp et al., 2012; Zweier et al., 2007). It is unknown whether these diseases are due to defects in neurogenesis, in mature differentiated cells, or both. Literature suggests that disrupted neurogenesis and/or neural differentiation may be partly responsible. For example, Tcf4 is expressed widely in the nervous system during mouse embryonic development and is also expressed in germinal layers (dentate gyrus, subventricular zone, and rostral migratory stream) of the postnatal mouse brain (Flora et al., 2007). *Tcf4* homozygous mutant mice are viable with normal overall brain morphology, but only live for 1–6 days (Flora et al., 2007).

These diseases and neurocognitive disorders may also be a result of dysfunction in postmitotic/differentiated neurons. To further understand the contributions of type I bHLH proteins in postmitotic neurons, we analyzed the effect of altering