Regulation of Expression and Activity of the bHLH-PAS Transcription Factor NPAS4

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Contents

Abstract

Development of the Central Nervous System (CNS) relies on complex transcriptional programs to specify distinct neuronal areas/cell types, and guide the formation of neuronal networks. Synaptic activity during post-natal brain development dictates the number and strength of synapses as well as promoting neuronal cell survival through activation of transcriptional programs. The establishment of synapses during this "critical period" of post-natal neuronal development and the local rearrangement, fine tuning and maintenance of synaptic connections into adulthood contributes to synaptic plasticity, memory, learning and cognitive function, while dysfunction in these processes is thought to contribute to a number of neuropsychiatric diseases. Studying transcription which underlies these events and disease states has been technically challenging due to the lack of gain and loss of gene expression systems to interrogate complex biological questions in primary neurons or the developing nervous system of rodents. As a result, despite clinical and anatomical data, the molecular mechanisms underlying neuropsychiatric disease or memory and learning remain poorly understood.

The basic-Helix-Loop-Helix (bHLH) – Per/Arnt/Sim (PAS) (bHLH-PAS) homology domain transcription factor Neuronal PAS factor 4 (NPAS4) is tightly coupled to neuron function by homeostatically regulating neuronal activity via stimulating formation of inhibitory synapses. NPAS4 expression is brain restricted and highly induced following neuronal depolarisation, paradigms of learning, seizure or ischemia. NPAS4 null mice are prone to seizures, hyperactivity, have defects in memory formation, social interaction, cognitive impairments, as well as age related neurodegeneration.

This thesis shows that NPAS4 expression is highly restricted to the CNS, in particular the cortex, by repressive activity of RE-1 Silencing Transcription Factor/Neuron-Restrictive Silencer Factor (REST/NRSF) in non-neuronal cells and stem cells. In addition, we also provide evidence

that microRNA-224 targets the NPAS4 3'UTR, which may contribute to regionalised NPAS4 expression in the brain. We identify human variants within NPAS4 and ARNT2 which disrupt NPAS4 function, which may have implications for neuropsychiatric disease. Using structural modelling and biochemical experiments we show that one of these variants disrupts dimerisation, providing insight into bHLH-PAS dimerisation mechanisms.

We also describe a new system for knockdown and ectopic expression which is broadly applicable for reliable, flexible and temporal control of gene expression to facilitate investigating gene function. This system incorporates single gateway compatible vector systems for lentiviral infection and Recombination Mediated Cassette Exchance (RMCE), the latter targeting the Collagen 1a1 (Col1a1) locus in germline competent embryonic stem cells. Using an optimised reverse tetracycline transactivator (rtTA) system with reduced background expression and increased sensitivity to doxycycline, we have shown that we can rapidly generate inducible overexpression and short hairpin RNA (shRNA) mediated knockdown cell lines with homogenous, inducible expression.

The work encompassed within this thesis investigates the molecular mechanisms underlying the restricted expression pattern of NPAS4, the contribution of human non-synonymous variants to NPAS4/ARNT2 transcription factor function, and the development of flexible, inducible and reversible gene expression systems for studying NPAS4 function *in vitro* and *in vivo*.

PhD Thesis Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to David Christopher Bersten and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below*) resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Publications

This thesis is based on the following publications and referred to in the text:

I. **Bersten DC**, Sullivan AE, Peet DJ, Whitelaw ML. bHLH-PAS proteins in cancer. Nat Rev Cancer. 2013 Dec;13(12):827-41.

II. **Bersten DC**, Wright JA, McCarthy PJ, Whitelaw ML. Regulation of the neuronal transcription factor NPAS4 by REST and microRNAs. Biochim Biophys Acta. 2014 Jan;1839(1):13-24.

III. **Bersten DC**, Bruning JB, Peet DJ, Whitelaw ML. Human Variants in the Neuronal Basic Helix-Loop-Helix/Per-Arnt-Sim (bHLH/PAS) Transcription Factor Complex NPAS4/ARNT2 Disrupt Function. PLOS one. 2014 Jan, DOI: 10.1371/journal.pone.0085768

IV. **Bersten DC**, Sullivan AE, Bhakti V, Li D, Thomas PQ, Bent S, Whitelaw ML, Inducible and reversible lentiviral and recombination mediated cassette exchange (RMCE) systems for controlling gene expression. Manuscript submitted *Nucleic Acids Research*

Conference oral presentations

REGULATION OF EXPRESSION AND ACTIVITY OF THE NEURONAL TRANSCRIPTION FACTOR NPAS4

Bersten D.C., Peet D.J. and Whitelaw M.L. Australian Society of for Biochemistry and Molecular Biology annual conference (**ComBio 2012)**

Additional publications

Bonnefond A, Raimondo A, Stutzmann F, Ghoussaini M, Ramachandrappa S, **Bersten DC**, Durand E, Vatin V, Balkau B, Lantieri O, Raverdy V, Pattou F, Van Hul W, Van Gaal L, Peet DJ, Weill J, Miller JL, Horber F, Goldstone AP, Driscoll DJ, Bruning JB, Meyre D, Whitelaw ML, Froguel P.

Loss-of-function mutations in SIM1 contribute to obesity and Prader-Willi-like features.

J Clin Invest. 2013 Jul 1;123(7):3037-41.