Investigation into the molecular function of the neuronal Hu RNA binding protein, HuCsv1

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Sincerely,
P.

Declaration of Originality

[*With the exception of this declaration*], this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Peter James McCarthy 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. 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, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed,

Peter James McCarthy

Abstract

Of the four Hu genes found in most vertebrates (HuA, HuB, HuC and HuD), all except HuA exhibit mRNA and protein expression that is essentially restricted to post-mitotic neurons of the developing and adult nervous systems. Spatial and temporal examination of individual "neuronal Hu" (nHu = HuB, HuC and HuD) proteins in brain tissue suggests nHu proteins may play a functional role during neuronal differentiation; as RNA-binding proteins, the nHu proteins may participate in gene regulatory events that are essential for acquisition of the neuronal phenotype.

We have identified a number of candidate mRNA targets of the nHu proteins. Our data suggest that the majority of these mRNAs interact with nHu proteins through sequences present in their 3′ untranslated regions (UTRs). From this 3′UTR target subset, several mRNAs were selected for further examination based on reported roles for their encoded proteins during axonogenesis, a critical developmental process during which nascent neurons grow and extend axons that eventually connect to and form synaptic connections with other neurons. The mRNAs chosen encode for cytoskeleton-modifying proteins; Cofilin, Vasodilator-Stimulated Phosphoprotein (VASP) and the Rho GTPase Cdc42.

The primary aim of the work reported in this thesis was to characterise the effect of interactions between the neuronal Hu protein HuC, and the CLIP-identified 3′UTRs listed above. To do this, the 3′UTR sequences were cloned into reporter vectors (both fluorescent and luciferase reporter-based) to produce reporter protein-encoding messages that included a putative target 3′UTR. These vectors were then used in co-transfection experiments with or without HuC and measurements of reporter protein and mRNA abundance obtained. Interestingly, despite initial speculation that HuC might be involved in directly regulating protein expression from target mRNAs, no significant effect of HuC on protein production from any of the 3′UTR-reporter mRNAs tested was observed. However and quite unexpectedly, measurement of 3′UTR-reporter mRNA abundance from co-transfection assays revealed a potential role for HuC in modulating alternative polyadenylation site choice for one of the CLIP-identified 3′UTR sequences. Regulation of mRNA polyadenylation site choice may be a novel mechanism by which nHu proteins post-transcriptionally control gene expression during neuronal development.