

Investigating Mechanisms of Post-transcriptional Gene Regulation in the Germ Cells of Zebrafish

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Abstract

In most organisms, the primordial germ cells are specified and set aside from the surrounding somatic tissues very early in development. Their ability to carry out a gene regulatory program quite distinct from the surrounding somatic cells, and their capacity to specify entire new organisms has made them a focus of many studies that seek to understand how specific transcriptional and translational programs contribute to cell fate.

Zebrafish, a vertebrate with external development of the embryo, is currently one of the best animal models for understanding the molecular basis of germ cell specification. Briefly, germ cell specification is dependent on maternally provided cytoplasmic determinants, termed the germ plasm. The germ plasm, is localised to areas of the embryo that will become the germ cells later in development by inheritance the germ plasm through cleavage divisions. A number of mRNA components of the germ plasm have been identified; interestingly many of them encode RNA-binding proteins, and almost all of them have invertebrate and mammalian orthologues. Evidence suggests that these maternally provided mRNA determinants are specifically maintained in the germ cells throughout embryonic development, and at least some of these gene products are essential for germ cell specification.

A number of studies have begun to elucidate the molecular mechanisms that allow germ cell specific maintenance of these mRNAs, and also to identify how maternally provided messages destined for the germ cells are destabilised and eliminated in the somatic tissues. For example, the germ cell specific mRNAs *nanos* and *TDRD7* are destabilised in somatic cells through interactions of the 3'UTR sequences with the microRNA miR-430. This miR-430-mediated repression is overcome in germ cells through the binding of an RNA-binding protein Dead end (DND) to distinct sites within the *nanos* and *TDRD7* 3'UTRs.

This thesis details a study of the zebrafish orthologue of *HuB*, a highly conserved RNA-binding protein with expression in neurons, testes and ovaries in adult vertebrates. In zebrafish, *HuB* mRNA is maternally provided, and is restricted to the germ cells by 24 hours of development; this is the first report to indicate expression of *HuB* in the germ cells of vertebrates, suggesting a possible role for *HuB* in germ cell development.

Through detailed mutagenesis studies, the *HuB* 3'UTR has been found to contain a set of four destabilising elements, which bring about somatic degradation of the mRNA, and a separate, 30-nucleotide motif that is responsible for germ cell specific stabilisation of the message. None of these identified destabilising elements are targets for miR-430, and thus they represent novel sequence elements for somatic message degradation in zebrafish. Through a candidate screening approach, DAZL, a germ cell specific RNA-binding protein, was identified as being capable of stabilising *HuB* mRNA. Furthermore, DAZL was shown to mediate this stabilisation of *HuB* mRNA by interacting, either directly or indirectly, with the 30-nucleotide stabilisation element that was identified in the *HuB* 3'UTR. This elucidation of the mechanisms of germ cell specific expression of the *HuB* mRNA is an important finding, for it reveals mechanisms of post-transcriptional regulation that are distinct from that of other germ cell specific mRNAs.

In summary, the identification of *HuB* as a germ cell specific mRNA, and the determination of the post-transcriptional mechanisms responsible for this specific expression is an important first step in understanding how *HuB* and other germ cell specific RNA-binding proteins contribute to germ cell development and function.

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 Sophie Wiszniak 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.

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xx