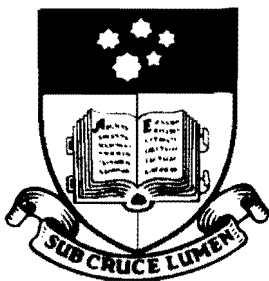


**CHARACTERISATION OF PSC1 AS AN ACIDIC RICH RS  
DOMAIN PROTEIN (ARRS) WITH A CONSERVED  
MAMMALIAN FAMILY MEMBER.**

Thesis submitted to the University of Adelaide for the degree of  
Doctor of Philosophy

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## SUMMARY

The Acidic Rich family of RS Domain proteins (ARRS) is defined by both the presence and arrangement of conserved domains within 2 family members. Conserved regions include an RS domain, zinc finger domain, RNA binding motif and a C terminal acidic rich region. Two conserved motifs within the RRM of ARRS proteins have been defined that are not found in the RRM of other RNA binding proteins. Peri implantation stem cell 1 (Psc1), the founding member of this family, was originally identified as a developmental marker differentially expressed between the Inner Cell Mass and primitive ectoderm of the mammalian embryo. Psc1 RNA is differentially up-regulated in the post gastrulation embryo and the adult, with high mRNA levels in lung, brain and kidney, and low level expression in other tissues.

Comparative analysis of Psc1 to RS domain proteins known to function in mRNA processing, such as SC35 and ASF/SF2, has shown it colocalises to characteristic nuclear speckles. However, in contrast to SR proteins, Psc1 localises to additional regions in the nucleus, not containing SR proteins, and to punctate regions in the cytoplasm termed cytospeckles. Further, in the absence of transcription, Psc1 localises to regions in the nucleus which exclude nuclear speckles.

Finally, unlike SC35 and ASF/SF2, which move rapidly in and out of nuclear speckles, FRAP assays show Psc1 is tethered within the nucleus. Analysis of Psc1 domain contribution to subcellular localisation and mobility shows the RRM to be both necessary and sufficient for Psc1 cytospeckle localisation and is responsible for the nuclear tethering of Psc1. The RS domain of Psc1 acts as a nuclear localisation signal and contributes to nuclear speckle localisation. The C terminal of Psc1 localises with microtubules and is proposed to mediate Psc1 cytoskeletal association.

The expression of the *Drosophila* acidic rich RS domain protein (NP609976) is developmentally regulated in the same manner as *Psc1*, it has a nuclear localisation profile identical to Psc1 and also localises to speckles in the cytoplasm, all of which support a conserved evolutionary role for Psc1 and the ARRS protein family in mRNA processing and trafficking both in the nucleus and in the cytoplasm.