

NUCLEAR TRANSPORT OF GLI TRANSCRIPTION FACTORS DURING SPERMATOGENESIS

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Development is highly regulated by complex signalling cascades. One such pathway is the Hedgehog (Hh) signalling pathway which plays an essential role in spermatogenesis. The Gli family of zinc finger TFs, consisting of Gli1, Gli2 and Gli3, are mediators of the Hh signalling cascade. Gli1 is an activator of Hh target genes, whereas Gli2 and Gli3 can undergo proteolytic cleavage and function as both activators and repressors. Little is known regarding the nuclear import pathway of these TFs. In this study, the mRNA expression pattern of all Gli family members in the developing mouse testis was compiled by *in situ* hybridisation and shown to have unique expression patterns. In the adult mouse testis, Gli1 mRNA was detected in spermatogonia through to round spermatids whereas Gli2 was only found in spermatogonia and spermatocytes. Very low levels of Gli3 mRNA were detected in all ages and cell types. Since little is known regarding the import pathway for Gli1, expression vectors containing different fragments of the N-terminus of Gli1 were created and used to perform transfection experiments and generate vectors for bacterial GFP-fusion protein expression. Transfection experiments into African green monkey kidney Cos-7 cells, and the murine spermatogenic cell lines, Gc-1 and Gc-2 using 3 different constructs localised the NLS(s) required to target Gli1 to the nucleus in the zinc finger DNA-binding domain of Gli1. Preliminary results for *in vitro* binding of bacterially expressed Gli1 indicated no binding by importin β 1 or β 3 but a weak interaction with the importin α/β heterodimer. This can be seen as the first step towards defining the nuclear import pathway for Gli1. The mechanisms by which Gli activity is modulated remain unanswered and the regulation of its nuclear entry may be an important means of doing so.