Chapter 3

The expression pattern of *lama1* during zebrafish development

3.1: Introduction

Mouse knock out and zebrafish mutant studies indicate that Laminin is an essential component of basement membranes (BM), and as a result, its absence causes embryonic death (Li et al. 2002; Miner et al. 2004). Laminin-111 (α1, β1, γ1) is the earliest Laminin to be expressed in the mouse and zebrafish embryo (Miner et al. 1997; Parsons et al. 2002; Sztal et al. 2011). Previous work in the lab showed that Laminin-111 is detected within the mouse myotomal BM (Bajanca et al. 2006), a structure critical for the correct migration and differentiation of myogenic progenitor cells that have delaminated from the dorsal medial lip of the somite. *Lama1*, encoding Laminin α1, is missing in somites of *Shh*$^{-/-}$ mice, resulting in the absence of myotomal BM and in the aberrant migration of myogenic progenitor cells to ectopic dorsal and ventral positions (Anderson et al. 2009). Specifically, there is a loss of *Lama1* expression in the somites and neural tube, but not in the pre-somitic mesoderm (PSM) and pro-mesonephros, in *Shh*$^{-/-}$ embryos (Anderson et al. 2009). Genetic and embryological analyses indicate that Shh signalling regulates *Lama1* expression in the somitic mesoderm, and that Laminin-111 is necessary and sufficient to initiate myotomal BM assembly (Anderson et al. 2009).

The expression pattern of *lama1* was previously reported in the zebrafish (Pollard et al. 2006; Zinkevich et al. 2006). However, these studies lack details, and in particular there are some discrepancies as to whether *lama1* is down regulated or not in somites after 24hpf (Pollard et al. 2006; Zinkevich et al. 2006).

Similar to mice, zebrafish also require *lama1* for normal development and survival (Semina et al. 2006). *Bashful* zebrafish, which carry a mutation in *lama1*, display a shortened body axis and defective notochord differentiation, in addition to abnormalities in the eye, anterior CNS, and the myotendinous junction (Paulus and Halloran 2006; Pollard et al. 2006; Semina et al. 2006; Sztal et al. 2012).

Therefore, *lama1* is required in BMs of both mice and zebrafish, suggesting conserved functions of this gene. I hypothesise that the expression pattern of *lama1* is conserved during evolution and is similar between the mouse and the zebrafish. I aimed to characterise the expression pattern of *lama1* in the wild-type zebrafish during embryonic development using in situ hybridisation. I also hypothesised that as in the mouse embryo, *lama1* expression in the somites and neural tube will be regulated by Shh signalling. To test this hypothesis, I have explored the expression of *lama1* in zebrafish lines carrying mutations in components of the Shh signalling pathway, including Smoothened mutants (*smu*), which lack Hh signalling, and Patched 1/2 mutants (*ptc1/2*), which have increased Hh signalling. I predicted that *smu* zebrafish would show a loss or down-regulation of *lama1* expression in the somites and neural tube, whilst *ptc1/2* mutant zebrafish would show an up-regulation of *lama1* expression in these two tissues.