Chapter 3

The expression pattern of \textit{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 Smoothenened 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.