AIMS

2.1. Aims of the Project

Skeletal muscle cells of the limb and body in the chick embryo arise from different progenitor cells. They arise differently and mature at varying embryonic stages. As shown earlier, MRFs are important in the differentiation of skeletal muscles can induce the expression of Mir-1/206 and Mir-133. Post-transcriptional control exerted by Mir-133 is important in myoblast proliferation and control exerted by Mir-1/206 is important in achieving myoblast differentiation. Balance between these two opposing effects result in proper development of the skeletal muscles.

The first aim of the project was to examine in detail the expression pattern of the muscle specific miRNAs, MiR-1/206 and MiR-133 in limb development. To do this, whole mount in situ hybridisation was to be carried out on chick embryos at embryonic stage HH 35. The limbs were then to be separated and sectioned along the transverse axis. These sections provided us with a detailed understanding of the muscles specific expression of these miRNAs and whether the miRNA expression is (slow of fast) fibre type specific.

Also shown earlier, MRFs can induce the expression of muscle specific MiR-1 and MiR-206 in the muscles of the body.

The second aim of this project was to investigate whether the MRFs can induce muscle specific miRNAs, MiR-1/206 and MiR-133, in the limb muscles of the chick embryo, in a similar fashion to that shown in the neural tube.

To test this, the chick embryos were to be injected with one of four RCAS viruses. Each of the RCAS viruses was designed to carry the sequence for one of the four MRFs.

- The RCAS virus carrying/expressing the MyoD sequence is referred to as RCAS-MyoD.
- The RCAS virus carrying/expressing the Myogenin sequence is referred to as RCAS-Mgn.
- The RCAS virus carrying/expressing the Myf5 sequence is referred to as RCAS-Myf5.
- The RCAS virus carrying/expressing the MRF4 sequence is referred to as RCAS-MRF4.

Concentrated RCAS virus particles were to be injected into the prospective limb bud at embryonic stage HH 14-15. This is the stage where the lateral dermomyotome cells start to delaminate and migrate into the limb bud.

The injected embryos were incubated until they reached embryonic stage HH25. By this stage, the myoblasts in the limbs start to differentiate and separate into two (dorsal and ventral) muscle masses as illustrated in figure 12 and 13. The RCAS virus once injected, infects the proliferating cells, propagates and then infects the adjacent cells. As a result, at the end of the incubation period, the RCAS virus spreads to both the muscle and non-muscle cells of the limb.

Once these infected embryos were collected they were tested for three transcripts with a whole mount *in situ* hybridisation procedure.

1. Presence of RCAS-virus, by testing for the RCAS-Gag transcripts.
2. Presence of ectopic MRF in the infected limb, by testing for the particular MRF transcripts.
3. Presence of ectopic Mir-1/206 and Mir-133, by testing for the particular mature MicroRNA sequence.

The combination of these observations would determine whether the MRF proteins can induce muscle specific miRNAs in the limb tissue.

To examine these aims, the methods as detailed in chapter 3 were used to conduct carefully planned experiments. The proceedings of the experiments are described and discussed in detail in chapters 4 and 5.