Chapter 8

Conclusions

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This thesis presents the results of extensive experiments into the functions of members of the *rab* family of GTPases in the zebrafish. Results for more than a third (13) of the 37 *rab* genes that have been investigated to date are presented in this thesis. The results demonstrate the essential nature of many of these genes for early embryonic development, with a loss-of function screen using antisense MO oligonucleotides leading to severe, and in some cases, highly specific, developmental defects.

Of the 13 *rabs* investigated in detail and described in this thesis, *rab3c1*, *rab28* and *rab1a3* show very specific defects when knocked down. *rab3c1* MO injected embryos display pigment defects, investigations into which demonstrate that these MO injected embryos are blind. *rab28* MO injected embryos show circular swimming behaviour, which may result from defects in balance, while knocking down *rab1a3* shows a reduction in pigmentation. These defects show that, far from being purely housekeeping genes, *rabs* can exhibit tissue specificity. This corresponds to the effect seen in mammals, where five *rabs* have shown very specific phenotypes, with two of these responsible for human disorders (Menasche et al., 2000; Verhoeven et al., 2003).

To date, members of the *rab1*, *rab6*, *rab7*, *rab8* and *rab11* families, as well as all the members of the *rab5* family – genes whose homologues in yeast are considered essential due to the ensuing lethality when absent – have been studied. Of these, only *rab5a2*, when knocked-down, causes death before 24hpf. Given the proposed housekeeping function of many of the *rab* genes, in particular those considered essential, it seems counter-intuitive that only *rab5a2* should show such a dramatic early lethality. This thesis elucidates a novel, essential and complex role for the maternal transcripts of *rab5a2* in Nodal signalling. The knocking down of *rab5a2* results in a severe reduction of *cyc*, Nodal-responsive genes and the dorsal marker *chd*. Overexpression of *rab5a2* results in locally up-regulated expression of *gsc* and *ntl* in the animal pole, in addition to endogenous expression in the margin, but had

little effect on *chd* expression. These results suggest that *rab5a2* is a vital component of the Nodal signalling pathway. The results of this study show no evidence for the restrictive clearance model, suggested by Scholpp (Scholpp and Brand, 2004), for the movement of Fgf8, in Nodal signalling. However, this cannot be ruled out as experiments using exogenous *sqt* and *cyc* to investigate the effect of overexpression or knock-down of *rab5a2* on Nodal signalling proved inconclusive. To address this, further studies using GFP tagged Nodals are necessary.

This thesis suggests a complex role for *rab5a2* in DV patterning, since knocking down *rab5a2* affects many DV patterning signals. Intriguingly, both dorsal promoting genes and some dorsal antagonists are affected by knocking down *rab5a2*, while other dorsal antagonists, such as *bmp2b*, are not affected. An important role is also established for *rab5a2* in epiboly, and so early zebrafish development. Evidence for this is provided by the large number of genes whose expression changed when *rab5a* was knocked down

In conclusion, the *rab* genes are an interesting group of genes whose function can specifically impact on many important developmental processes. Although many of these genes show similar phenotypes, some show interesting and unique phenotypes that deserve further study. The unique phenotype of *rab5a2* has shown this gene to be essential for early development and Nodal signalling with an interesting role in DV patterning, one again worthy of further study.