# Chapter 4

## Discussion and Further Work

## 4.1 Screening the Zebrafish Rabs

The *rab* genes have classically been thought of as housekeeping genes because of their ubiquitous expression and vesicle trafficking role (Gurkan et al., 2005; Le Gallic and Fort, 1997). It is easy to assume that, given this ubiquitous expression, loss of a given Rab function would not yield a specific phenotype. However, this is not necessarily the case, a few *rabs* have been implicated in mammalian disorders such as Griscelli's syndrome (Menasche et al., 2000) or Charcot-Marie-Tooth type 2B neuropathy (Verhoeven et al., 2003). The specificity of *rabs*, in addition to some control at the gene expression level, is achieved by post-translational protein modification and the actions of protein regulators i.e. RabGDI. It is, therefore, important to investigate each *rab* to identify any specific role. The continuation of the Rab screen has resulted in the identification of a further 13 Rabs (bringing the total number of *rabs* screened to 37) that, when knocked-down, show defects in normal development. In many of these cases, the defects are very similar and include reduced head size and defects to the tail. However, some *rabs* show more individual phenotypes, including pigmentation defects, slowed development and a failure to hatch.

### 4.1.1 Pigmentation Defects

The knocking down of the Rab genes *rab3c1* and *rab1a3* resulted in defects in pigmentation. While *rab3c1* MO injected embryos showed a thicker layer of pigment the *rab1a3* MO injected embryos showed the opposite with a reduction in pigmentation.

#### 4.1.1.1 Rab3c1

In mammals Rab3c localizes to synaptic vesicles and cycles on and off the synaptic vesicle membrane with the exocytic release of neurotransmitters. Rab3c dissociates from the synaptic vesicles after the stimulation of exocytosis (Fischer von Mollard et al., 1994) and is thought to perform an important regulatory role in exocytosis rather

than direct involvement (Geppert et al., 1997; Nonet et al., 1997). This synaptic localization may explain the phenotype seen in the *rab3c1* MO injected embryos. At 24hpf, the brain is severely disrupted with forebrain and mid brain structures ill defined, by 5dpf these fish show pigmentation defects and are unable to respond to visual stimulus. When touched, the *rab3c1* MO injected fish were capable of moving their eyes which suggested the fish were blind and unable to see the stimulus, rather than incapacitated and unable to respond. The pigmentation defect in the *rab3c1* MO injected embryos can be explained by their failure to respond to light as control embryos which are capable of subtly altering their chromatophores to blend into their environment (Logan et al., 2006). In bright sunlight or against light backgrounds, zebrafish can aggregate their melanosomes becoming considerably lighter, while in the dark or against a dark background, they can disperse the melanophores and become darker. As the MO injected embryos are unable to see their environment, they are constantly dark adapted so their melanophores are always dispersed, resulting in them appearing darker (Logan et al., 2006). In addition, the *rab3c1* MO injected embryos have no visible swim bladder. This may be due to the swim bladder not developing or not inflating. The abnormal eye morphology in the MO injected fish - small eyes with protruding lenses - may be the cause of the blindness, possibly via a reduced retina, a problem with the lens or other eye components. Malformation or disruption to the optic nerve or visual centres of the brain is also a viable explanation since Rab3c is known to be involved in synaptic transmission (Fischer von Mollard et al., 1994). In the adult rat brain, staining for Rab3a, with which Rab3c has been shown to be localized (Fischer von Mollard et al., 1994), showed no localisation in the optic tract (Xu et al., 1998). However, expression of *rab3c1* in zebrafish shows expression throughout the brain including the post optic areas (Campos, 2004).

A screen in *Drosophila* seeking to identify potential targets for retinal calmodulin proteins found one of the groups of potential targets included proteins with domains similar to the Rab3 GDP/GTP exchange factors (Alvarez et al., 2003). Interestingly although Rab3c itself has never been associated with human disease and disorders its regulators have. Homozygous inactivating mutations in the GTPase activating protein Rab3GAP has been shown to be responsible for Warburg Micro syndrome (WARBM1) (Aligianis et al., 2005). WARBM1 is a severe autosomal recessive

disorder that is characterized by developmental abnormalities of the eye (including microcornea, congenital cataracts and optic atrophy) and the central nervous system (including microcephay). It is thought that defects in Rab3GAP causes a failure of the exocytic pathway to release ocular and neurodevelopmental trophic factors (Aligianis et al., 2005). A more recent paper showed that Martsolf syndrome, which has overlapping characteristics with WARBM1, also has defects in RAB3GAP. RAB3GAP is a heterodimeric protein with one catalytic (RAB3GAP1) and one noncatylitic subunit (RAB3GAP2). Warburg microsyndrome shows defects in RAB3GAP1, while Martsolf syndrome shows defects in the second (Aligianis et al., 2006).

Rab escort protein REP1 is the zebrafish homologue of the human *choroideremia* gene and the first characterized REP. The human disease choroideremia shows X-linked degeneration of the retinal pigment epithelium, resulting in affected males developing night blindness in their teens with progressive loss of peripheral vision and then complete blindness. Loss of function of the gene c*horoideremia* found on the X chromosome is responsible (Cremers et al., 1992). Interestingly, a zebrafish mutant for REP1 shows startling similarities to *rab3c1* MO injected embryos. At 5dfp the REP1 mutants had uninflated swim bladders, noticeably smaller eyes and irregular eye pigmentation. The REP1 mutants also showed a patchy, discontinuous distribution of iridophore pigment cells. Electron microscopy showed that the retinal layers were disrupted in the mutants. At 6dpf, oedema was observed around the heart and abdomen. In addition, REP1 mutants had defects in hair cells including the lateral line and the inner ear (Starr et al., 2004).

There is still some concern over the incomplete penetrance of the phenotype observed in *rab3c1* MO injected embryos which could be due to several factors. The first theory is that the phenotype seen in the *rab3c1* MO injected embryos isn't the result of knocking down *rab3c1* alone. It is possible that some of the embryos may have a mutation in other genes only uncovered on knocking down *rab3c1*. A second, more likely possibility is that there is a mutation in the *rab3c1* gene itself. To confirm this it will be necessary to sequence the gene in the *rab3c1* MO injected phenotypic and non-phenotypic siblings and compare.

#### 4.1.1.2 Rab1a3

The *rab1a3* MO injected embryos showed less pigmentation than controls. This phenotype appears to be due to fewer melanophores rather than abnormal distribution of melanosomes within the melanophores. However, the melanosomes in the remaining melanophores of the *rab1a3* mutant embryos appeared to have aggregated. One possibility for this reduction in melanophores is that the cells don't migrate properly from the neural tube, resulting in fewer melanophores reaching their destination. This may result from a defect in neural crest cells. Dr Isabel Campos showed a similar phenotype in the *rab* which she named *rab1b*. Unfortunately, once the zebrafish genome was sequenced, the MO used for this *rab* was shown to partially knock down two additional *rab1* genes. However, the clone which Dr Campos used for the expression pattern of *rab1b* has equally close homology to *rab1a* as it does for *rab1b*, showing 98% identity to the *rab1a3* clone. Therefore, it is probable that these genes are the same. The expression pattern for *rab1b* showed localization in the chordamesoderm, polster, neural crest and blood. The localization in the neural crest is consistent with the phenotype seen in *rab1a3* MO injected embryos. Defects in neural crest cells have been seen in zebrafish to be responsible for the loss of pigment (Kelsh et al., 1996; Parichy et al., 1999; Southard-Smith et al., 1998).

In the zebrafish mutant *colourless* (*cls*) there is extensive loss of pigment cells and enteric nervous system, with large reductions in sensory and sympathetic neurones and putative satellite glia and Schwann cells (Dutton et al., 2001; Kelsh and Eisen, 2000). This phenotype suggests that *cls* functions in specification, proliferation or survival of progenitors for all nonectomesenchymal crest derivatives (Dutton et al., 2001). The *cls* gene encodes a *sox10* homologue, loss of which causes neural crest cells to take ectomesenchymal fates rather than form non-ectomesenchymal fates. If cells do form non-ectomesenchymal fates, they generally fail to migrate and do not overtly differentiate, dying by apoptosis between 35 and 45 hours post fertilisation (Dutton et al., 2001). The melanophore defects in *cls* mutants are thought to be mostly explained by disruption of expression of *microphthalmia (Mitf). Mitf* is a basic helix-loop-helix/leucine zipper transcription factor, known to be required for development of eye and crest pigment cells in the mouse. Interestingly, the human

disorder Waardenburg-Shah syndrome shows reduced enteric nervous system and reduced pigment cell number (Dutton et al., 2001).

An additional explanation for the reduction in melanocytes in *rab1a3* morphant embryos may be a defect in migration. A possible explanation for this defect in migration could be defective cell adhesion, since this has been shown to be required for cell migration (Ulrich et al., 2005). In chick, during delamination in neural crest cells, *N-cadherin* is down-regulated in migrating neural crest (Bronner-Fraser et al., 1992; Nakagawa and Takeichi, 1995; Nakagawa and Takeichi, 1998), whilst *cadherin-6B*, which is expressed in the dorsal neural tube is also down-regulated (Nakagawa and Takeichi, 1995; Nakagawa and Takeichi, 1998). These expression patterns suggest that cadherins mediate strong cell contacts in the neuroepithelium and must be down-regulated for neural crest cells to become migratory (Halloran and Berndt, 2003), suggesting a role for cadherins and cell adhesion in the distribution of melanophores. However, the zebrafish N-cadherin mutant, *parachute*, has no defects in pigmentation (Lele et al., 2002). The zebrafish mutant *sparse* encodes a type III receptor tyrosine kinase, kit, which is expressed in melanocytes and is required, cell autonomously, for melanocyte dispersal and migration from the neural tube (Parichy et al., 1999). The *sparse* mutant exhibits only half the normal complement of melanocytes (Kelsh et al., 1996; Nasevicius and Ekker, 2000; Odenthal et al., 1996).

The *rab1a3* MO injected embryos, in addition to showing reduced melanophore numbers, showed increased aggregation in the remaining melanophores. To ascertain whether the melanophores in theses embryos are unable to diffuse their meloanosomes, it would be interesting to place the *rab1a3* MO injected and control MO injected embryos into either caffine or adrenalin and compare: the aggregation of melanosomes can be achieved by addition of adrenalin, while dispersion can be achieved by addition of caffeine (Rodionov et al., 1998).

### *4.1.2 Slowed Development*

Four of the Rabs in the screen showed slowed development and were deformed as early as shield stage. Such early phenotypes could be down to problems with convergence extension and dorsoventral patterning or epiboly.

In the epiboly mutant *hab*, epiboly begins normally in the blastula and early gastrula stage but, by 70% to 80%, epiboly mutants begin to arrest and dissociate, with their blastoderm peeling off the yolk. When the zygotic and maternal genomes are heterozygous (ZMD) for *hab,* the embryos complete epiboly an hour after their wild type siblings (McFarland et al., 2005). In addition, morphogenesis of the neural tube is abnormal, with gaps forming in the midline during segmentation stages. At later stages, ectopic rows of neurons form in the widened spinal cord and hindbrain (McFarland et al., 2005). The *hab* mutant is caused by a mutation in the zebrafish homolog of the adhesion protein E-cadherin (Shimizu et al., 2005b). E-cadherin is required for the epiblast cells from the interior layer to sequentially move into the exterior layer, where they become restricted to that layer; and participate in subtle cell shape changes that further expand the blastoderm. In *hab* mutants, cells that intercalate into the exterior layer do not change cell shape or become restricted, with many of these cells moving back into the interior layer. *hab*/E-cadherin is necessary for the cell rearrangements that spread the teleost blastoderm over the yolk (Shimizu et al., 2005b). Although the *hab* mutant shows slowed development, the phenotype is quite different from those observed in the slowed development *rabs.* The severest *hab* mutants died before 24hpf, with cells peeling off the yolk – a phenotype not seen in any of the *rab* loss of function experiments in this chapter. The *hab* mutants also show asynchronous movement of cells down the yolk and a large streak of accumulated cells on one side of the embryo. In the *rab* loss of function embryos, this asynchronous movement is not evident and, although there is some cell accumulation in these embryos, it is not as extensive as in the *hab* mutants. This suggests that the phenotypes seen in *rab* loss of function are for the most part unlikely to be due to an effect on epiboly.

The process of convergence and extension occurs during epiboly and it is thought that these movements must be interdependent, as epiboly is slowed in *Xenopus laevis* by treatments that block convergence (Hikasa et al., 2002).The zebrafish *trilobite (tri)* mutant, however, shows reduced convergence and extension movements, while epiboly and involution are not affected (Hammerschmidt et al., 1996a; Sepich et al., 2000; Solnica-Krezel et al., 1996). Since convergence/extension cell movements contribute to lengthening of the embryo (Kimmel et al., 1995), the *tri* mutant embryo is shorter and its somites are wider than in a wild-type embryo. These mutant embryos show normal organizer formation, tissue patterning, and overall development (Sepich et al., 2000; Solnica-Krezel et al., 1996). The convergence movements of lateral cells in *tri* mutants are normal from shield through yolk plug closure, but are reduced, compared with wild-type, by the one somite stage (Sepich et al., 2000). A second convergence/extension mutant *knypek* is required for convergence movements of lateral cells and convergence extension movements of dorsal tissue. The mutants show reduced body length and a shortened malformed tail. The *knypek* gene product regulates cellular movements but not cell fate specification, with the convergent extension movement defects in *knypek* associated with abnormal cell polarity, as mutant cells fail to elongate and align medio-laterally. The *knypek* locus encodes a member of the glypican family of heparan sulfate proteoglycans. Glypican is required during vertebrate gastrulation, as a positive modulator of non-canonical Wnt signalling, to establish polarized cell behaviours, underlying convergent/extension movements (Topczewski et al., 2001). All of the *rab* loss of function experiements that showed slowed development also, showed reduced body length. *rab1a4* and *rab18* showed a more pronounced reduction than *rab11a1*, which is consistent with a defect in convergence and extension. The *tri* mutant showed thicker somites, whereas the somites in these *rabs* appeared normal thickness, although they were U-shaped rather than the chevron shape seen in controls. The *tri* mutant also showed convergence extension defects after the yolk plug closure stage, whereas the *rab* loss of function embryos showed defects in gastrulation at shield stage. Although both *tri* and *knypek* mutants show cyclopia, the head defects seen in the *rab* MO injected embryos are not consistent with this phenotype; they show a more severe phenotype, with smaller head and eye structures. This suggests that altered convergence and extension movement defects are either not responsible for

the defects seen in these *rab* loss of function embryos or that they are accompanied by other defects.

Defects in epiboly and convergence/extension alone don't explain the defects seen in these slow developing *rab* MO injected embryos but a combination of both factors may explain some of the phenotypes seen. The early arrest mutants such as *zombie, spectre* and *speed bump* show head defects similar to those seen in the *rab* MO injected embryos, in addition to a reduction in body axis and defects in the tail (Kane et al., 1996b). However, these arrest mutants do not arrest until 80% epiboly at the earliest, which is inconsistent with the slowed development in the *rab* MO injected embryos apparent at shield stage. Since Rab proteins are responsible for vesicle trafficking, it is possible that these *rabs* might be involved in both epiboly and convergence extension in addition to other as yet unknown process.

### *4.1.3 Hatching Gland*

Zebrafish embryos hatch from their chorions, using a combination of processes. The osmotic pressure of the perivitelline fluid within the chorion increases, while the hatching gland releases enzymes that digest the chorion membrane. The chorion is thus weakened and the embryo can use muscular movements (eg. a flip of the tail), to release itself from the chorion (Buznikov and Ignat'Eva, 1958). Hatching gland cells produce secretory granules that contain the hatching enzyme. These secretory granules increase in number until the hatching gland disappears after hatching (Buznikov and Ignat'Eva, 1958)

In zebrafish, the hatching gland is composed of two cell populations: mesodermally derived hatching gland cells, which express the *hatching gland* gene (*hgg*), and ectodermally derived support cells (Kimmel et al., 1990). The hatching gland is derived from the polster, an accumulation of cells found anterior and below the forebrain of the embryo at tail bud stage. The polster, in turn, is derived from the anterior mesendoderm. Differentiation of the polster and, later, the hatching gland cells, is controlled by specific gene regulation, a process programmed early in embryogenesis (Inohaya et al., 1995; Inohaya et al., 1999). There are many genes

expressed in the pre-polster, the best characterised of these is *Cathepsin L* (*CatL* or *hgg1*), a protease secreted by the hatching gland. Also expressed in the pre-polster, is *cyclase associated protein- 1* (*Cap1*), which is thought to be required for the regulation of actin dynamics within pre-polster cells, influencing cell behaviour during the generation and migration of the polster (Daggett et al., 2004).

An important family of transcription factors in the formation of the polster are the zebrafish Kruppel-like factors (*zKLF4*). *zKLF4* is a zinc finger family of transcription factors, expressed early during polster formation, which are required for further specification of the polster into the hatching gland (Kawahara and Dawid, 2000; Kawahara and Dawid, 2001). KLF4 is one of the earliest markers of the polster and lateral plate mesoderm (LPM), and marks the fate of the hatching gland, blood, and vasculature (Amatruda and Zon, 1999; Oates et al., 2001), with z*KLF4* having a critical role in erythroid cell differentiation in zebrafish (Kawahara and Dawid, 2000; Kawahara and Dawid, 2001). If *zKLF4* is knocked-down, the hatching gland is absent and the embryos do not hatch (Gardiner et al., 2005), although the embryos were normal until this stage, including those cell types derived from the same parent, such as heart and anterior macrophages. If the embryos were manually dechorionated, they developed normally and survived into adulthood.

The four *rab* genes that showed a failure to hatch when knocked-down, also showed a variety of other phenotypes: most notably, pericardial oedema. It is possible thereofore that the hatching defect seen in these *rab* MO injected embryos is due to an effect on the polster or anterior mesoderm. It is, also, possible that these *rabs* affect specification of the hatching gland from the polster. However, due to the multitude of other phenotypes, it is unlikely that these *rabs* affect just the hatching gland cells. The *rab11b2* MO injected showed the hatching gland cells to have accumulated at two points on the yolk, rather than the uniform stripe of cells seen in controls, suggesting a defect in the dispersal of the hatching gland cells. In addition, this accumulation of cells appeared to be dying, which could be due to a number of factors, including apoptosis due to the cells being mis-dispersed or hatching gland cells being poisoned by disrupted secretion. Three out of four of the MO injected *rabs* that showed the hatching defect were from the Rab11 family. It is, therefore, possible that these Rabs may function in the same pathway.

All of the *rab* MO injected embryos with hatching gland defects also showed defects in the tail structures, with *rab11b1, rab11b2 and rab6a* showing the severest defects. These tail defects could be responsible for the inability of MO injected embryos to hatch, since even with a fully functional hatching gland, the embryos would be unable to break their way out of the chorion as their tail movements would be compromised. It is, therefore, possible in such cases that the hatching gland has little to do with the inability to hatch. It is more likely, though, that there is a defect in the hatching gland in some, if not all, of the *rab* genes, especially *rab11b2* MO injected embryos, which show an abnormal hatching gland. This defect in the hatching gland may then be aggravated by the defects in tail structure, resulting in the embryos inability to hatch. To establish if the reduction in hatching success is the result of defects in the hatching gland, expression of genes associated with the gland, such as *hgg1*, could be assayed. While to establish if muscle defects in the tail could be responsible, phalloidan staining could be employed.

### *4.1.4 Swimming Defects*

Many of the MO injected embryos showed some sort of tail defect and, therefore, had abnormal swimming behaviour. The *rab28* MO injected embryos, however, appeared largely normal, compared to many of the other *rab* MO injected embryos in the screen. There was some brain cell death at 24hpf and, by 48hpf, pericardial oedema. However, the most striking defect was the modified swimming behaviour these embryos showed. Some of these MO injected embryos had kinked tails but many of them, other than a slightly shortened body axis, showed normal tail structures. The *rab28* MO injected embryos swam in a circular pattern.

There are many possible explanations for the swimming defect in the *rab28* MO injected fish, including muscle abnormalities, defects in the notochord, basement membrane or neuromuscular junctions and impaired balance.

Embryos with defects in neuromuscular junction usually show short swimming response. *twitch once* mutant embryos, when touched, respond with one or two

swimming strokes, instead of an escape response. This response is called use-dependent fatigue and has been shown to be a consequence of a mutation in the tetratricopeptide domain of muscle rapsyn. This mutation inhibits the formation of subsynaptic acetylcholine receptor clusters. This loss of receptor clusters results in reduced synaptic strength, is augmented by a postsynaptic depression not seen at normal neuromuscular junctions, and results in use-dependent muscle fatigue (Ono et al., 2002). The *shocked* (*sho*) mutant also exhibits an aborted escape response (Granato et al., 1996). *shocked* (*sho*) is a mutation which causes motor deficits attributable to CNS defects. Mutant embryos display reduced spontaneous coiling of the trunk, diminished escape responses when touched, and an absence of swimming. The shocked mutant shows a mutation in the *slc6a9* gene that encodes a glycine transporter (GlyT1). *glyt1* is expressed in the hindbrain and spinal cord, which are regions known to be required for generation of early locomotory behaviours (Cui et al., 2005). A lack of muscle acetylcholinesterase in the mutant *ache* shows slowed synaptic current and so sustained contractions on both sides of the tail (Behra et al., 2002). Embryos with muscle defects, such as dystroglycan MO injected embryos, respond to touch but appear to be less flexible than controls. By 48 hpf, *dystroglycan* MO injected embryos are curved and dystrophic, compared with controls, and move in an uncoordinated fashion (Parsons et al., 2002b). In *lama2* MO injected embryos, there is defective organisation of the muscle tissue and they responded poorly to touch, with uncoordinated movements (Parsons et al., 2002b). None of these defects show comparable swiming behaviour to that seen in the *rab28* MO injected embryos, suggesting the neuromuscular junction, notochord or muscle tissue may not be responsible for the defective swimming behaviour seen in these MO injected embryos. In order to ascertain if this is the case, expression of genes required for notochord, such as *laminins* or *coatomers*, could be assayed, while defects in muscle structure can be investigated by phalloidin. Defects in the neuromuscular junction could be identified by expression analysis of neuronal markers and by pharmaceutical methods, such as addition of acetylcholinesterase inhibitors.

Fish with defects in balance show characteristic swimming behaviour, with the fish swimming in circular motions, resulting in the name “circler” mutants (Nicolson et al., 1998). The causes of balance defects include defects in ear and the lateral line. The inner ear contains two macular organs, the saccule and utricle, which are found

in all vertebrates. The saccule and utricle are pouch-like vestibules which contain a bed of hair cells coupled to calcium carbonate crystals. In fish, the crystals coalesce to form a single large polycrystalline mass called an otolith. Forces that result in the movement of the otolith are transduced to the underlying hair bundles (Nicolson, 2005), with defects in the otoliths or the hair cells causing defects in balance. The gene *starmaker* is needed for otolith formation. Knocking down this gene results in a small fraction of MO injected embryos displayed circling behaviour (Sollner et al., 2003). Defects in hair cells in zebrafish can affect both the ear and the lateral line, since the lateral line is a system of superficial canal hair cells that detects water movements. Glial cells have been implicated in preventing premature development of lateral line hair cells (Grant et al., 2005; Lopez-Schier and Hudspeth, 2005). The swimming defect in these *rab28* MO injected embryos seems quite similar to the defects seen in fish with ear or balance defects. This does not though explain the oedematous region around the heart.

Defects in hair cells could result from defects in all forms of cilia. Monocilia have been proposed to establish the left-right (LR) body axis in vertebrate embryos by creating a directional fluid flow that triggers asymmetric gene expression. Cilia inside Kupffer's vesicle are motile and create a directional fluid flow just prior to the onset of asymmetric gene expression in lateral cells. Ciliated KV cells are required during early somitogenesis for subsequent LR patterning in the brain, heart and gut (Essner et al., 2005). This may explain the brain heart and swimming abnormalities.

### *4.1.5 Non-Specific Defects*

The compex defects seen in the two *rab* MO injected embryos in this group, such as brain cell death, oedema and tail defects, were also seen in many of the other MO injected embryos. The brain and tail structures are under the control of many pathways. Therefore, it is difficult to ascertain which pathways have been disrupted by these *rabs* being knocked-down. It is necessary to look at the data obtained for homologues of these *rabs* in other models

Amoung the first *rab* genes uncovered in rat brain was the homologue of *ytp1*, *rab1*. There have now been two mammalian *rab1* genes identified *rab1a* and *rab1b.* Both mammalian *rab1* genes have been suggested to regulate transport of cargo between the ER and the Golgi apparatus (Palokangas et al., 1998; Plutner et al., 1991; Sannerud et al., 2003; Tisdale et al., 1992). Rab1 in rat brain is seen in neuroblasts and glioblasts (Ayala et al., 1989). This expression may explain the cell death death seen in *rab1a2* MO injected embryos, in addition to that seen in *rab1a4* and *rab1a1*. Interestingly, in yeast, Ypt1p associates with cytoplasmic α-synuclein and elevated expression of Rab1, protected against α-synuclein induced dopaminergic neuron loss in animal models of Parkinsons disease (Cooper et al., 2006).

Rab1a and Rab1b have been found to be highly expressed in human cardiac tissue. In addition, Rab1b has been shown to be highly expressed in murine cardiac tissue. Cardiac specific overexpression of Rab1a in cardiac tissue of mice resulted in a dilated cardiomyopathy that resulted in premature death at six weeks of age. Rab1a overexpression revealed that Golgi stacks and surrounding transitional vesicles were markedly enlarged. This shows a role for *rab1* in heart developmeant and may explain the pericardial oedema in *rab1a1, rab1a2* and *rab1a4*. If the heart is defective in these MO injected embryos, circulation is reduced and oedema may result. However, there was no obvious defect, other than the oedema, in the heart of these *rab1* MO injected embryos but it is possible circulation may have been reduced.

In human cell tissue culture the inactive form of Rab1b has been shown to block forward transport of cargo and induces Golgi disruption (Alvarez et al., 2003). The phenotype is analogous to that induced by brefeldin A (BFA) and causes resident Golgi proteins to relocate to the ER, as well as inducing redistribution of ER-Golgi intermediate compartment proteins. The COPI (Coatomer complex ) machinary was shown to be compromised by the release of beta-COP into the cytosol. Inactive Rab1b is reversed by expressing known mediators of COPI recruitment suggesting that Rab1b function influences COPI recruitment. Further evidence is provided by the finding that cells expressing the active form of Rab1b are resistant to BFA. (Lazar et al., 1997). The notochord defect, seen in the *rab1a2* MO injected embryos, may be the the result of the *rab1a* affecting COPI. The zebrafish mutants *sneezy,*

*happy,* and *dopey* encode the α, β and β' subunits of COPI. These mutants all show defects in their notochord. Coatomer activity is required for normal chordamesoderm differentiation, perinochordal basement membrane formation and pigmentation (Coutinho et al., 2004). Although associations have been seen for Rab1b and COPI all zebrafish *rab1s* share high sequences homology with both *rab1a* and *rab1b*. It is, therefore, possible that any of the zebrafish Rab1s may function in a similar way to mammalian Rab1b.

*rab20* has been shown to be expressed in the kidney tubule and intestinal epithelial cells in mice (Curtis and Gluck, 2005). Electron microscopic studies have revealed that Rab20 is located on endocytic structures underlying the plasma membrane, suggesting that they play a role in endocytosis/recycling (Lutcke et al., 1994). However, this is the extent of the current knowlage on *rab20*. It is, therefore, difficult to discuss the mechanisms that could be involved in *rab20* function and those that are defective in the *rab20* MO injected embryos. This problem led Dr Matthew Clark to develop a small inexpensive microarray for the routine analysis of molecular phenotype. In the future, microarray analysis of this and the other *rabs* may provide valuable information on these lesser studied *rabs* as well as new insights into those that are better characterised.