Chapter 6

Microarray analysis of Rab5a2

6.1 Introduction

Microarrays are a powerful tool which can be used to compare, not only the expression of thousands of gene within a sample but, also, between multiple samples. The arrays comprise 'spots' of DNA, RNA or oligonucleotides (probes), which correspond to genes, immobilized on slides or chips made from glass, silicone or plastic. A sample of RNA or cDNA is labelled with a fluorescent dye and hybridized to the microarray. The 'spots' on the slide then bind any complimentary RNA (or cDNA) in the sample in a dose dependent manner. The expression of these genes can then be quantified.

6.1.1 Why Microarray Rabs?

There are currently over 80 zebrafish Rabs, some of these as investigated in this thesis have very specific phenotypes (Chapter 3.). However, many show similar and complex phenotypes which include brain cell death, shortened AP axis and irregular somites. Others have shown no morphological phenotype. The similar and complex phenoypes seen in many of the Rabs makes it very difficult to identify any specific pathway which is affected in MO injected embryos while the presence of MO injected embryos with essentially wild type phenotypes, substantially compounds the problem. In these Rabs which show no phenotype a pathway may be affected, however, it is possible the loss of this Rab in ideal conditions is compensated for. Microarrays provide an efficacious method of screening all these Rabs and elucidating which pathways they affect.

6.1.2 The Microarray

The microarray used in the following experiment was a custom made oligonucleotide array designed specifically to investigate zebrafish developmental genes. Each slide contained 1898 separate oligos which were 65 base pairs in length and were carefully selected so that the 65 bases were unique to the particular gene they were targeting. The genes on the array included XPAT transcripts from ZFIN, approximately 300

handpicked transcripts chosen by the Stemple lab, 213 transcripts thought to be involved in cell – cell signalling chosen by the Wright lab at the Sanger Institute and 109 housekeeping transcripts. These oligos were then immobilized onto a glass slide by an array spotting robot; each oligo was spotted twice to ensure greater reproducibility. This custom oligoarray was probed using two dyes enabling the control RNA samples to be labelled with one dye and hybridized to an array slide whilst the experimental RNA samples were labelled with a second and hybridized to the same array slide (Figure 6.1.1). Placing both control and experimental samples on the same array slide results in reduced experimental variation. To ensure any experimental variations resulting from differences in the dyes is minimised, the dyes were swapped resulting in one slide with Cy5 labeled control and Cy3 labeled experimental RNA and a second slide with Cy3 labeled control and Cy5 labeled experimental RNA.

6.1.3 The Experiment

In order to obtain samples for the array 300, single cell stage embryos were collected from three pairs of parent fish resulting in three groups of 300 embryos. Replicate parent fish came from the same line to reduce variation. Each clutch of 300 embryos is split into two groups with 150 embryos in each dish. One of the groups from each parent is injected with 6ng of control MO. The second is injected with 5ng of *rab5a2* MO. The embryos are left to develop at 28°C until they reached 30% epiboly, at which point 65 embryos are then removed from each of the control embryo injected groups and placed in a 1.5ml Eppendorf tube. The embryos were washed three times with zebrafish egg water. All the water is removed and the embryos are frozen in dry ice for 20minutes before being stored at -80. The same procedure is observed for the *rab5a2* MO injected embryos. At shield stage a further 65 embryos were removed from both the control injected embryo groups and the *rab5a2* MO injected embryos. The method employed for the 30% epiboly stage embryo is repeated for these samples. The remaining embryos are left to develop until 24hpf and their phenotype checked ensuring that there are no anomalies in these embryos and that they show the phenotype expected for these embryos. Embryos were frozen at 30% epiboly and shield stage enabling comparisons to be made between the former stage at which

there is little visual difference between control and MO injected embryos and shield stage when the phenotype becomes apparent in the *rab5a2* MO injected embryos.

Figure 6.1.1: Image capture of one of the custom made oligonucleotide microarray slides showing hybridization of control and *rab5a2* samples as two colour data. (blue = cy5, red = cyc3 , other colours are a combination of the two samples)

RNA is isolated directly from the frozen samples by homoginising in trizol and the RNA quantified. Given the early stage of these embryos it was necessary to amplify the RNA (see Methods section 2.3.3).

6.2 Results

6.2.1 Establishing an appropriate fold change.

The fold change for each gene that changes in the microarray experiment is plotted by the computer programme Genespring. The programme will then only show genes that change above the fold threshold, in the case of Figure 6.2.1 this is a two fold change. The blank area between the lines represents the majority of the genes while those points outside the lines represent those genes that changed by two fold or more. Due to the large number of genes that changed at the two fold level the fold change threshold was increased to three fold.

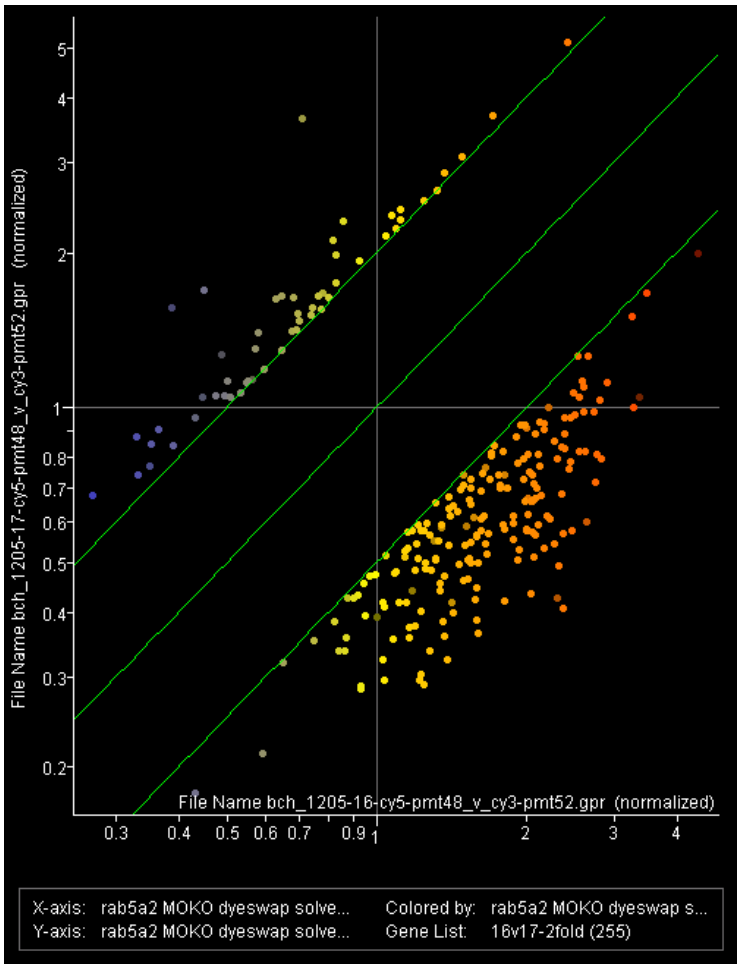

Figure 6.2.1 : Graph plotted in the microarray analysis programme Genespring showing the number of genes that changed more than two fold when *rab5a2* MO injected embryos were compared to control embryos.

6.2.2 The top 50 genes that increased and decreased in rab5a2 MO injected embryos when compared to controls.

After analysis by Genespring the data was tabulated with the accession number from NCBI, the oligo number and the fold change of that oligo. The accession number was then identified and the corresponding gene tabulated. Since most genes have two oligos the list was examined for a second oligo and the fold change for both averaged. Dr Wright's oligos were also checked for duplicates with known genes on the slide, if these matched the fold change was again averaged. Due to the large number of genes that changed more than 3 fold the top 50 genes that increased and the top 50 genes that decreased at each stage were tabulated with their average fold change, the name of the gene and the number if oligos for that gene that changed (Tables 5.2.1-5.2.4).

6.2.2.1 Expression changes at 30% Epiboly Stage

In the 30% epiboly stage embryos at the 3 fold stringency 382 genes changed in the *rab5a2* MO injected embryos when compared to control embryos. 201 genes showed a decrease in activity in *rab5a2* MO injected embryos compared to controls while 181 showed an increase in gene activity in *rab5a2* MO injected embryos when compared to controls. The top 50 genes that decrease and the top 50 genes that increase between *rab5a2* MO injected embryos and control embryos at the 30% epiboly stage are presented in Table 6.2.1 and 6.2.2 respectively.

6.2.2.2 Expression changes at Shield Stage

In the shield stage embryos at the three fold stringency 426 genes changed in the *rab5a2* MO injected embryos when compared to control embryos. 211 genes showed a decrease in activity in *rab5a2* MO injected embryos compared to controls while 215 showed an increase in gene activity in *rab5a2* MO injected embryos when compared to controls. The top 50 genes that decrease and the top 50 genes that increase between *rab5a2* MO injected embryos and control embryos at shield stage are presented in Table 6.2.3 and Table 6.2.4 respectively.

Av. fold change	No oligo's	Description
0.13	1	T-box gene 16
0.17	3	trophoblast glycoprotein-like
0.19	2	bone morphogenetic protein 4
0.22	1	zic family member 3 heterotaxy 1 (odd-paired homolog)
0.22	1	keratin 4
0.23	2	SRY-box containing gene 2
0.25	1	ATPase, Na+/K+ transporting, alpha 1b polypeptide
0.26	2	T-box transcription factor TBX6
0.26	2	zgc:101612
0.27	2	fibroblast growth factor 8
0.28	2	U1 small nuclear ribonucleoprotein polypeptide A
0.29	1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5
0.30	1	Unknown
0.30	2	zgc:92414
0.30	2	Type I cytokeratin, enveloping layer
0.32	1	CCAAT/enhancer binding protein alpha
0.32	1	caudal type homeo box transcription factor 4
0.32	1	tyrosine 3-monooxygenase
0.33	2	chordin
0.33	1	fibroblast growth factor 24
0.33	1	methyl-CpG binding domain protein 3b
0.34	2	bonnie and clyde
0.34	2	LIM homeobox 1b
0.35	2	Ved
0.35	1	fibroblast growth factor 19
0.36	1	mannosidase, beta A, lysosomal
0.36	2	protocadherin 8
0.37	1	tyrosyl-tRNA synthetase
0.37	2	gastrulation brain homeobox 1
0.37	1	peroxisomal biogenesis factor 3
0.38	2	mki67 interacting nucleolar phosphoprotein (human) – like
0.38	3	deltaD (dld),
0.39	2	zgc:101000
0.39	2	transgelin 2
0.39	1	AHA1, activator of heat shock ATPase homolog 1, like
0.40	1	hairy and enhancer of split related-7
0.40	2	solute carrier family 3, member 2 like
0.40	2	Vent
0.40	2	lymphocyte cytosolic plastin 1
0.40	2	ubiquitin-activating enzyme E1-domain containing 1
0.40	2	Wnt-11 protein precursor
0.40	2	Rab14
0.40	1	iroquois homeobox protein 1, a isoform 1
0.41	2	CB967 5- similar to Filamin A
0.41	1	glutamate-ammonia ligase
0.42	1	minichromosome maintenance protein 3
0.42	1	tumor protein p73-like isoform alpha 1
0.42	1	SRY-box containing gene 3
0.43	1	cysteine and glycine-rich protein 1
0.43	1	Homeobox protein Hox-B2a (Hox-B2)

Table 6.2.1: The fold change of the top 50 genes that decreased in *rab5a2* MO injected embryos at the 30% epiboly stage compared to control embryos.

Av. fold change	No oligo's	Description
6.70	2	T-box 24
6.04	2	v-fos FBJ murine osteosarcoma viral oncogene homolog
5.99	2	forkhead box I1
4.49	1	unc-45 homolog B (C. elegans) (unc45b),
3.93	1	muscle segment homeobox E
3.91	2	claudin g (cldng),
3.71	2	Insulin gene enhancer protein ISL-2 (Islet-2).
3.69	2	Myoblast determination protein 1 homolog (Myogenic factor 1)
3.55	1	enolase 3, (beta, muscle)
3.53	2	heat shock protein 47
3.50	2	Homeobox protein Dlx6a (DLX-6)
3.43	2	matrix metalloproteinase 13
3.32	1	DIG0228_268
3.26	2	hemoglobin alpha embryonic-1 (hbae1),
3.22	1	septin 9
3.11	2	insulin-like growth factor binding protein 1
3.10	2	Ribosome binding protein 1 homolog (dog)
3.06	2	H1 histone family, member X
3.01	2	Microphthalmia-associated transcription factor a (mitfa),
3.00	1	friend leukemia integration 1
3.00	2	Hypothetical protein
3.00	2	iroquois homeobox protein 7
2.99	2	Eomesodermin homolog
2.89	1	Aminolevulinate, delta-, synthetase 2
2.87	1	endothelium-specific receptor tyrosine kinase 2 (tie2),
2.83	1	hypothetical protein LOC405860
2.67	1	Ras homolog gene family, member E
2.65	1	CB1077 5- similar to Myosin Vb,
2.65	1	hemoglobin beta embryonic-1
2.64	1	SRY-box containing gene 31,
2.63	2	deltaA
2.61	1	myogenic factor 6
2.61	1	Forkhead box B1.1
2.56	2	suppressor of fused homolog (Drosophila) (sufu),
2.55	2	similar to CCCH zinc finger protein C3H-2
2.54	1	HHGP protein
2.53	1	Hypoxia-inducible factor 1 alpha inhibitor
2.52	1	hairy-related 5
2.49	1	bone morphogenetic protein 2b
2.46	2	transforming growth factor, beta receptor II (tgfbr2),
2.45	1	Bcl2-like
2.44	2	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c
2.43	2	LIM homeobox 8
2.41	2	four and a half LIM domains (fhl),
2.37	1	eyes absent homolog 1 (eya1),
2.34	2	dachshund a (dacha),
2.34	1	Ictacalcin
2.34	1	CUG triplet repeat, RNA-binding protein 1
2.32	1	growth associated protein 43 (gap43),
2.32	2	muscle-specific beta 1 integrin binding protein 2

Table 6.2.2: The fold change of the top 50 genes that increased in rab5a2 MO injected embryos at the 30% epiboly stage compared to control embryos.

Av. fold change	No oligo's	Description
0.07	2	T-box gene 6
0.07	1	zic family member 1 (odd-paired homolog) (zic1),
0.09	2	membrane protein, palmitoylated 1 (mpp1)
0.14	2	Carbonic anhydrase (Carbonate dehydratase)
0.17	3	Trophoblast glycoprotein-like
0.17	2	myeloid ecotropic viral integration site 3
0.20	1	myeloid ecotropic viral integration 1 (meis1),
0.20	1	LTP4
0.21	2	GLI2a
0.21	2	Zgc:92414
0.21	2	myogenic factor 5
0.23	2	Macrophage stimulating 1 (hepatocyte growth factor-like)
0.23	2	type I cytokeratin, enveloping layer
0.23	2	Zgc:101612
0.23	1	midkine-related growth factor b
0.24	2	chordin (chd),
0.24	1	Keratin 4 (krt4),
0.24	2	Mki67 (FHA domain) interacting nucleolar phosphoprotein (human) – like
0.25	1	Caudal type homeo box transcription factor 4 (cdx4),
0.25	2	Beta-2-microglobulin precursor
0.26	2	forkhead box C1b (foxc1b),
0.26	2	protocadherin 8 (pcdh8),
0.27	1	zic family member 3 heterotaxy 1 (odd-paired homolog)
0.27	2	Rab14
0.27	1	tyrosine 3-monooxygenase
0.27	1	mutL homolog 1, colon cancer, nonpolyposis type 2
0.28	2	Clone CB926 5- similar to Gem-associated protein 5
0.28	1	minichromosome maintenance protein 3
0.29	2	Hypothetical protein LOC378998
0.29	2	Bone morphogenetic protein 4
0.29	1	seryl-tRNA synthetase (sars),
0.30	1	Kinesin family member 11(kif11),
0.30	2	Oep
0.31	2	U1 small nuclear ribonucleoprotein polypeptide A
0.31	1	DIG0410_1903
0.31	2	Ved
0.31	2	Lymphocyte cytosolic plastin 1
0.31	2	Roundabout homolog 3 (robo3),
0.32	2	cDNA clone CB473 5
0.32	2	Sine oculis homeobox homolog 3b
0.33	1	Apolipoprotein A-I precursor (Apo-AI) (ApoA-I)
0.33	2	Sp5 transcription factor-like
0.33	3	delta D (dld)
0.34	2	hypothetical protein LOC550434
0.35	1	fibroblast growth factor 19
0.35	2	interleukin 17 receptor D (il17rd),
0.36	2	Sonic hedgehog protein precursor (SHH) (VHH-1)
0.36	1	3-beta-hydroxysteroid dehydrogenase
0.36	2	Hypothetical protein.
0.36	1	retinol dehydrogenase 10

Table 6.2.3: The fold change of the top 50 genes that decreased in rab5a2 MO injected embryos at the shield stage compared to control embryos.

Av. fold change	No oligo's	Description
11.89	1	homeo box B5a
8.90	2	claudin g
7.37	1	MAD homolog 3
7.06	2	neogenin 1
6.46	1	forkhead box B1.1
5.98	1	tyrosyl-tRNA synthetase
5.86	2	protein tyrosine phosphatase, non-receptor type 2, like
5.64	2	v-fos FBJ murine osteosarcoma viral oncogene homolog
5.37	1	sp8 transcription factor
5.14	2	forkhead box I1
4.95	1	dachshund a
4.37	2	ribosome binding protein 1 homolog (dog)
4.22	2	T-box 24
4.08	2	suppressor of fused homolog
3.91	1	Homeobox protein Hox-B5b (Hox-B5-like) (Zf-54)
3.89	1	enolase 3, (beta, muscle)
3.87	2	Insulin gene enhancer protein ISL-2 (Islet-2)
3.64	1	orthodenticle homolog 5
3.52	2	TSC22 domain family 2
3.50	2	zgc:86701 (zgc:86701),
3.50	1	ras homolog gene family, member E
3.43	2	selenoprotein P, plasma, 1b
3.43	1	ATPase, Na+/K+ transporting, alpha 1a.2 polypeptide
3.41	2	musashi homolog 2
3.40	2	Ena-vasodilator stimulated phosphoprotein
3.39	2	linker histone H1M
3.36	2	fused toes homolog
3.36	2	H1 histone family, member X
3.29	3	epithelial V-like antigen 1
3.19	2	Homeobox protein Dlx5a (DLX-4)
3.16	2	Heat shock protein 9B
3.15	2	Epididymal secretory protein E1 precursor
3.15	2	phenylalanine hydroxylase
3.12	2	similar to CCCH zinc finger protein C3H-2
3.10	2	jun B proto-oncogene
3.10	2	unc-45 (C. elegans) related
3.09	1	jagged 1b
3.07	2	zgc:101900 (zgc:101900)
3.07	2	DIX domain containing 1
3.05	1	septin 9
3.04	1	Paired-like homeodomain transcription factor 2a
3.01	2	insulin-like growth factor binding protein 1
2.94	2	dachshund b
2.92	2	translocating chain-associating membrane protein
2.82	3	deltaB
2.79	1	endothelium-specific receptor tyrosine kinase 2
2.77	2	Eomesodermin homolog
2.73	2	Homeobox protein Dlx6a (DLX-6)
2.68	1	T-box 20
2.68	2	Ribonucleoside-diphosphate reductase M2 chain

Table 6.2.4: The fold change of the top 50 genes that increased in rab5a2 MO injected embryos at the shield stage compared to control embryos.

6.2.3 Validation of Results

RT-PCR was used to validate the results of the microarray. The remainder of the uncoupled RNA from the microarray was used in conjuction with fluorescent taqman oligonucleotides for some of the genes that were shown to change and others that were not. The oligos used were for the genes *chordin*, *patched 2.1*, *transferrin receptor(tr)*, *wnt8a*, *fgf8*, *ntl*, *gsc*, *bmp4*, *bmp2*, *lfty1*, *lfty2*, *sqt*, *copa*. The data for these was then plotted in a box and whisker plot showing the fold difference between *rab5a2* MO injected embryos and control embryos. Since not all the genes could be put on a single plate, three different plates and three different dilutions were made. Therefore the housekeeping gene *tr* is plotted three times (blue boxes on Figure 6.2.3 and Figure 6.2.4) on each graph, to evaluate the reproducibility of each dilution plate. The spread of the data seen in *tr* receptor expression (Figure 6.2.3 and Figure 6.2.4) for each plate dilution made it difficult to evaluate the differences between each plate. All the *tr* receptor expression data did fall within the same range but this range was extensive. The 30% epiboly data range for *tr* receptor was less extensive and suggests that at least for the 30% epiboly data each plate dilution was comparable to the other plate dilutions (Figure 6.2.3). *tr* and *copa* are described as housekeeping genes suggesting that their expression does not change (Batista et al., 2004). The microarray data showed that at 30% epiboly the expression of *transferrin a*, a *tr* ligand, was slightly increased (appendix) however there was no change at shield stage. RT-PCR data for *tr* expression both at 30% epiboly and (Figure 6.2.3) at shield stage (Figure 6.2.4) is inconclusive with no obvious fold change. *copa* showed little change in expression, however the median for the 30% data set fell below the one fold change boundary suggesting some weak support for a decrease in gene expression (Figure 6.2.3) although this is inconclusive. The microarray data showed a slight increase in *copa* gene expression in *rab5a2* MO injected embryos at the 30% epiboly stage; however there was no change at shield stage (appendix).

The RT-PCR data set for *bmp2b* spans an extensive range at the 30% epiboly stage, thus proving inconclusive (Figure 6.2.3). The microarray data shows a large increase in *bmp2b* expression in *rab5a2* MO injected with the gene appearing in the top 50 genes that increase at the 30% epiboly stage (Table 6.2.2). At shield stage the

microarray data continues to show an increase in *bmp2b* gene expression but to a lesser extent (appendix). The RT-PCR data for *bmp2b* at shield stage however appears not to change (Figure 6.2.4).

chd and *bmp4* both showed dramatic decreases in gene expression in *rab5a2* MO injected embryos in the RT-PCR data both at 30% epiboly and shield stage (Figure 6.2.3 and Figure 6.2.4). This correlates with the data seen in the microarray where both *chd* and *bmp4* appear in the top 50 genes that decrease at both the 30% epiboly stage and shield stage (Table 6.2.1 and Table 6.2.3). The decrease in *chd* expression also corresponds to the abolition of *chd* expression seen in *ish* analysis reported in chapter 4. *fgf8* also shows a decrease in gene expression of *rab5a2* MO injected embryos in the RT-PCR data at both the 30% epiboly stage and shield stage (Figure 6.2.3 and Figure 6.2.4) corresponding to the substantial decrease in *fgf8* gene expression seen in the microarray data at the 30% epiboly stage (Table 6.2.1). The microarray data did not show any difference for *fgf8* expression at shield, however, this could be a result of the high fold change threshold set.

The nodal target genes *gsc* and *ntl* showed a trend towards decreased expression in *rab5a2* MO injected embryos in the RT-PCR data set at both the 30% epiboly stage (Figure 6.2.3) and shield stage (Figure 6.2.4). *ntl* expression was also reduced in the microarray data at the 30% epiboly stage (appendix), while *gsc* and *ntl* expression have been shown by *ish* analysis to be reduced by *ish* analysis in *rab5a2* MO injected embryos (Chapter 5 Figure 5.2.2). The nodal target gene *flh* was also reduced in *rab5a2* MO injected embryos in chapter 5 (Figure 5.2.2) in addition to being reduced in the 30% epiboly stage microarray data (appendix). Interestingly the expression of nodal *sqt* in *rab5a2* MO injected embryos did not reach the three fold change threshold in the microarray data and the RT-PCR data is inconclusive however there is a weak trend towards decreased expression at the 30% epiboly stage (Figure 6.2.3).

Finally *wnt8* and *wnt11* both showed decreased expression in *rab5a2* MO injected embryos in the microarray data. *wnt11* showed a significant decrease at the 30% epiboly stage (Table 6.2.1) but did not reach the fold change threshold at shield stage, however expression of *wnt11* at shield stage in the *rab5a2* MO injected

embryos was shown by *ish* analysis to be decreased (Figure 6.2.2). *wnt8a* expression was decreased in both 30% and shield stage MO injected embryos in the microarray data (appendix) and the RT-PCR data (Figure 6.2.3 and Figure 6.2.4).

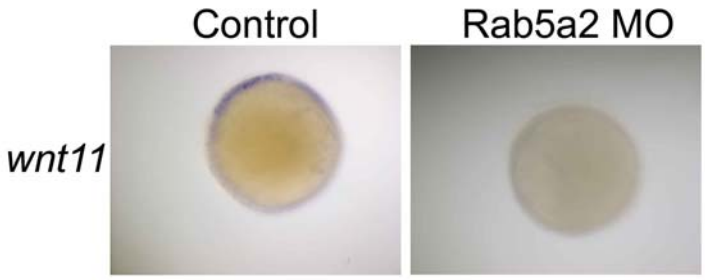

Figure 6.2.2: *ish* analysis of *wnt11* expression in *rab5a2* MO injected embryos compared to control embryos at shield stage.

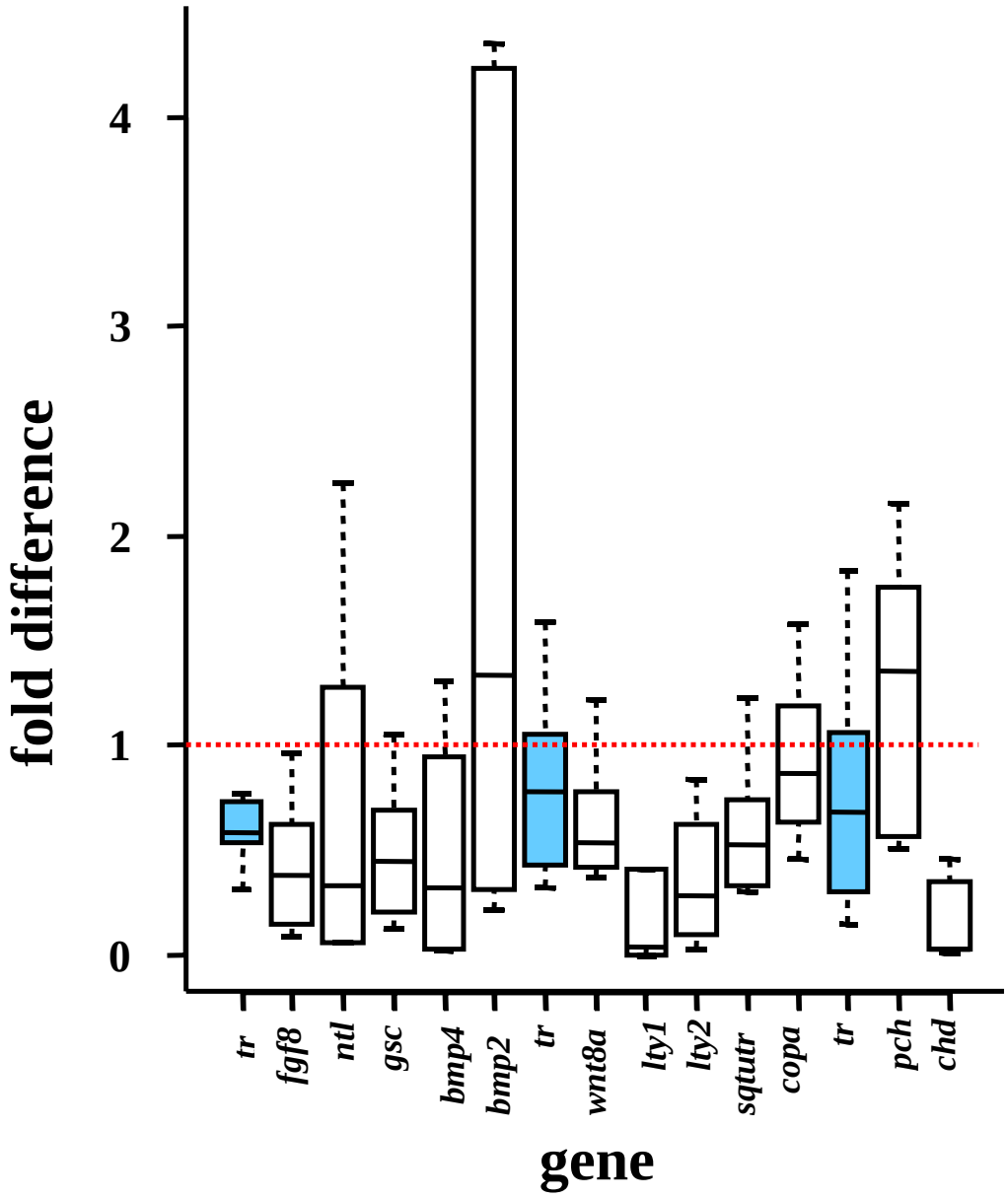

Figure 6.2.3.: Box and whisker plot showing the fold change of gene expression in *rab5a2* MO injected embryos when compared to control embryos at the 30% epiboly stage. Where the upper and lower bounds of the box represent the upper and lower quartiles respectively, the line that bisects the box represents the median and the whiskers extend 1.5 times the inter-quartile range beyond the 25% and 75% quartiles (boxes). The red dotted line represents the point at which there was no change in expression and the blue boxes show *tr*.

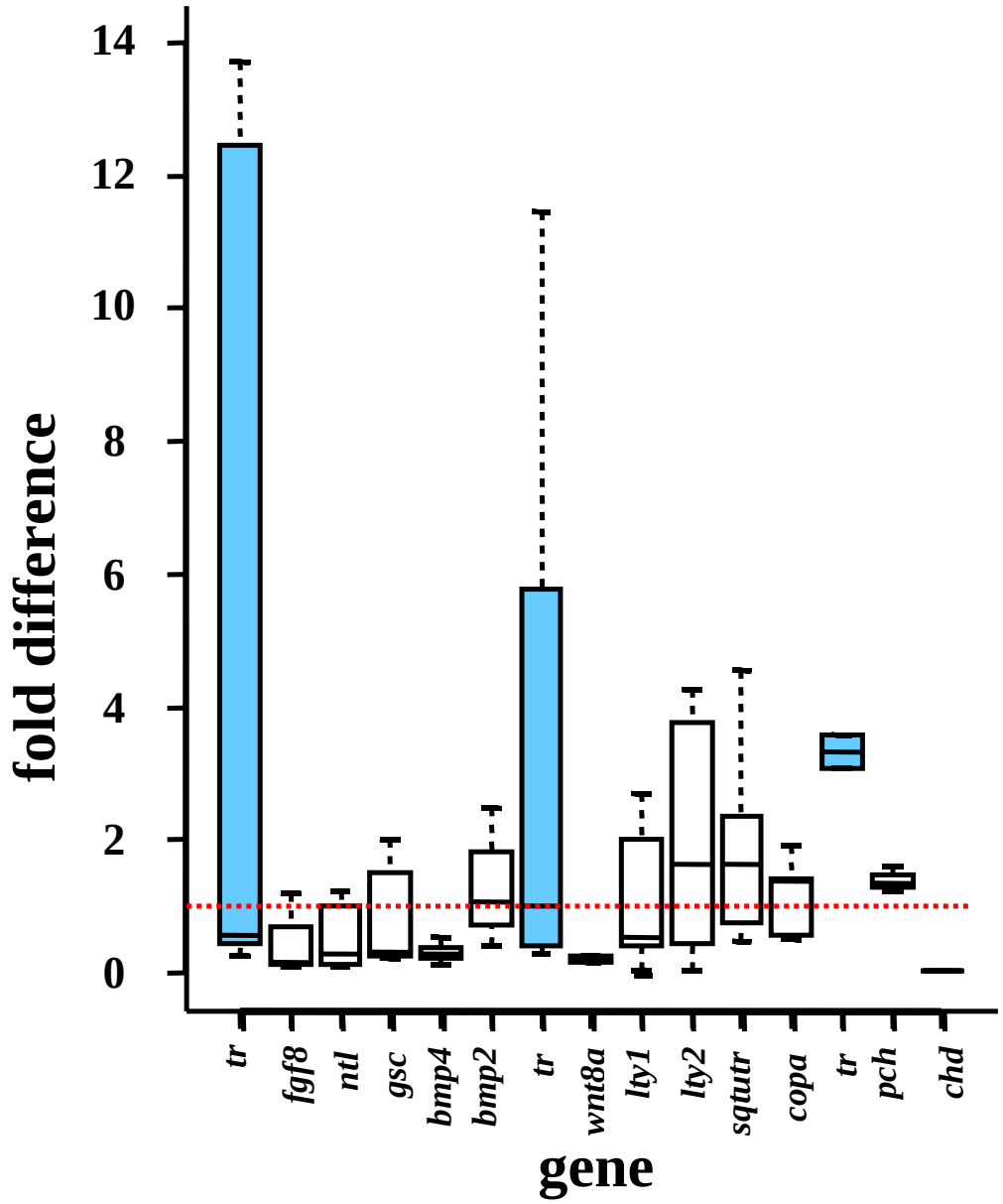

Figure 6.2.4: Box and whisker plot showing the fold change of gene expression in *rab5a2* MO injected embryos when compared to control embryos at shield stage. Where the upper and lower bounds of the box represent the upper and lower quartiles respectively, the line that bisects the box represents the median and the whiskers extend 1.5times the inter-quartile range beyond the 25% and 75% quartiles (boxes). The red dotted line represents the point at which there was no change in expression and the blue boxes show *tr*.

6.2.4 Identifiction of groups of genes with similar function whoose expression changed with knock down of rab5a2.

The top 50 genes that increased at the 30% epiboly stage were grouped according to their gene ontology (GO) terms (www.geneontology.org). This is a database of genes from different animals that have been given standardized terms to describe their function, location and biological process. This enables all known genes with know functions, locations and biological processes to have standardized terms enabling faster and more accurate comparisons between genes and pathways and animals. The top 50 genes that decreased and increased at the 30% epiboly stage and the top 50 that decreased and increased at the shield stage were also grouped according to their GO terms. These groups were then displayed in pie charts (Figures 6.2.5 – 6.2.8).

6.2.4.1 Groups of genes that decreased at 30% epiboly stage

The majority of the genes that decreased at the 30% epiboly stage had either no gene ontology or the gene function was unknown. The majority of genes with known function are involved in transcription whilst genes involved in DV patterning are also significantly represented (Figure 6.2.5).

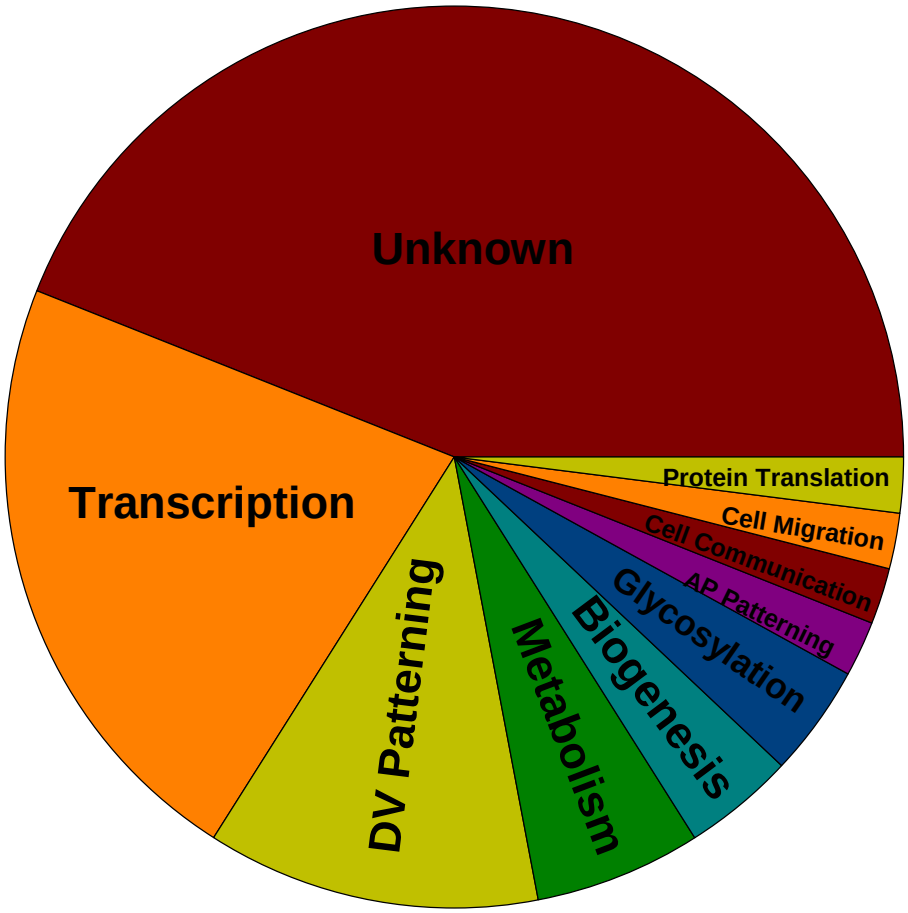

Figure 6.2.5: Pie chart showing the gene ontology biological processes of the top 50 genes that decreased in *rab5a2* MO injected embryos at the 30% epiboly stage.

6.2.4.2 Groups of genes that increased at 30% epiboly stage

The majority of genes that increased were either unknown or had unknown function in the gene ontology database. As with the genes that decreased the majority of genes with known gene ontology function are involved in transcription. Interestingly ranked joint forth are genes involved in DV patterning (Figure 6.2.6).

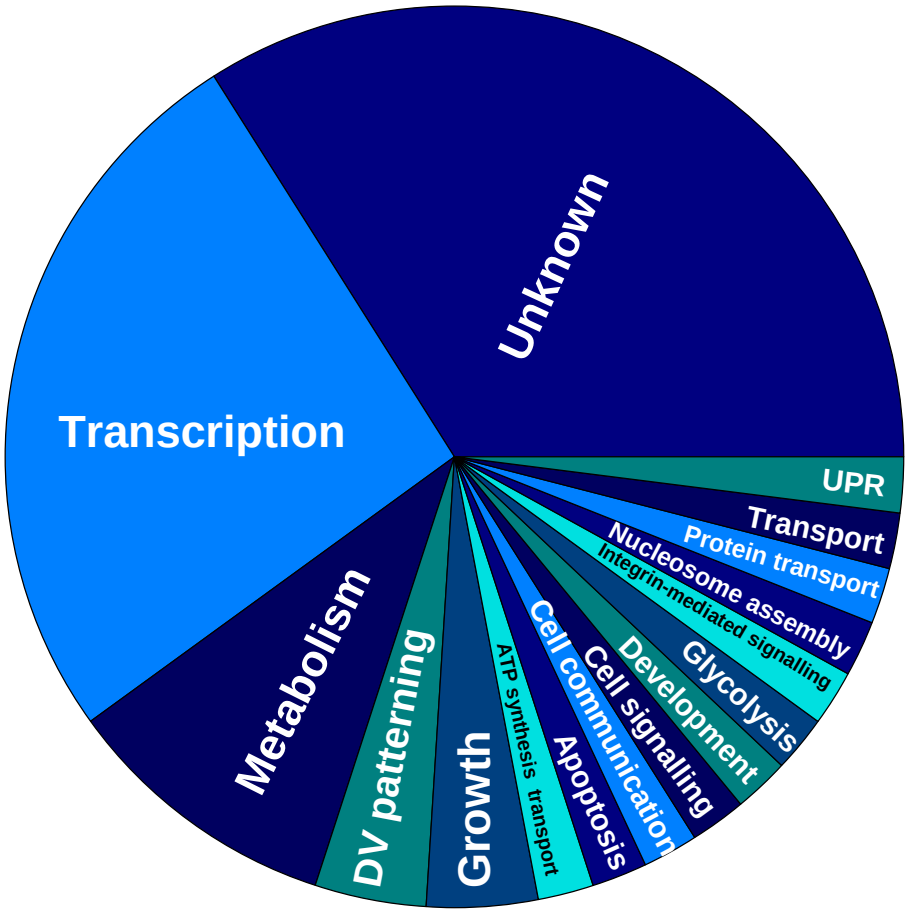

Figure 6.2.6: Pie chart showing the gene ontology biological processes of the top 50 genes that increased in *rab5a2* MO injected embryos at the 30% epiboly stage.

6.2.4.3 Groups of genes that decreased at shield stage

In the top 50 genes that decreased at shield stage the majority had unknown function in the gene ontology data base or were unknown genes. Interestingly the majority of known genes that decreased at shield stage were implicated in DV patterning while those genes involved in transcription were ranked third (Figure 6.2.7).

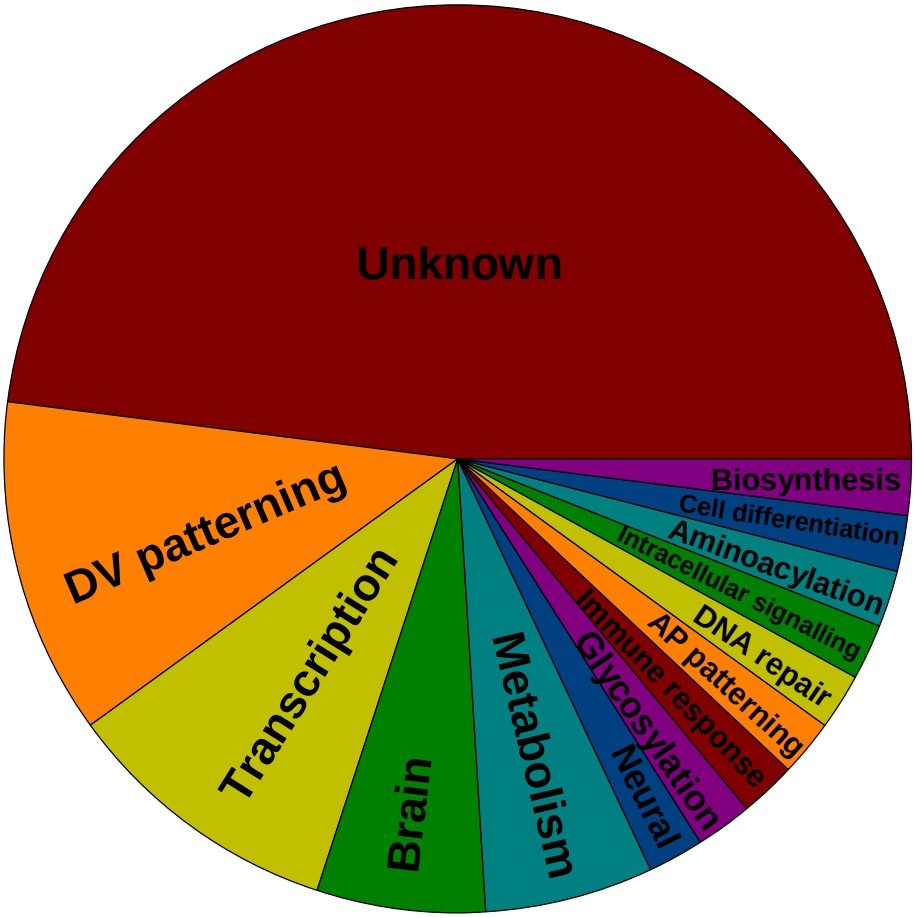

Figure 6.2.7: Pie chart showing the gene ontology biological processes of the top 50 genes that decreased in *rab5a2* MO injected embryos at shield stage.

6.2.4.4 Groups of genes that increased at shield stage

In the top 50 genes that increased at shield stage the majority again had unknown function in the gene ontology data base or were unknown genes. The majority of known genes that increased appear to be implicated in transcription, with those involved in protein processing ranking third. Interestingly the number of genes involved in DV patterning at shield stage, compared to 30% epiboly stage, appeared to have fallen resulting in the DV patterning genes being ranked at the bottom (Figure 6.2.7).

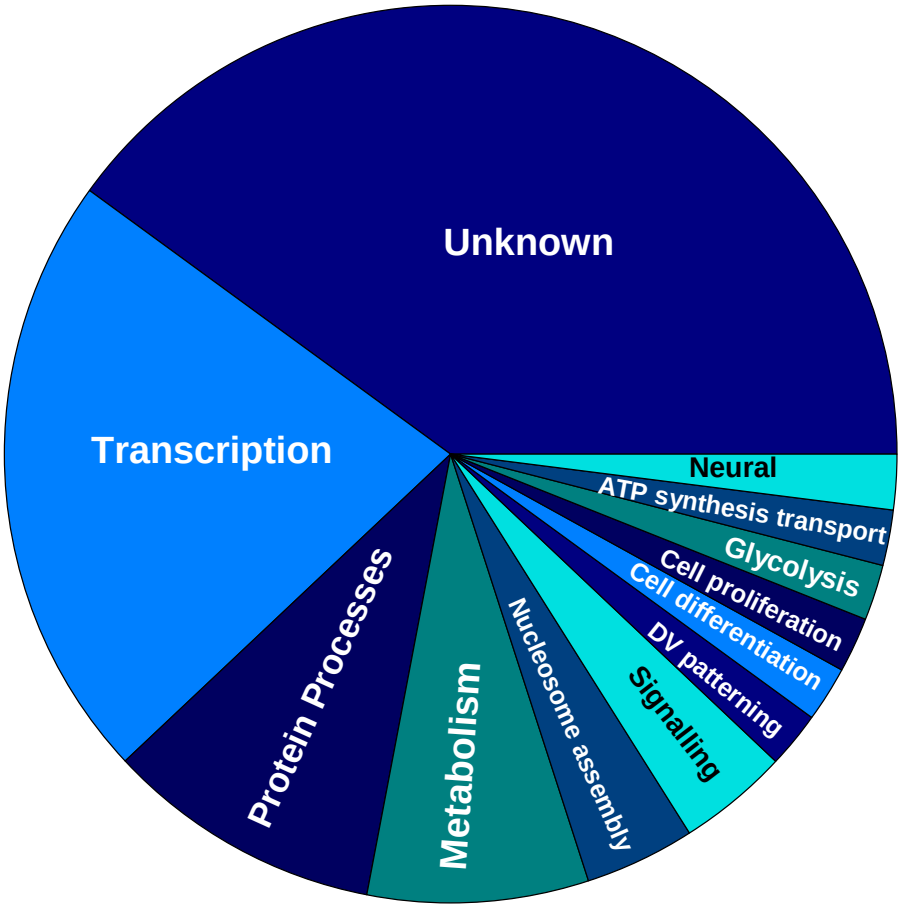

Figure 6.2.8: Pie chart showing the gene ontology biological processes of the top 50 genes that increased in *rab5a2* MO injected embryos at shield stage.