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Modelling oral-facial-digital syndrome type 1, a human ciliary disorder, in zebrafish

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Oral-facial-digital syndrome type 1 (OFD1) is an X-linked disease in which hemizygous males die prenatally; affected females display facial dysmorphisms, cleft lip and palate, digital and central nervous system malformations, and polycystic kidneys. At the cellular level, we previously proved that OFD1 protein is a core centrosomal component and localizes to basal bodies of primary cilia in renal epithelia. To study OFD1 function in development, we used morpholino technology in zebrafish. We obtained morphants characterized by hydrocephalus and pericardial edema (50%), bent body axes (30%), randomization of brain and abdominal viscera laterality (25%), and otolith anomalies. Less frequently pronephric cysts also occurred. A similar phenotype in zebrafish has been described following targeting of ciliary proteins (e.g. inversin or intraflagellar transport proteins), supporting a role of OFD1 in ciliary function. Kupffer's vesicle in fish is a transient structure, thought to have a similar role in determining laterality as the mammalian embryonic node. In OFD1 morphants, we find that Kupffer's vesicles do form but that their cilia appear slightly stunted. Our new data firstly demonstrate that OFD1 deficiency profoundly perturbs development and secondly suggest that OFD1 protein is required for morphological maturation and function of cilia.

The cytoskeletal gene *Ablim1* is expressed asymmetrically across the left-right axis and can be activated in a *Nodal*-independent manner in the left lateral plate mesoderm

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We have carried out a microarray-based screen comparing left and right embryonic tissues and identified actin-binding lim protein 1 (*Ablim1*) as being asymmetrically expressed. *Ablim1* has previously been shown to bind to the cytoskeleton and to affect cell morphology. It has been argued to be an adapter protein bringing lim-binding proteins to the cytoskeleton. We demonstrate that *Ablim1* is asymmetrically expressed in the left lateral plate at E8.5 and further shows a highly dynamic and asymmetric expression pattern at the node between E7.5 and E8.5. *Ablim1* is the first structural gene to be implicated in mammalian left–right (L–R) establishment. *Nodal* is upstream of all known left-sided genes in mammals and is expressed in the left lateral plate at E8.5. Analysis of *Ablim1* expression in known L–R mutants shows that *Ablim1* can be activated in the absence of *Nodal* in the left lateral plate. This demonstrates that a *Nodal*-independent left-sided signal must exist. Analysis of *Ablim1* node expression reveals that nodal flow and calcium signalling, normally argued to direct patterning information away from the node, also affect events internal to the node. We present a model that integrates current knowledge of the stages of nodal flow with our observations.

Genetic interactions between *Gas1* and *Shh* during early craniofacial development

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The *Growth arrest-specific gene 1* (*Gas1*) encodes a GPI-linked transmembrane glycoprotein, originally identified by its ability to arrest the cell cycle in mouse fibroblasts. *In vitro* studies on cultured somites have implied that Gas1 can physically bind to *Sonic hedgehog* (*Shh*) protein and inhibit Shh-induced growth. Shh plays a critical role during early craniofacial development, being required for normal division of the forebrain and the establishment of facial symmetry. Loss of Shh signalling in both mice and humans can lead to defects in midline patterning of the face and holoprosencephaly. Our analysis of *Gas1* mutant mice reveals the presence of multiple craniofacial defects characteristic of mild holoprosencephaly, including cleft palate, maxillary hypoplasia, fused maxillary incisors and anomalies of the pituitary gland. This suggests that Shh transduction might be impaired in the absence of Gas1 function, a finding confirmed by the presence of reduced *Ptc1* transcription in the fronto-nasal region and palatal processes of *Gas1* mutant embryos. Positive interaction between these two molecules was further reiterated by genetic analysis; the loss of a single *Shh* allele in a *Gas1* mutant background produced a more severe defect of the facial midline, including the presence of only a single external nostril.

Lack of the murine homeobox gene Hesx1 leads to a posterior transformation of the anterior forebrain

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The homeobox gene *Hesx1* is an essential repressor that is required within the anterior neural ectoderm for normal forebrain development in mouse and humans. Combining genetic cell labelling and marker analyses, we demonstrate that the absence of *Hesx1* leads to a posterior transformation of the anterior forebrain during mouse development. Our data suggest that the mechanism underlying this transformation is the ectopic-catenin signalling within the activation of Wnt/*Hesx1* expression domain in the anterior forebrain. When ectopically expressed in the developing mouse embryo, *Hesx1* alone cannot alter the normal fate of posterior neural tissue. However, conditional expression of *Hesx1* within the anterior forebrain can rescue the forebrain defects observed in the *Hesx1* mutants. This rescue experiment has revealed a differential sensitivity of the telencephalon and eyes to *Hesx1* dosage. The research presented here provides new insights into the function of *Hesx1* in forebrain formation.

This work is supported by the Wellcome Trust.

***Foxg1* and the informative relevance of the Foxg1-Dlx5-Pax6 network in cephalogenesis**

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Fundamental to this project is the further elucidation of the developmental importance of *Foxg1*, a member of the *Fox* winged-helix/fork-head transcription factor gene family, during cephalogenesis. *Foxg1* (formerly *BF1*) has been shown to be critical for prosencephalic development; here we show that the loss of function of *Foxg1* in mice is critical during cephalogenesis for both prosencephalic and skull development. Thus we support the notion of *Foxg1* being instrumental in the specific set of interactions that informs the coordination and integration of the brain and skull. The early expression pattern of *Foxg1* in the anterior neurectoderm and rostral surface ectoderm leads us to the presentation of a second aim of this research, i.e. an investigation of the genetic interactions of *Foxg1* with *Dlx5* and *Pax6*, transcription factors likewise expressed early in development in the overlapping regions of the neural and non-neural ectoderm and known for their relevance in brain and skull development.

This work is supported by the Royal Society and an EU Marie Curie Early Stage Research Training Fellowship.

Tooth phenotype on the Tbx1 mouse model for DiGeorge syndrome

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DiGeorge syndrome is associated with the deletion of a portion of the 22q11 chromosome in humans. This syndrome is characterized by abnormalities in tissues developing from the pharyngeal apparatus. Patients with this syndrome display, amongst others, craniofacial anomalies including ear malformation, short mandible and cleft palate. Some of these patients also display enamel hypoplasia along with enamel hypocalcification. The portion of the deleted 22q11 chromosome has numerous candidate genes, but recent studies suggest *Tbx1* as the most likely candidate. There is a mouse model with a null mutation on the *Tbx1* gene that mimics the DiGeorge syndrome phenotype. In this study we look at the relationship between *Tbx1* and tooth development at the stage of enamel formation. *Tbx1* expression is restricted to the epithelial component of tooth primordia and appears to mark the epithelial cells destined to give rise to the enamel-matrix-producing ameloblasts. Here we show the importance of *Tbx1* in the regulation of genes involved in tooth development and differentiation. *Tbx1* mutant teeth grown to maturity in kidney capsules display a tooth phenotype. Taken together these results suggest a relationship between *Tbx1* and tooth development and mineralization.

Elevated maternal expression of the imprinted *PHLDA2* gene is associated with low birth weight

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The identification of genes that regulate fetal growth will help establish the reasons for intrauterine growth restriction (IUGR). Most autosomal genes are expressed biallelically, but some are imprinted, expressed only from one parental allele. Imprinted genes are associated with fetal growth and development. The growth of the fetus *in utero* relies on effective nutrient transfer from the mother, to the fetus via the placenta. The expression levels of three imprinted genes – the paternally expressed insulin growth factor 2 (*IGF2*), the mesoderm-specific transcript isoform 1 (*MEST*) and the maternally expressed pleckstrin-homology-like domain, family A, member 2 (*PHLDA2*) – and the polymorphically imprinted insulin-like growth factor 2 (*IGF2R*) gene are all known to have roles in fetal growth and were studied in the placentae of 200 white European, normal-term babies. Quantitative expression analysis with real-time PCR showed the maternally expressing *PHLDA2* but not the paternally expressing *IGF2* and *MEST* nor polymorphic maternally expressing *IGF2R* placental levels to have a statistically significant effect on birth weight (BW). *PHLDA2* expression levels are negatively correlated with size at birth. These data implicate *PHLDA2* as an imprinted gene important in fetal growth and also as a potential marker of fetal growth.

Postnatal metabolic consequences of prenatal overexpression of *Dlk1/Pref1* in mouse

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Genomic imprinting is a form of epigenetic regulation that causes a subset of genes to be expressed predominantly from only one of the two parental chromosomes. Imprinted genes are involved in the control of prenatal and postnatal growth, brain function and energy homeostasis. *Dlk1/Pref-1* is a maternally repressed imprinted gene on mouse chromosome 12. *Dlk1* is highly expressed in the embryo and is downregulated in most adult tissues. *Dlk1*-deficient mice exhibit increased adiposity, showing the importance of *Dlk1* in inhibiting adipogenesis. We decided to alter dosage of this gene *in vivo* to understand more about its function. We generated transgenic *Dlk1* mice carrying a 70 kb BAC transgene. Hemizygous transgenic embryos (WT/TG) express twofold more *Dlk1* than normal littermates, equivalent to levels if *Dlk1* were

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not imprinted. Adult WT/TG animals exhibit reduced accumulation of adipose tissue and lean mass. Reduced adiposity might be caused by increased metabolic rate of these tissues and not by a developmental deficit. Loss of adiposity is reflected in significantly depressed levels of serum leptin associated with increased food intake. Mice overexpressing *Dlk1* thus have an impaired ability to store energy as fat. In the free-fed state, levels of glucose are lower suggesting enhanced insulin sensitivity. Interestingly, levels of *Dlk1* protein in adult tissues do not differ significantly between WT/TG and WT/WT adult animals, suggesting a major causal role for *Dlk1*/*Pref-1* overexpression prenatally in the adult metabolic phenotypes.

Characterization of the imprinted non-coding RNA *Kcnq1ot1* and its developmental role in targeting imprinted gene silencing

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The mouse IC2 cluster of imprinted genes contains the ncRNA *Kcnq1ot1* gene. It is expressed from the paternal allele, but repressed on the maternal allele by germline DNA methylation of its promoter. This cluster has an important role in fetal growth regulation with epimutation leading to Beckwith–Wiedemann syndrome in humans. All other imprinted genes in this cluster are paternally repressed, some ubiquitously and others in the placenta. Prematurely truncating the ncRNA results in loss of paternal silencing of the surrounding genes, suggesting that either the *Kcnq1ot1* RNA or the transcription of *Kcnq1ot1* acts in *cis* to repress these genes by targeting them with repressive histone modifications. Here we have characterized *Kcnq1ot1* to understand how it epigenetically silences gene expression in *cis*. We show that the *Kcnq1ot1* RNA is 121 kb in length, unspliced, and localized to the nucleus, suggesting a role in the regulation of gene expression. *Kcnq1ot1* is paternally expressed from the 2-cell embryo, prior to the establishment of repressive histone modifications of placentally imprinted genes, suggesting significant mechanistic parallels with X-inactivation. Surprisingly, *Kcnq1* (antisense to *Kcnq1ot1*) also shows imprinted expression in the 2-cell embryo and so may be initially repressed by a different mechanism. As the ncRNA is long and nuclear, we suggest that it may exert its regulatory role on genes in *cis* by physical 'coating' of the imprinting cluster.

Investigating the link between retrotransposed genes and genomic imprinting

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Retrogenes are functional gene duplicates formed by the reverse transcription of a cellular RNA in the germ line, followed by integration of the resulting cDNA into the genome. While a possible link between retrotransposition and genomic imprinting has been discussed previously, the nature of this link has not been defined in detail. In an attempt to address this, we performed a systematic screen for retrogenes among mouse genes known to undergo genomic imprinting. At three of the loci identified, CpG islands overlapping the retroposed exons undergo methylation during oogenesis but are unmethylated in sperm. Inter-species comparative sequence analyses indicate that the differentially methylated CpG islands originated either during or after the retroposon integration events, suggesting that the insertions were linked to the acquisition of imprinting at these three loci in ancestral mammals. Common features of these epigenetically distinct retrogenes were employed in the identification of a novel imprinted locus on mouse chromosome 2. Imprinting is conserved in the human genome for the three retrogenes that originated prior to the divergence of rodents and primates, suggesting that selective constraints have acted to maintain parental-origin-specific expression at these loci.

The UniProt Knowledgebase: a useful resource for developmental biology

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The UniProt Knowledgebase (UniProtKB) provides the scientific community with a single, centralized, authoritative resource for protein sequences and functional information. It consists of two sections: UniProtKB/Swiss-Prot is manually curated to ensure

high-quality information content while UniProtKB/TrEMBL is a preliminary section enriched with automated classification and annotation. The manual curation process involves extracting experimentally validated data from scientific literature and combining these with the results of a range of sequence analysis programs to provide a complete overview of both sequence and functional information. UniProtKB also acts as a focal point of database interconnectivity by providing links to more than 70 other biological resources. The UniProt Knowledgebase offers a number of features that are specifically useful in the context of developmental research and is a valuable resource for those working in the field. The database can be accessed at http://www.uniprot.org.

p21^{WAF1} expression in the developing embryo

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Normal patterning and morphological development requires the precise coordination of cell proliferation, differentiation and programmed cell death. To control these processes a large complex network of regulatory genes has evolved to specify when and where in the developing embryo cell proliferation, differentiation and cell death occur. The cyclin-dependent kinase inhibitor p21 has many roles within the cell and has been implicated in many processes including cell cycle arrest, cell death and cell differentiation. As p21 is involved in these processes which occur through development it would be expected that p21 would be expressed within the developing embryo. By combining the use of p21-LacZ transgene mice and OPT scanning we have demonstrated the three-dimensional expression profile of p21^{WAF1} during development. Crossing these mice to various knockout lines will further our knowledge of p21 regulation during development.

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Valproic acid-induced skeletal malformations: associated gene expression cascades

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Valproic acid (VPA) is an anticonvulsant drug widely used therapeutically for a variety of neurological conditions, including epilepsy. VPA is also well known for its teratogenic potential in both humans and experimental animal models. Nevertheless, the mechanisms underlying VPA's anticonvulsant efficacy or its teratogenicity remain to be elucidated. In order to study valproic-acid-induced teratogenic effects, two approaches were utilized. In the first project, gene-expression profiles were analysed, whereas the second study was focused on histone acetylation status. Analysis of cDNA microarray indicated that several ontological groups (histone deacetylase complex, GTPases, cell proliferation, apoptosis and cytoskeletal) have significantly enriched gene expression changes in response to the teratogenic insult. The histone deacetylase (HDAC) enzymes participate in the nucleosome structure control. Several studies showed that VPA is a strong inhibitor of HDAC activity in cell and animal models, producing histone hyperacetylation. Using antibody anti-hyperacetylated histone H4 we showed that VPA exposure *in utero* induced hyperacetylation of embryonic proteins, specifically those localized in the caudal neural tube and in the somites, the main target organs of VPA teratogenic effects. The results of these studies suggest that VPA induces congenital malformations through gene-expression alterations by chromatin structure misregulation.

Discovery of a novel GNB3 mutation that causes the retinopathy globe enlarged phenotype and possibly hypertension in chickens

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The autosomally recessively inherited condition retinopathy globe enlarged (rge), was recently mapped to chicken chromosome 1. Rge chickens first experience visual problems at 3 weeks of age and lose all vision by 8 weeks of age. The ubiquitously expressed GNB3 protein, which mapped to the same rge locus, was identified as an obvious candidate gene. Following sequence analysis of GNB3 exons, we identified an in-frame 3 bp deletion, which results in the deletion of a single aspartic acid residue at codon 153 (del153D) in rge-affected birds. Following blotting studies, using a GNB3-specific antibody, we demonstrated a significant decrease ($\sim$70%) in GNB3 protein and a resultant decrease in cAMP levels in retina, liver, heart and kidney, in affected rge/rge birds. There was a significant increase in cGMP levels in the retinas of rge-affected chickens, resulting in a down-regulation in the visual transduction pathway. The decrease in cAMP levels has previously been shown to be associated with tissue oedema. Our results may therefore help to explain the characteristic enlarged-globe phenotype in rge chickens. We are currently investigating whether our severe del153D mutation has a similar biological affect to the common and milder human 825C>T GNB3 mutation, which predisposes individuals to hypertension.

Characterization and regulation of the human *TBX22* promoter

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Although cleft palate is a common birth defect, little is known about its aetiology. The T-box transcription factor *TBX22* is one of the few genes established as a major cause with missense, nonsense, frameshift and splice site mutations identified in patients with X-linked cleft palate and ankyloglossia (CPX). Characterization of the transcript and putative promoter sequences identified a novel upstream uncoding exon and two alternative transcripts encoding an identical open reading frame. Promoter activity was observed only in the distal promoter. The minimal promoter sequence contains several putative T-box binding sites and we demonstrate that TBX22 itself has repressor activity on this promoter. Truncated constructs map the repressor domain to the N-terminal region while the C-terminal fragment contains an activation domain. Although the repressing activity of TBX22 is dominant in this system, post-translational modifications might be the switch between TBX22 acting as a transcriptional repressor or activator. These results suggest *TBX22* transcription might be autoregulated or regulated by other T-box proteins with overlapping expression domains. Full-length TBX22 constructs containing naturally occurring missense mutations lose the ability to repress promoter activity. The novel upstream exon and promoter could be a candidate region for novel mutations in CPX-like patients without coding region mutations.

Neuregulin3 signalling in epithelial and mammary gland development

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Neuregulin3 (Nrg3) mediates the specification of mammary placodes in mice. Nrg3 is a member of the neuregulin family of secreted and membrane-attached growth factors and is a ligand for the receptor tyrosine kinase, Erbb4. The aim of this research is to further characterize the role of Nrg3 signalling in mammary and epithelial morphogenesis. We have generated transgenic mice expressing Nrg3 under the control of the human keratin 14 promoter. The K14 promoter directs the expression of the Nrg3 transgene to the basal epithelia and hair follicles from E9.5 onwards. All 13 transgenic founders have displayed striking skin and epithelial appendage phenotypes, including the mammary glands. These results show that Nrg3 signalling can regulate cell fate during early mammary gland morphogenesis and suggest that Nrg3 can also regulate other pluripotent epidermal cell populations.

Work in our laboratory is supported by Breakthrough Breast Cancer.

The Teashirt 3 transcription factor marks a novel renal tract lineage and enhances development of the mammalian ureter and kidney papilla

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In *Drosophila*, teashirt (tsh) is expressed in mesodermally derived stellate cells as they undergo mesenchymal-to-epithelial transition, integrating into the renal tubule. We found that all three mammalian Tsh orthologues (Tshz1–3) are expressed throughout metanephrogenesis, with Tshz3 transcript levels falling markedly postnatally. We used heterozygous mice with LacZ knocked-into the *Tshz3* coding sequence to further study Tshz3 expression. At the inception of the metanephros we observed β-galactosidase expression exclusively in loosely associated cells around the ureteric bud stem: thereafter, differentiating ureteric smooth muscle and a subset of stromal cells in the metanephric papilla expressed the transgene; in adults, rare medullary interstitial stromal cells expressed Tshz3. Homozygous, null mutant late-gestation mice displayed gross hydronephrosis with attenuation of smooth muscle in the renal pelvis and proximal ureter. Additionally, the kidney papilla failed to grow, with downregulation of local BMP4 expression. We suggest that *Tshz3*-positive cells mark a 'third lineage' in metanephric development, in addition to ureteric bud and nephrogenic mesenchyme. Precursors in this third lineage promote lower renal tract smooth muscle differentiation and also populate niches in medullary stroma to nurture growth of papillary tubules.

Identification of a new mutant with spina bifida and other birth defects

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Birth defects remain the greatest cause of infant mortality in the Western world, yet their genetic aetiology remains largely undiscovered. During ongoing mouse ENU mutagenesis programmes, several new mutants have been created that exhibit a range of birth defects. The *hitchhiker* mutant is characterized by multiple developmental abnormalities, including exencephaly, spina bifida, oedema and polydactyly. Using a positional cloning strategy we have identified the causative mutation in *hitchhiker* as a splicing defect in the *Tulp3* gene.

This work is funded by the Medical Research Council with support from the Genetics Society.

Identification of a novel gene for primary ciliary dyskinesia

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Primary ciliary dyskinesia (PCD), a member of the ciliopathies (incidence 1 : 20 000), is a genetically heterogeneous autosomal recessive disorder associated with dysmotility of 'motile' cilia and sperm flagella due to ultrastructural abnormalities. It is progressive and affects the respiratory tract, middle ear and reproductive organs, characterized by recurrent respiratory tract infections, sinusitis, bronchiectasis and subfertility. Patients also exhibit left–right laterality defects arising from defects in embryonic nodal cilia. Following a genome-wide linkage scan we identified a new locus for PCD in two consanguineous Arabic families on chromosome 6p21.1 (maximum multipoint lod score of 6.7). Candidate gene sequencing revealed a homozygous single amino acid deletion in all seven affected individuals in *RSP9*, a gene encoding a ciliary structural protein homologous to the *Chlamydomonas reinhardtii* radial spoke head protein. The affected patients have a rare ultrastructural defect of intermittent absence of the ciliary central pair microtubules. This is an unusual form of PCD since none have laterality problems and their respiratory cilia retain a normal beat frequency with a circular beat pattern similar to that of embryonic nodal cilia, but which is ineffective for mucociliary function. Screening of a larger cohort of PCD families for *RSP9* mutations is in progress and *in situ* probes and morpholinos targeting the gene in mice and zebrafish have been developed in an effort to characterize its function in relevant tissues.

The *Drosophila* microtubule-associated protein Futsch is phosphorylated by Shaggy/Zeste-white 3 at a homologous GSK3 beta phosphorylation site in MAP1B

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The *Drosophila* homologue of the microtubule-associated protein MAP1B is encoded by the *futsch* locus. The deduced protein Futsch is about twice the size of MAP1B and shows high homology in the N- and C-terminal domains. Futsch co-localizes with microtubules and is necessary for the organization of the microtubule cytoskeleton during axonal growth and synaptogenesis. To further analyse the functional relevance of Futsch as a MAP1B-like protein, we performed a molecular analysis of the conserved protein domains. Using a number of antisera, we show that, unlike MAP1B, Futsch is expressed as a single protein. The function of MAP1B is in part regulated by phosphorylation mediated by kinases that include casein kinase 2 and glycogen synthase kinase 3β (GSK3β). We show here that at least one GSK3β phosphorylation site of MAP1B is conserved in Futsch and that this site can be phosphorylated by GSK3β and its *Drosophila* homologue, Shaggy (Sgg). To test the functional relevance of these findings we generated a number of minigenes and assayed their ability to rescue the phenotype of *futsch* mutants. Our data highlight some differences between MAP1B and Futsch but demonstrate that important structural and functional aspects are conserved between fly and vertebrate members of this protein family.

Time-lapse confocal imaging of wound healing in the murine corneal epithelium

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The corneal epithelium is maintained by a constant centripetal migration of cells. In X-inactivation mosaics, striping patterns develop in the corneal epithelium between 6 and 8 weeks of age, often forming whorling patterns towards the centre of the cornea. These stripes represent streams of clonally related cells migrating inwards from stem cells at the periphery of the cornea. When the corneal epithelium is injured the surrounding epithelial cells migrate on the corneal stroma to cover the defect. In whole-eye organ culture, a 1 mm central wound involving the full thickness of the epithelium is fully healed by around 18 hours after wounding. At this point the wound area is covered by an intact epithelial layer one or two cells thick. X-gal staining of healed, centrally wounded X-inactivation eyes reveals that striping patterns are reconstituted during wound healing. In GFP mosaics the healing process can be imaged using time-lapse confocal microscopy. It is demonstrated for the first time that clones remain contiguous throughout their migration. Healing of peripheral wounds was observed to form *de novo* whorling patterns, revealing that basal cells in the epithelium can migrate both away from and towards the limbal region.