*Genet. Res., Camb.* (2015), vol. 97, e1. © Cambridge University Press 2015
doi:10.1017/S0016672314000214


*Abstracts of papers presented at the 25th Genetics Society's Mammalian Genetics and Development Workshop held at the Institute of Child Health, University College London on 7th November 2014.*

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## The role of cell cycle regulators in neural tube closure

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During neurulation, the neural plate bends around the median hinge point (MHP), overlying the notochord. MHP cells spend more time in G1/S-phase of the cell cycle than lateral cells, and this is associated with adoption of a wedged cell shape due to interruption of interkinetic nuclear migration. Clustering of wedge shaped cells in the MHP drives midline bending. To determine the mechanism underlying the prolonged cell cycle in the MHP, we examined the expression of cell cycle inhibitors, and found the cyclin-dependent kinase inhibitors (CDKIs), p21 and p57, expressed in midline cells. p21 null mice, which exhibit median hinge point formation, show upregulation of p57 in midline cells, supporting our hypothesis of a redundant relationship between p21 and p57 during neural plate bending. Detailed cell cycle analysis in vivo and in vitro is being undertaken to examine if loss of cyclin-dependent kinase inhibitors, by genetic ablation and electroporation, leads to shortening of G1/S-phase in cells of the median hinge point, thereby disrupting cell wedging and neural plate bending.

## Basal progenitors in the zebrafish neural tube: investigating their origin and spatial organisation

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Basal neuronal progenitors (BPs) divide at a distance from the ventricle and are a major source of neurons during mammalian neurogenesis. BPs are thought to responsible for the expansion of the cerebral cortex during mammalian evolution, but are also present in non-cortical regions and non-mammalian species. BP have not been widely studied in zebrafish however *vsx1* has been shown to label BPs in the zebrafish neural tube (Kimura et al, 2008). Our aim is to characterize the generation and distribution of BP during zebrafish embryonic development.

Standardization methods and 3D realignment techniques have allowed us to map the spatial distribution of BPs in the spinal cord and hindbrain of embryos 24, 36, 48 and 72 hours post fertilisation. In the spinal cord, a small, constant number of basal divisions is observed, the majority of which express *vsx1*:GFP. In the hindbrain, non-apical divisions can be seen in basal and subapical locations and diversify as the embryos develop. This suggests that, similar to mammalian brains, diverse subpopulations of neural progenitors may be generated at different stages of zebrafish embryonic development. We will investigate this further using live imaging, which may provide us with new insights into the function and generation of BPs.

## Role of HIRA in cardiovascular development

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The development of the cardiovascular system is regulated by sequential, time controlled pathways. Transcription is partly regulated by variant histones which have attracted interest in the regulation of chromatin organisation. HIRA has been widely described as a replication independent chaperone of H3·3 (a variant of Histone H3). I investigated the role of HIRA in the development of the cardiovascular system in mice. I have used tissue specific conditional knockouts such as cardiogenic mesoderm

(*Mesp1*cre), cardiomyocytes (*Nkx2·5*cre) and second heart field (*Mef2c*cre).

I observed several phenotypes in the *Mesp1Cre* conditional *Hira* mutant embryos which had VSDs and oedema at E15·5. Using Optical Projection tomography (OPT) I reconstructed the Pulmonary Trunk of my *Nkx2·5*cre mutants and observed a Tetralogy of Fallot like phenotype at E15·5. Both were embryonic lethal. However *Mef2cCre* mutants displayed no apparent abnormalities thus demonstrating a specific role of *Hira* during heart development as it is required in the First Heart Field. In addition, I performed a RNA-seq at E11·5 and E12·5 using *Mesp1Cre* cardiac RNA and have demonstrated that HIRA interacts specifically with BRG1 (Brahma-related gene) and WHSC1 (a histone methyltransferase) in the heart at E12·5 and E14·5. Together these experiments will shed light on the genes mostly affected by the lack of HIRA and its epigenetic regulation during cardiovascular development.

**Lineage analysis to determine stem cell locations in the mouse corneal and limbal epithelia**

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We are using a lineage tracing approach to resolve an ongoing debate about the location of stem cells that maintain the corneal epithelium during normal homeostasis. One long-standing hypothesis is that the stem cells are located in the limbus, at the corneal periphery. These limbal epithelial stem cells (LESCs) are believed to produce transient amplifying cells, which move centripetally to the centre of the cornea in the basal layer and replenish the suprabasal layers. According to this model, LESCs maintain the cornea during normal homeostasis, becoming more active following wounding to repair the tissue. This LESC hypothesis has been challenged by an alternative corneal epithelial stem cell (CESC) hypothesis, which proposes that corneal epithelium is normally maintained entirely by stem cells located throughout the corneal epithelial basal layer and that LESCs only contribute to wound repair. Preliminary lineage tracing experiments, using $ROSA^{mT/mG}$ and ROSA26-LacZ reporter lines crossed to tamoxifen inducible CAGG-CreER, produced stripes of labelled cells in the corneal epithelium. After short chase periods, stripes were restricted to the periphery but, after 14-16 weeks, stripes were longer and extended to the centre. Although some stripes were discontinuous, most appeared to originate from the limbus, as predicted by the LESC hypothesis.

**Class 3 semaphorins and neuropilins selectively organise boundary caps at the embryonic CNS-PNS interface**

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Boundary caps (BC) are neural-crest-derived cell clusters on the surface of the brainstem and spinal cord that mark the dorsal root entry zone (DREZ) of sensory nerves and motor nerve exit points (MEPs) during embryogenesis. BCs are thought to guard the interface between the peripheral and central nervous systems (PNS/CNS), allowing axons to cross whilst constraining cell bodies of neurons and other cell types, and have been suggested as a possible regenerative therapy. Few genetic pathways have been identified that control BC organisation or function. We demonstrate that SEMA3A and its receptor NRP1 is selectively required for BC clustering and organisation at the DREZ, whilst SEMA3 F and NRP2 are specifically required for this process at MEPs. These defects are mirrored by specific defects in axon patterning. Loss of both SEMA3A/3 F or NRP1/2 leads to large ectopic boundary cap-like structures, which associate with ectopic axons. In addition, culturing neural crest cells with DRG induces expression of a boundary cap marker, suggesting that neuronal/axonal presence may induce the differentiation of presumptive BC. Our results suggest that SEMA3A and SEMA3 F cooperate through NRP1 and NRP2 to restrict BC cells to axon exit and entry points to ensure appropriate BC function.

**Defective glycine cleavage system causes neural tube defects and non-ketotic hyperglycinemia**

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https://doi.org/10.1017/S0016672314000214 Published online by Cambridge University Press

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The glycine cleavage system (GCS) in mitochondria provides one-carbon units to folate one-carbon metabolism (FOCM) through the decarboxylation of glycine. The FOCM, in turn, provides one-carbon units for important cellular functions such as nucleotide synthesis and methylation, and has been implicated in the aetiology of neural tube defects (NTDs). Mutations in the GCS are known to cause non-ketotic hyperglycinemia (NKH), which presents with elevated glycine in cerebrospinal fluid, plasma and urine, seizures and respiratory failure resulting in neonatal death, brain malformations, and mental retardation. We generated loss-of-function mice models of *Gldc* (a component of the GCS) and found partially penetrant NTDs in homozygous mutants, accompanied by retarded growth and development and altered folate profiles. Supplementation with formate (a source of one-carbon units) rescues the NTDs and normalizes growth and folate profiles. Downstream of FOCM, proliferation is found to be reduced in the cranial neural tube. Non-NTD mutants survive to birth but exhibit features of NKH such as elevated glycine in body fluids, early post-natal lethality, and hydrocephalus.

**Mutations in PLK4, encoding a master regulator of centriole biogenesis, cause microcephaly, growth failure and retinopathy**

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Centrioles are essential for ciliogenesis. However, mutations in centriole biogenesis genes have been reported in primary microcephaly and Seckel syndrome, disorders without the hallmark clinical features of ciliopathies. Here we identify mutations in the master regulator of centriole duplication, the PLK4 kinase, and its substrate TUBGCP6 in patients with microcephalic primordial dwarfism and additional congenital anomalies including retinopathy, extending the human phenotype spectrum associated with centriole dysfunction. Furthermore, we establish that different levels of impaired PLK4 activity result in growth and cilia phenoptyes, providing a mechanism by which microcephaly disorders can occur with or without ciliopathic features.

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**Cdk5rap2 function during zebrafish neurogenesis**

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Primary microcephaly is a human brain disease that develops during embryonic gestation and it is characterized by a small brain phenotype. Several centrosome-associated proteins that have been linked with this disease (*Cdk5rap2, Aspm, Wdr62*, etc.) may regulate many aspects of cell division. It has been suggested that microcephalies arise from disruption of progenitor divisions and premature cell cycle exit. The aim of the present work is to investigate the role of CDK5RAP2 during brain development and neuronal differentiation in zebrafish embryos.

To abrogate *Cdk5rap2* function antisense oligo morpholinos predicted to block protein translation and splicing between exon 4 and 5 were injected into 1-cell stage embryos. We analysed brain size, rate of neurogenesis and apical/basal progenitor divisions in the telencephalon of zebrafish embryos injected with control and CDK5RAP2 morpholinos. The results show that Cdk5rap2 plays an important role in brain growth in zebrafish. Reduction of Cdk5rap2 function results in a microcephalic brain phenotype with a decrease in neurogenesis and a reduction in the number of cells in division. These findings suggest that Cdk5rap2 may promote the normal development of zebrafish brain through the regulation of cell cycle progression.

**The role of Fgf10 in the regulation of epithelial progenitors during the initial stages of murine salivary gland development**

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There is an increasing need to identify factors that regulate progenitors in salivary glands due to their

https://doi.org/10.1017/S0016672314000214 Published online by Cambridge University Press

application in regeneration as a treatment of xerostomia. We suggest that Fgf10 maintains the identity and promotes the expansion of early epithelial progenitors during salivary gland development. We identified Sox9 as an early potential epithelial progenitor marker in submandibular glands (SMG) expressed in a subset of oral epithelium that will invaginate to form the gland. These $Sox9^{+}$ cells are reduced in a Fgf10 dose dependent way with $Fgf10^{+/-}$ mice exhibiting a hypoplastic bud by E11·5 and $Fgf10^{-/-}$ mice absence of bud formation. Although Sox9 expression is induced in the placode of $Fgf10^{-/-}$ SMGs it initiates later in development (E11·5 instead of E11·0) and is down-regulated by E12·5 followed by further reduction in progenitor cell proliferation, induction of apoptosis and early expression of ductal differentiation markers. A similar result is observed in *ex vivo* SMG slice culture experiments where inhibition of FgfR or Erk signalling at E11·0 leads to Sox9 down-regulation. Conversely, treatment of SMG slice cultures with Fgf10 beads results in up-regulation of Sox9 in the tongue epithelium. These results suggest a positive regulation between Fgf10 signalling, Sox9 expression and epithelial proliferation.

**Investigating the complex transcriptional relationship at the Mcts2/H13 imprinted locus**

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Genomic imprinting is an important process regulated by epigenetic mechanisms which ensures a mostly monoallelic expression of certain genes. The Mcts2/H13 locus represents a classic example. Mcts2 is a retrogene, which has been retrotransposed into the fourth intron of the host gene H13. Mcts2 has a maternally methylated CpG island associated with its promoter and is paternally expressed. H13 generates at least five transcripts, all of which initiate from a single promoter, but which differ in their use of polyadenylation sites. Transcription from paternal alleles, which lack methylation at the Mcts2 promoter, generates truncated *H13* transcripts that undergo internal polyadenylation. On maternal methylated alleles, the Mcts2 promoter is inactive and H13 transcript elongation proceeds to downstream polyadenylation sites. This mechanism is intimately coupled to the processes of alternative splicing. The third exon of H13 includes a weak splice donor site and is characterised, on the maternal allele, by high levels of H3K36me3. We show in this study that Setd2 is responsible for H3K36me3 in mouse fibroblasts and that an overall decrease of such post-translational modification affects the synthesis of H13 transcripts.

**Genome-wide mapping of meiotic recombination and chromosome segregation outcomes in human oocytes & embryos.**

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Risk of chromosomally imbalanced pregnancies increase exponentially in women over 35 years old. Crossovers between homologous chromosomes (homologs) have an important role in ensuring their correct segregation at the first meiotic division. Lowered or altered patterns of crossovers have been proposed to contribute to age-related aneuploidy in human oocytes. Here, we utilise all three products of human female meioses (embryo/oocytes and their corresponding polar bodies) to generate genome-wide maps of crossovers and chromosome segregation patterns (Meiomaps). Only through analysis of all three meiotic products can the origin of chromosome segregation errors be traced. This analysis reveals a novel reverse segregation pattern in which both homologs separate their sister chromatids at meiosis I, followed by non-random segregation of the two non-sisters at meiosis II. This reverse segregation pattern contributes significantly towards aneuploidy in oocytes from women of advanced maternal age. We also

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https://doi.org/10.1017/S0016672314000214 Published online by Cambridge University Press

detect selection for higher recombination rates in the female germline and report chromosomal drive for recombinant chromatids at meiosis II. These 'Meiomaps' thus provide new insights into the origin of human aneuploidy and selection pressures on recombination rates in the human female germline.

### Epigenetic Regulation at MLL1 target genes

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The mixed-lineage leukaemia 1 (MLL1) protein is a histone methyl-transferase that deposits the gene activating H3-K4 trimethyl mark, and is often mutated in leukaemia. MLL1 acts as a large complex, and is associated with a number of cofactors, but the mechanisms regulating the histone methyl-transferase activity remain unclear. Here we examine the role of Msk1, a downstream kinase of the MAP-kinase pathway, in regulating MLL1 activity. Msk1 is known to act as a histone H3S10 kinase, a modification found to stimulate MLL1's methylation activity in vitro.

Here we use a number of approaches to characterise the biochemical and functional interaction(s) between MLL1 and Msk1. Initial studies using a differentiation model based on a haematopoetic stem cell line, indicated that these regulators act a coordinated manner at MLL target genes. We demonstrate that MLL1 and Msk1 can be co-immunoprecipitated and ChIP-seq shows that their patterns of genomic binding correlate strongly, suggesting a direct functional interaction. In transient MLL1 and Msk1 knock-down cells, known MLL1 target genes were down-regulated, and at a global level ~3900 genes changed in the same manner. This down-regulation is associated with changes in histone modification at target genes, suggesting that histone cross-talk between MLL1 and Msk1 acts as a means of gene regulation. These findings suggest that MLL1 can be regulated by the extracellular environment via the MAP kinase pathway and Msk1.

### Factors Affecting the Severity of Sickle Cell Anaemia

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Extreme variation is observed between Sickle Cell Anaemia patients. In mild cases patients live largely unaffected lives, whereas in severe cases patients can experience multiple strokes and organ failure during childhood. Genetic factors are associated with this variation, including polymorphisms that increase foetal haemoglobin levels, however, these do not account for all of the variation observed. Using exome sequencing of severe and mild patients, we aim to identify novel modifier genes and variants. Top candidates will be validated in over 400 SCA patients, and *in vitro* functionality of variants will be investigated using CRISPR genomic editing. Phenotypic discordancy has also been observed between monozygotic twins, and we hypothesise that epigenetic variation affects SCA phenotype. Nucleated erythroid progenitors have been expanded from peripheral blood, facilitating the investigation of gene expression and epigenetic marks in the SCA affected cell type. This technique will be used to interrogate differences in gene expression between monozygotic twins discordant for SCA phenotypic severity. Identifying novel factors, both genetic and epigenetic, that influence the severity of the SCA phenotype is critical in advancing our understanding of disease aetiology, and may highlight potential therapeutic targets, or form the basis of a clinical test to predict symptomatic severity.

### Diphyodonty versus monophyodonty: the peculiar case of *Monodelphis domestica*

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Most mammals have two sets of teeth (diphodonty), a deciduous dentition replaced by a permanent dentition. Mice in contrast only have one set of teeth (monophodonty). We aimed to understand the differences between dental organs with different replacement capacities in the same animal: the short-tailed opossum (*Monodelphis domestica*). The opossum, like the mouse, is monophyodont, with the exception of the third premolar, which is replaced once.

Odontogenesis is initiated in the oral epithelium by the formation of the dental lamina, a ribbon-like epithelial structure from which first-generation teeth develop. Replacement teeth develop in association with the successional lamina, an extension of the dental lamina. Three-dimensional reconstructions of histological sections revealed the successional lamina initiated at

https://doi.org/10.1017/S0016672314000214 Published online by Cambridge University Press

multiple tooth positions, however the laminas on non-replacing teeth started to regress, associated with reduced proliferation, increased apoptosis, and breakdown of the basement membrane. In other animals, the dental lamina has been shown to express the putative stem cell marker Sox2. Comparative molecular analyses of the third premolars and the canines showed reduced Sox2 protein in the successional lamina of the canine compared to the robust expression in the third premolar. Such differences may underlie the regenerative potential and survival of the successional dental lamina.

**RhoA-dependent disassembly of actomyosin is necessary for mouse spinal neural tube closure**

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The cytoskeleton is widely considered essential for neurulation and yet mouse spinal neural tube closure can occur despite disruption of actin microfilaments by Cytochalasin D. To investigate this apparent contradiction, we treated mouse embryos in culture with a panel of cytoskeletal inhibitors. Preventing actomyosin cross-linking, F-actin assembly or myosin II contractile activity had no effect on spinal neural tube closure. In contrast, inhibiting Rho kinase signalling or blocking F-actin disassembly resulted in spinal neural tube defects, with apical F-actin accumulation and abnormal adherens junctions in the neuroepithelium. *Cofilin 1*-null embryos closely yielded a similar phenotype, supporting a key role for actin turnover. Blebbistatin could rescue the neural tube closure defects caused by ROCK inhibition. This study identifies a requirement for precise regulation of RhoA/Rho kinase/LIM kinase/cofilin signalling in mouse spinal neurulation and shows that, while actomyosin assembly and myosin ATPase activity are not limiting factors for mouse spinal neural tube closure, there is an essential role for actin turnover and actomyosin disassembly within the neuroepithelium.

**Molecular and functional characterisation of ciliopathy genes using proteomics and CRISPR mouse models**

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Cilia perform diverse functions during mammalian development and adult homeostasis. Whilst previous research has provided a list of components required to build and maintain cilia, how these genes and their encoded proteins function as part of interaction networks to confer specialised functions to cilia subtypes remains poorly understood.

We aim to investigate the roles of two ciliary proteins, HEATR2 and ZMYND10, previously described as axonemal dynein assembly factors required for cilium motility. Loss of either of these factors in humans causes a motile ciliopathy called Primary Ciliary Dyskinesia. We have generated CRISPR mouse models to query their roles during development. To gain a mechanistic insight, we are performing proteomic studies.

Our IP/MS studies reveal distinct interactomes for both proteins suggesting HEATR2 and ZMYND10 function at distinct stages during axonemal dynein complex assembly/trafficking. Preliminary findings will be presented. Furthermore, preliminary phenotyping data from the CRISPR mutant mice as PCD disease models will also be presented.

Despite the phenotypic similarities between *HEATR2* and *ZMYND10* PCD patients, our IP/MS studies reveal the molecular complexity of axonemal dynein assembly/trafficking. The distinct CRISPR null allele phenotypes of these mutant mice further support different functional requirements of these ciliary genes during development.

**Truncation of RASSF7 induces centrosome amplification; a role in cancer?**

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RASSF7 is a Ras-association domain containing protein which localises to the centrosome, is required for completing mitosis and whose expression is upregulated in a range of cancers. Investigating the function of RASSF7 will help us to gain a deeper understanding of mitosis and whether increased levels of RASSF7 protein can promote cancer formation. To achieve this aim, I am focusing on understanding the role of the four domains of RASSF7 protein in mediating its subcellular localization. GFP fusion proteins were expressed in early *Xenopus laevis* embryos

and showed that the coiled-coil domain is responsible for the centrosomal localization of RASSF7. Interestingly, removing the C-terminal domain caused accumulation of GFP-RASSF7 at the centrosomes. In addition, this lead to enlargement of the centrosomes, an increase in centrosome number and increased cell death later in development. This may suggest that increased centrosomal levels of RASSF7 could promote tumourigenesis, as centrosome amplifications/ defects are common in tumours.

### The implications of reduced *Mthfd1l* expression in the folic acid-resistant *curly tail* mouse

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Spina bifida is a severe developmental defect arising from a failure of spinal neural tube closure. They comprise around half of neural tube defects (NTDs), which affect approximately 1 per 1,000 pregnancies worldwide. A major advance in the prevention of spina bifida was the finding that maternal perinatal folic acid supplementation can reduce the risk of having an affected pregnancy by around 70%. However, about 30% of affected pregnancies are folic acid 'resistant' and there is currently no preventative treatment available for these pregnancies.

The *curly tail* mouse (*ct*) is a model for folic acid-resistant NTDs, in which 15–20% of homozygous mutant mice develop spina bifida. The major causative in *ct* mice corresponds to a hypomorphic allele of *Grhl3*. However, the frequency of NTDs is strongly influenced by genetic background, implying the presence of modifier genes. We identified reduced expression of the gene *Mthdf1l* in *ct* embryos compared to partially congenic wild-types. *Mthfd1l* which encodes an enzyme of mitochondrial folate 1-carbon metabolism – the metabolic cycle to which folic acid contributes. Mthfd1L produces formate and we have found reduced formate levels in the plasma of *ct* mice. Supplementation of pregnant *ct* mice with sodium formate decreased the incidence of spina bifida by 70% and also increased the litter size. Analysis of folate containing metabolites in neurulation stage embryos has revealed a change in prolife between treated and untreated embryos. Comparison of untreated *ct* embryos with stage matched wild-type embryo sharing a similar background also revealed differences which formate treatment partially recovers. These observations implicate reduced supply of one-carbon units in causation of NTDs.

### High Throughput Imaging and Phenotyping of Homozygous Lethal Mouse Lines at MRC Harwell

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The International Mouse Phenotyping Consortium will generate 5000 conditional KO mouse lines by 2015 and make them available to the research community together with a broad phenotypic characterisation and the expression profile of the targeted locus. MRC Harwell is responsible for producing, distributing and analysing 500 of these lines. We will present a pilot for a pipeline of work focusing on the characterisation of the embryonic lethal lines, including the identification of the window of lethality, gene expression at E12·5 and acquisition of morphological data by OPT and microCT. MRC Harwell has recapitulated known phenotypes validating the imaging techniques used. We are now building an exciting repository of novel mutant lines covering a broad range of developmental systems.