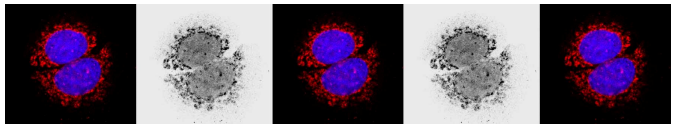
13th Annual Australian Cell Cycle Workshop Katoomba NSW

8-9 November, 2010



Co-hosted by the
Garvan Institute and CMRI

We would like to thank our sponsors for supporting the 13th Annual Cell Cycle Workshop.

SILVER SPONSORS

http://www.olympus.com.au/

http://www.biosci.com.au/

http://www.gelifesciences.com

http://www.edwardsco.com.au/

http://www.pacificlab.com.au/

http://www.thermofisher.com.au/

http://www.qiagen.com

http://www.garvan.org.au

http://www.anzscdb.org/

BRONZE SPONSORS

http://www.bd.com/anz/

http://www.genesearch.com.au/

www.johnmorris.com.au/

CONFERENCE INFORMATION

Thankyou for attending the 13th Annual Australian Cell Cycle Workshop. The Australian Cell Cycle Workshop is an annual meeting that brings together the Australian cell cycle community to discuss their latest research in an informal and relaxed setting.

LOCATIONS FOR CONFERENCE EVENTS

All conference events will be held in the Carrington Hotel.

Pre-conference drinks: Lounge Bar

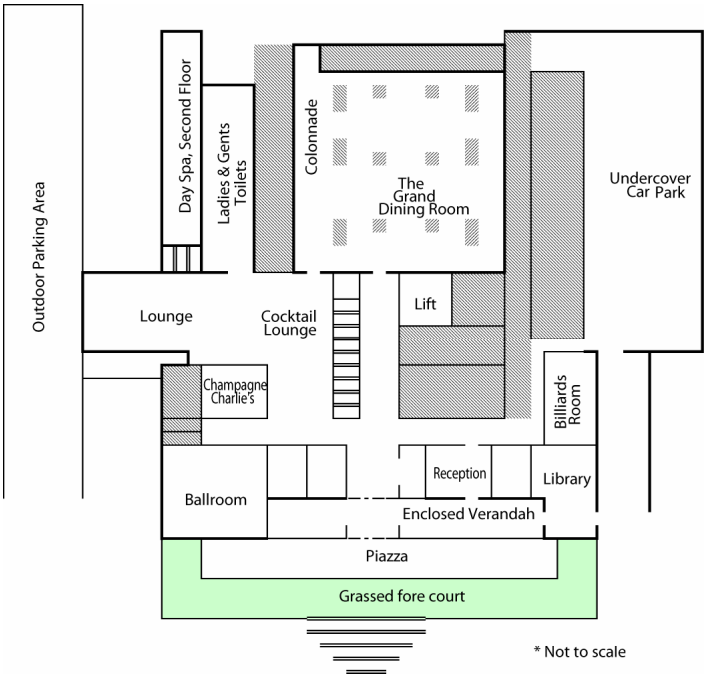
Registration : Ballroom

Talk Sessions: Ballroom

Morning Tea, Lunch and Afternoon Tea: Enclosed Verandah

Trade Display: Library (adjacent to the verandah)

Conference Dinner: The Grand Dining Room



The 13th Annual Cell Cycle Workshop is co-hosted by the Garvan Institute and CMRI.

Garvan Institute: Liz Musgrove, Liz Caldon, Marcelo Sergio, Jane Barraclough, Christine Lee, Jo Scorer, Andrew Stone

CMRI: Megan Chircop, Rose Boutros

PROGRAM

SUNDAY, 7th November

5.00-7.00pm	Pre-conference drinks will be held at the Carrington Hotel in the Lounge bar. Registration will be available at this time.

MONDAY, 8th November

Registration

8.00-8.50am	There will be a registration desk at the rear of the Ballroom.

8.50am – 10.30am
Session 1 – G_1 and Growth
Session Chair: Liz Musgrove

8.50-9.00am	*Liz Musgrove* *Introduction and welcome*
9.00-9.30am	Patrick Humbert The Scribble/Dlg/Lgl polarity module in the control of mammalian development and cancer
9.30-9.50am	Jason Coombes The role of a specific G_1-regulatory actin filament population defined by the tropomyosin isoform Tm5NM1 in oncogenesis
9.50-10.10am	Jaclyn Quin A novel inhibitor of ribosomal RNA gene transcription identifies coupling of PolI transcription to multiple cell cycle checkpoints and senescence
10.10-10.30am	Leonie Quinn Growth pathways controlling transcription of the *myc* oncogene

10.30am – 11.00am
Morning Tea + Trade Display
Verandah and Library

11.00am – 1.10pm
Session 2 – Centrosomes
Session Chair: Megan Chircop

11.00-11.20pm	Charlotte Smith Endocytic proteins are required for efficient progression through mitosis
11.20-11.50pm	Jessie Jeffrey Centrobin regulates interphase microtubule dynamics and the assembly of functional mitotic spindles
11.50-12.10pm	Rose Boutros Identifying centrin 2 as a novel cdk2 substrate
12.10-1.10pm	Steve Doxsey Centrosomes and midbodies: emerging roles in asymmetric division, stem cells, 'cancer stem' cells and ciliopathies

1.10pm – 2.00pm
Lunch + Trade Display
Verandah and Library

2.00pm-3.40pm
Session 3 – Transcription and Cancer
Session Chair: Maija Kohonen-Corish

2.00-2.30pm	Nicole Cloonan MicroRNAs and the mammalian cell cycle
2.30-2.50pm	Elaine Sanij UBF controls nucleolar integrity-checkpoint mechanism for cell cycle progression
2.50-3.20pm	Megan Chircop Dynamin inhibitors are a new class of anti-mitotic agents with anti-cancer properties
3.20-3.40pm	Liz Musgrove The role of G_1/S checkpoint regulators in endocrine resistance in ER-positive breast cancer

Afternoon Tea
3.40pm – 4.00pm
Verandah and Library

4.00pm – 5.30pm
Session 4 – Mitosis
Session Chair: Jörg Heierhorst

4.00 – 4.30pm	Andrew Burgess The Greatwall of mitosis
4.30 – 4.50pm	Audrey Lian Mitotic localisation and function of IQGAP1 during glioblastoma cell division
4.50 – 5.10pm	Brian Gabrielli Cyclin A/cdk2 dependent Plk1 association with adenomatous poylposis coli regulates mitotic spindle anchoring
5.10 – 5.30pm	Maggie Ma Sorting nexin 9 is required for mitotic progression

7.00pm
Conference Dinner
Grand Dining Room

TUESDAY, 9th November

9.00am – 10.30am
Session 5 – DNA replication and protein turnover
Session Chair: Patrick Humbert

9.00-9.20am	Nicholas Hoch A non-catalytic function for a checkpoint kinase during normal DNA replication
9.20-9.40am	Lakshmi Gopinathan The functions of cyclin A2 in DNA replication and mitosis
9.40-10.00am	Liz Caldon Cyclin E2 increases genomic instability due to failed degradation in the S phase of cancer cells
10.00-10.30am	Boris Sarcevic Mechanisms of mono- and poly-ubiquitination: ubiquitination specificity depends on compatibility between the E2 catalytic core and amino acid residues proximal to the lysine

Morning Tea
10.30am-11.00am
Verandah and Library

11.00am – 12.40pm
Session 6 – DNA Damage
Session Chair: Brian Gabrielli

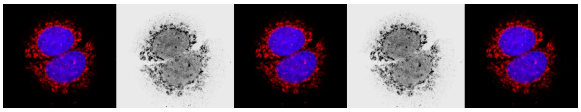
11.00-11.30am	Derek Richard hSSB1, the sensor of double strand DNA breaks?
11.30-11.50pm	Laurent Pangon MCC is a novel target of the DNA damage response
11.50-12.20pm	Christopher Jolly Cell cycle regulation of uracil base excision repair (BER) in hypermutating B cells
12.20-12.40pm	Daniel Speidel High resolution cell cycle and ploidy analysis in tissues: a valuable tool for the *in vivo* analysis of experimental animal models and patient biopsies

Lunch
12.40pm – 1.30pm
Verandah and Library

1.30pm – 3.00pm
Session 7 – Animal Models
Session Chair: Ross Hannan

1.30-1.50pm	Christina Heinlein Analysis of stress induced cell cycle alterations in tissues-*in vivo*
1.50-2.20pm	Galina Schevzov Regulation of the G_1 phase of the cell cycle by a specific population of actin filaments defined by tropomyosin 5NM1
2.20-2.50pm	Jörg Heierhorst A breathtaking phenotype: double life of a DNA repair protein as an essential regulator of lung organogenesis
2.50- 3.00pm	Megan Chircop *Closing Remarks*

ABSTRACTS



INVITED SPEAKER: STEPHEN DOXSEY

CENTROSOMES AND MIDBODIES: EMERGING ROLES IN ASYMMETRIC DIVISION, STEM CELLS, 'CANCER STEM' CELLS AND CILIOPATHIES

Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA.

Midbodies and centrosomes are two of a rare category of organelles that are present in the cytoplasm and are not membrane bounded. Both play critical roles in cell division. The midbody (MB) is a singular organelle formed between daughter cells during cell division and is required for their final separation in late cytokinesis. Recent studies show that MBs persist in cells long after division, but the fate of these so called midbody derivatives (MBds) is unclear. Our work shows that mitotic midbodies segregate asymmetrically to the daughter cell with the older centrosome. They selectively accumulate in stem cells in mouse and human tissues, in cultured stem cells and induced pluripotent cells. Differentiation of stem cells is accompanied by MBd loss, and involves NBR1-mediated autophagic degradation. NBR1 binds the MB protein CEP55 providing a molecular link between MBds and the autophagy pathway. Like stem cells, subpopulations of cancer cells accumulate MBds. These MBd-accumulating cells evade autophagosome encapsulation of MBds and thus exhibit low autophagic activity. In contrast, normal dividing or differentiated cells do not accumulate MBds and mediate MBd degradation through NBR1. Functional analysis show that MBds contribute to stem cell properties and the tumor-like features of cancer 'stem cells'. These results identify MBd-accumulation as a novel feature of stem cells and cancer 'stem' cells generated by unique mechanisms including asymmetric inheritance and evasion of receptor-mediated autophagy.

Centrosomes contribute to spindle organization and orientation in mitosis and mediate primary cilia assembly in noncycling cells. Proteins essential for cilia assembly also localize to centrosomes in mitosis, but their potential mitotic functions are unknown. We now show that depletion of the intraflagellar transport protein IFT88 induces mitotic defects in cultured cells and zebrafish embryos. IFT88 interacts with two microtubule (MT) nucleating components in mitotic cells, γ tubulin and EB1. Following IFT88 depletion, these proteins decrease at centrosomes/spindle poles concomitant with a reduction in astral MT nucleation, spindle misorientation and disruption of the cell division plane. IFT88 forms a complex with cytoplasmic dynein1. Both proteins are found in cytoplasmic particles together with γ tubulin, EB1 and MT bundles. GFP-IFT88 localizes to these particles/MT bundles, which move vectorially toward spindle poles in a microtubule- and dynein-dependent manner. Inhibition of IFT88 blocks this dynein-dependent movement. We conclude that IFT88 is part of a molecular motor-based transport system involved in transporting MT bundles and MT nucleating proteins to spindle poles. This unanticipated mitotic function of a cilia protein reveals a new molecular mechanism for cilia proteins in the orientation of the mitotic spindle and the plane of cell division and has important implications for the etiology of ciliopathies.

THE GREATWALL OF MITOSIS

Andrew Burgess, Aicha Gharbi-Ayachi, Suzanne Vigneron, Jean-Claude Labbé, Thierry Lorca and Anna Castro

Universités Montpellier 2 & 1, CRBM, CNRS UMR 5237, IFR 122, 1919 Route de Mende, 34293 Montpellier cedex 5, France

Historically entry, maintenance, and exit from mitosis were directly related to cyclin B/CDK activity. Thus entry and maintenance depended on cyclin B/CDK1 activation, while exit required deactivation via cyclin B degradation. However recent evidence has demonstrated that in addition to cyclin B/CDK1 activation, phosphatases, in particular PP2A must be inhibited for cells to enter into and maintain mitosis. However what inhibited PP2A at mitotic entry, and how this was coordinated with the activation of cyclin B/CDK1 was unknown. We recently identified the kinase Greatwall (Gwl) as a critical mitotic regulator of PP2A. In Xenopus egg extracts, Gwl depletion results in a rapid exit from mitosis even in the presence of high cyclin/CDK activity, due to a reactivation of PP2A. Furthermore, in human cells, we identified MAST-L (microtubule-associated serine/threonine kinase-like protein) as the functional orthologue of Gwl. The endogenous protein is primarily nuclear, but also localises at centrosomes, the mitotic spindle, and mid-body. SiRNA knockdown blocked human cells in G2, while partial depletion resulted in cells entering into an aberrant mitosis with multiple defects. This correlated with premature dephosphorylation of cyclin B/CDK1 substrates, inactivation of the SAC, and subsequent exit from mitosis with severe cytokinesis defects. Inhibition or co-knockdown of PP2A rescued the loss of CDK1 substrate phosphorylation. Finally we show that Gwl inhibits PP2A by phosphorylating the Arpp19/ENSA proteins, which promotes its association with and inhibition of PP2A activity. These results demonstrate that Gwl is critical for maintaining a correct kinase/phosphatase balance to ensure mitosis occurs with complete fidelity.

IDENTIFYING CENTRIN 2 AS A NOVEL CDK2 SUBSTRATE

Rose Boutros, Brian Gabrielli, Bernard Ducommun, Phillip Robinson, Megan Chircop

Cell Signalling Unit, Children's Medical Research Institute, The University of Sydney, 214 Hawkesbury Road, Westmead, NSW 2145, Australia.

The centrosome duplication cycle is synchronised with DNA replication in interphase. Upon mitotic entry, the two centrosomes separate forming the bipolar mitotic spindle. Misregulation of the pathways that regulate centrosome duplication results in centrosome aberrations and errors in chromosome segregation. These defects are considered to contribute to neoplastic transformation.

Centrosome duplication is regulated by CDK2 in complex with cyclins E and A, which is in turn activated by the CDC25 family of phosphatases. We have previously demonstrated that CDC25B localises to the centrosome throughout interphase to control centrosome duplication during S phase and the nucleation of microtubules in interphase cells. CDC25B is overexpressed in many human cancers. We have found that its overexpression in cancer cell lines causes centrosome amplification and aberrant nucleation of microtubules, resulting in severely abnormal mitotic spindles. This occurs through over-activation of a centrosome-specific pool of CDK2, subsequently causing misregulation of centrosome proteins. In particular, centrosomal CDC25B directly controls the level of the key structural centrosome component, centrin 2. Centrin 2 encodes a CDK consensus motif and cyclin binding domain. We are therefore investigating whether centrin 2 represents a novel CDK2-cyclin substrate in centrosome biogenesis, and whether its misregulation through aberrant CDC25-CDK2 activity causes centrosome defects contributing to chromosome instability and neoplastic transformation.

CYCLIN E2 INCREASES GENOMIC INSTABILITY DUE TO FAILED DEGRADATION IN THE S PHASE OF CANCER CELLS

Caldon C.E., Sergio, C.M., Sutherland, R.L. and Musgrove E.A.

Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

Overexpression of cyclin E1 accelerates progression into S phase of the cell cycle and promotes genomic instability in cancer cells. Cyclin E1 is regulated via periodic expression and degradation: expression peaks at the G1/S phase transition, and it is then rapidly degraded during S phase after recognition by the SCF(Fbw7) ubiquitin ligase. In this study we identify crucial differences in the regulation of cyclin E2 and cyclin E1. We show that cyclin E1 and E2 are expressed in different phases of the cell cycle of cancer cells, with cyclin E2 protein expression maintained during S phase while cyclin E1 was rapidly degraded after S phase entry. This was due to a failure of Fbw7 to effectively target cyclin E2 for proteosomal degradation, leading to prolonged S phase stability. Cyclin E1 induces genomic instability when it is expressed in S phase, which led us to assay whether cyclin E2 is a particularly effective inducer of genomic instability due to its prolonged S phase expression. We show that constitutive overexpression of either cyclin E1 and E2 hinders the progression of cells through mitosis, where this disruption is likely to induce genomic instability. Moreover, the overexpression of cyclin E2 induced micronucleation, a marker of genomic instability, at a greater rate than cyclin E1. Cyclin E2 has previously been assumed to be functionally redundant with cyclin E1. However, we have identified that cyclin E1 and E2 are independently regulated in cancer cells, and that cyclin E2 may be a stronger promoter of genomic instability due to its constitutive S phase expression.

DYNAMIN INHIBITORS ARE A NEW CLASS OF ANTI-MITOTIC AGENTS WITH ANTI-CANCER PROPERTIES

Megan Chircop (nee Fabbro)

Children's Medical Research Institute, The University of Sydney, 214 Hawkesbury Road, Westmead, NSW 2145, Australia.

Inhibitors of mitotic proteins are one of the new classes of chemotherapeutic agents emerging in clinical trials for the treatment of cancer. Such targeted inhibitors are selective for mitotic protein kinases, e.g. Aurora kinase and Polo like kinase and mitotic kinesin motor proteins, e.g. KSP. The requirement of membrane trafficking proteins in the latter stages of mitosis has started to emerge. These proteins could also be amenable to the development of inhibitors for cancer therapy. One such protein is the endocytic protein dynamin II (dynII). We have described several series of small molecule inhibitors of dynamin that inhibitor its GTPase activity and prevent receptor-mediated endocytosis. I will discuss that inhibitors of dynamin are (1) the first compounds to exclusively block the final stage of mitosis, cytokinesis, without affecting progression through any other stage of the cell cycle, (2) induce apoptotic cell death via a mitochondrial-dependent pathway only in cells that failed cytokinesis, (3) combination treatment with antagonists against the pro-survival factor, bcl-2, causes cells to become increasingly sensitive to dynamin inhibitor-mediated cell death and (4) dyn inhibitors have minimal effect on non-tumourigenic fibroblast cells. These specific toxicity and anti-proliferative properties of dyn inhibitors indicate that dynII is a novel target for pharmacological intervention for the treatment of cancer.

MICRORNAS AND THE MAMMALIAN CELL CYCLE

Nicole Cloonan[1], Brian Gabrielli[2], Liz Musgrove[3], and Sean M. Grimmond[1].

[1]Queensland Centre for Medical Genomics, Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia, 4072 [2]Diamantina Institute, The University of Queensland, St Lucia, QLD, Australia, 4072 [3]Garvan Institute, Darlinghurst, NSW, Australia, 2010

MicroRNAs (miRNAs) are crucial components of the regulatory circuitry of cells, modulating target mRNA activity and controlling key biological processes such as cellular proliferation and apoptosis. Due to both the small size and mismatch tolerance of miRNA-mRNA interacting regions, individual miRNAs can potentially bind hundreds of mRNA targets. Despite this apparent ambiguity in targeting, miRNAs can drive specific biological phenotypes by targeting a network of highly interacting, functionally related genes. By using the resolution provided by deep-sequencing of miRNAs, and integrating other “omic” scale data with systems biology approaches, we are able to gain significant insight into the miRNA biology of the mammalian cell cycle and the dysregulation of these miRNA networks in cancer.

THE ROLE OF A SPECIFIC G1-REGULATORY ACTIN FILAMENT POPULATION DEFINED BY THE TROPOMYOSIN ISOFORM TM5NM1 IN ONCOGENESIS

Coombes, JD[1], Schevzov G[1], Kan FC-Y[2], MacKenzie KL[2], Gunning P[1].

[1]Oncology Research Unit, School of Medical Sciences, University of New South Wales [2]Cancer Cell Development Group, Children's Cancer Institute Australia

G1-phase progression is dependent on an intact actin cytoskeleton. However, actin filament populations are heterogeneous and the functions of specific filament populations have remained largely unknown. Specific functional populations of actin filaments can be identified by their association with over 40 different isoforms of Tropomyosin (Tm). We have identified a specific actin filament population defined by Tm5NM1 that mediates G1-phase progression. All cancer cells analysed to date adopt a unique and distinct expression profile of Tm isoforms and the expression of Tm5NM1 in particular has been reported to show a strong correlation with cancer. Together with this association and the proposed function of Tm5NM1 as a cell cycle mediator, we aimed to investigate the role of this filament population in oncogenesis. We have immortalised and transformed embryonic fibroblasts from Tm5NM1-knockout mice via retroviral transduction with cDNAs encoding SV40 Large T antigen and oncogenic H-Ras and analysed their growth in culture. We found that, compared to controls, immortalised fibroblasts devoid of Tm5NM1 (Tm5NM1 -/-) have impaired proliferation in serum-free growth conditions. In soft-agar anchorage-independent cultures, immortalised Tm5NM1-/- fibroblasts generated smaller colonies. Additionally, cells cultured under high confluency displayed phase bright cytoplasmic vacuoles, indicative of a stress related response. These data are consistent with defects in the signalling leading to G1-phase progression, and would predict an impairment of tumour growth *in vivo*. Experiments are currently underway to quantify the effect of Tm5NM1 actin filaments on the growth of transformed fibroblasts in a mouse tumour model. We conclude that Tm5NM1-containing actin filaments may mediate the actin-based promotion of cancer cell proliferation.

CYCLIN A/CDK2 DEPENDENT PLK1 ASSOCIATION WITH ADENOMATOUS POYLPOSIS COLI REGULATES MITOTIC SPINDLE ANCHORING

Vanessa Oakes, Brittney Harrington, Stephanie Huong Le, Heather Beamish, Brian Gabrielli

The University of Queensland Diamantina Institute, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia.

Mutations of the adenomatous poylposis coli (APC) protein are major contributors to tumour initiation and progression in several tumour types. These mutations can affect APC function in mitotic spindle anchoring to the cell cortex and orientation. We have previous shown that the mitotic spindle anchoring and orientation function of APC is regulated by cyclin A/cdk2 phosphorylation of APC Ser1360. Here we report that the phosphorylation of APC Ser1360 acts as a binding site for Plk1. Inhibition of Plk1 or over expression of the polobox domain of Plk1 resulted in an identical spindle anchoring defect as cyclin A depletion. Cyclin A depletion also affected the localisation of Plk1 in mitotic cells and decoration with the mitotic phospho-antibody MPM2. However, Plk1 inhibition and polobox domain over expression also affected normal bipolar spindle formation a phenotype which has not been associated with cyclin A /cdk2 depletion/inhibition or APC mutation, indicating that cyclin A/cdk2 is only potential regulating a subset of Plk1 functions in mitosis.

THE FUNCTIONS OF CYCLIN A2 IN DNA REPLICATION AND MITOSIS

Lakshmi Gopinathan, Kasim Diril, and Philipp Kaldis

Institute of Molecular and Cell Biology (IMCB), 61 Biopolis Drive, Singapore 138673

Cyclin A2 is believed to be an integral component of the cell cycle, and its essential role in cell proliferation is evidenced in the embryonic lethality associated with cyclin A2 knockout (KO) mice. Unpredictably, mouse embryonic fibroblasts (MEFs) derived from cyclin A2 conditional knockout (CKO) mice proliferate normally when cyclin A2 is ablated. Detailed studies using cyclin A2 CKO mice generated in our laboratory have revealed that cyclin A2-null MEFs display delayed exit from the S-phase and decreased immortalization rates. To assess the relative contributions of Cdk1 and Cdk2 in partnering with cyclin A2, Cdk1 CKO and Cdk2 KO mice were crossed with cyclin A2 CKO mice, followed by analyses of MEFs generated from these double knockout (DKO) mice. Cdk2/cyclin A2 DKO MEFs entered senescence prematurely with substantial increase in G2/M population and re-replication. Growth rates of these DKO MEFs were significantly impaired, but prolonged passaging did eventually lead to immortalization. Thus, the proven compensatory function of Cdk1 in the absence of Cdk2 appears to be compromised in Cdk2/cyclin A2 DKO MEFs. We are currently analyzing Cdk1/cyclin A2 DKO MEFs.

A BREATHTAKING PHENOTYPE: DOUBLE LIFE OF A DNA REPAIR PROTEIN AS AN ESSENTIAL REGULATOR OF LUNG ORGANOGENESIS

Sabine Jurado[1,2], Ian Smyth[3], Bryce van Denderen[1,2], Nora Tenis[1], Andrew Hammet[1,¶], Kimberly Hewitt[1], Jane-Lee Ng[1], Carolyn J. McNees[1,¶], Sergei V. Kozlov[4], Hayato Oka[5,¶], Masahiko Kobayashi[6], Lindus A. Conlan[1], Timothy J. Cole[3], Ken-ichi Yamamoto[6], Yoshihito Taniguchi[5,¶], Shunichi Takeda[5], Martin F. Lavin[4,7], and Jörg Heierhorst[1,2]

[1]St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia, [2]Department of Medicine, St. Vincent's Hospital, The University of Melbourne, Fitzroy, Victoria, Australia, [3]Departments of Biochemistry and Molecular Biology, and Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia, [4]Queensland Institute of Medical Research, Herston, Queensland, Australia, [5]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [6]Cancer Research Institute, Kanazawa University, Ishikawa, Japan, [7]Central Clinical Division, University of Queensland, Royal Brisbane Hospital, Herston, Queensland, Australia.

Zn^{2+}-finger proteins comprise one of the largest protein superfamilies with diverse biological functions. The ATM substrate Chk2-interacting Zn^{2+}-finger protein (ASCIZ; also known as ATMIN and ZNF822) was originally linked to functions in the DNA base damage response and has also been proposed to be an essential cofactor of the ATM kinase. Here we show that absence of ASCIZ leads to *p53*-independent late embryonic lethality in mice. *Asciz*-deficient primary fibroblasts exhibit increased sensitivity to DNA base damaging agents MMS and H_2O_2, but *Asciz* deletion or knock-down does not affect ATM levels and activation in mouse, chicken or human cells. Unexpectedly, *Asciz*-deficient embryos also exhibit severe respiratory tract defects with complete pulmonary agenesis and severe tracheal atresia. Nkx2.1-expressing respiratory precursors are still specified in the absence of ASCIZ but fail to segregate properly within the ventral foregut, and as a consequence lung buds never form and separation of the trachea from the oesophagus stalls early. Comparison of phenotypes suggests that ASCIZ functions between Wnt2-2b/ß-catenin and FGF10/FGF-receptor 2b signaling pathways in the mesodermal/endodermal crosstalk regulating early respiratory development. We also find that ASCIZ can activate expression of reporter genes via its SQ/TQ-cluster domain *in vitro*, suggesting that it may exert its developmental functions as a transcription factor. Altogether, the data indicate that, in addition to its role in the DNA base damage response, ASCIZ has separate developmental functions as an essential regulator of respiratory organogenesis.

ANALYSIS OF STRESS INDUCED CELL CYCLE ALTERATIONS IN TISSUES - *IN VIVO*

Christina Heinlein[1], Antony Braithwaite[1] and Daniel Speidel[1]

[1]*Children's Medical Research Institute; Westmead, Australia*

Exposure of cells and whole organisms to gamma radiation, UV light, chemical agents and oxidative stress causes DNA damage. Cell cycle alterations, such as arrest in the G1 and G2 phases are known to be principal mechanisms by which cultured cells respond to such exogenous damage. Although there are detailed studies analysing the role and significance of cell cycle arrest for genomic stability in tissue culture, very little is known about cell cycle alterations as a stress response in tissues *in vivo*.

We have recently developed a flow cytometry based technique enabling us to assess stress induced cell cycle effects in different organs and tissues. Using this technique we have analysed cell cycle alterations over time after exposure of living mice to gamma radiation and tissue to other stress stimuli.

Data show that stress induced cell cycle effects do occur *in vivo*. These effects differ significantly among various tissues and are also dose dependent. Strikingly, cell cycle effects as stress response appear not to be restricted to proliferating tissue only but can also be observed in organs that are considered to be quiescent.

A NON-CATALYTIC FUNCTION FOR A CHECKPOINT KINASE DURING NORMAL DNA REPLICATION

Nicolas Hoch, Alessandro Fazio, Andrew Hammet and Jörg Heierhorst

St. Vincent's Institute, Melbourne, VIC, Australia

Aberrant DNA replication is a major source of genome instability and can, thus, lead to cancer onset and progression. The replication checkpoint is an extensively studied tumor suppressor pathway involved in preventing replication fork stalling and/or collapse after DNA damage. However, little is known about its functions during replication of undamaged chromosomes. Similarly to the corresponding pathway in mammalian cells, the budding yeast Chk2-like kinase Rad53 is activated upon phosphorylation of its N-terminal serine-glutamine/threonine-glutamine cluster domain (SCD1) by the central checkpoint kinases Mec1/Tel1 (ATR/ATM) in response to DNA damage. Here we show that Rad53 SCD1 phosphorylation also has crucial kinase-independent functions during DNA replication in the absence of exogenous DNA damage. Mutation of all four phospho-acceptor threonines in this region to unphosphorylatable alanines leads to spontaneous DNA damage and checkpoint activation during normal S-phase, presumably due to increased replication fork stalling at intrinsically difficult-to-replicate loci. Consistently, we show that SCD1 phosphorylation is highly increased during normal DNA replication, although Rad53 kinase activation cannot be detected under these conditions. Importantly, a *rad53* kinase-dead mutant does not share most of the phenotypes observed for SCD1 mutant alleles during basal DNA replication, strongly suggesting a kinase-independent function of SCD1 phosphorylation sites. Our results support a model in which a scaffolding function of SCD1 phosphorylation promotes replication fork stability, thus preventing spontaneous DNA damage arising during an unchallenged round of replication.

THE SCRIBBLE/DLG/LGL POLARITY MODULE IN THE CONTROL OF MAMMALIAN DEVELOPMENT AND CANCER.

Lorey Smith, Nathan Godde, Imogen Elsum, Ryan Galea, Allison Ogden, Lukas E. Dow, Sarah M. Russell, Helena E. Richardson, Patrick O. Humbert

Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia.

A Genetic screen in Drosophila for regulators of Cyclin E identified scribble, discs large (dlg) and lethal giant larvae (lgl) as key epithelial polarity regulators with mutation in any of these genes resulting in loss of polarity, overproliferation and multilayering of epithelial cells leading to 3D-tumourous overgrowth, and in the presence of activated Ras, invasion and metastasis. Evidence from cancer patients suggests that Scribble/Dlg/Lgl could act as tumour suppressors in some epithelial cancers with low levels and/or mislocalization of mammalian Scribble, Dlg and Lgl homologues correlating with increased tumour invasiveness and malignancy. The actual role of mammalian Scribble/Dlg/Lgl complex in development and tumourgenesis however remains poorly understood.

We have used a combination of in vivo animal analysis, biochemical studies and RNAi screening to address the functional role of Scribble complex in mammalian cells. To bypass the neonatal lethality of existing mouse mutants, we have generated a novel conditional allele of Scribble in the mouse. Our initial studies indicate that loss of Scribble results in deregulated proliferation and tissue homeostasis in multiple epithelial tissue types and we will discuss how Scribble may impact on this process in view of 1) our recent demonstration that Scribble can regulate the Ras-Raf-MAPK pathway both in mammals and Drosophila, and 2) our recent identification through an RNAi screen of a novel functional interaction between mammalian Scribble and the asymmetric division regulatory complex of LGN/pins. We propose that Scribble, Dlg and other associated polarity proteins are signalling regulators involved in a new pathway controlling epithelial tumour progression in mammals.

CENTROBIN REGULATES INTERPHASE MICROTUBULE DYNAMICS AND THE ASSEMBLY OF FUNCTIONAL MITOTIC SPINDLES.

Jessie Jeffery[1], Armando van der Horst[2], Ilya Grigoriev[3], Nicholas Hamilton[4.5], Robert Parton[4,6] V. Nathan Subramaniam[7], Anna Akhmanova[3], Kum Kum Khanna[1]

1: Signal Transduction Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia, 2: University Medical Center, Utrecht, Netherlands., 3: Erasmus Medical Center, Rotterdam, Netherlands, 4: The University of Queensland, Institute for Molecular Bioscience, Brisbane, Queensland, Australia, 5: ARC Centre of Excellence in Bioinformatics, The University of Queensland, Brisbane, Australia, 6: The University of Queensland, Centre for Microscopy and Microanalysis, Brisbane, Queensland, Australia, 7: Membrane Transport Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

The centrosome is critical for the nucleation and organisation of microtubules in many cell types. Here we describe the role of the centrosomal protein, Centrobin, in stabilising the microtubule network throughout interphase and regulating spindle formation during mitosis. We provide the first evidence for Centrobin as a suppressor of microtubule nucleation. Centrobin-depleted cells initiate microtubule nucleation more rapidly than control cells and exhibit a two-fold increase in EB1 comets nucleating from the centrosome concomitant with an increase in growth and shrinkage frequencies. Centrobin-depleted cells also have less soluble tubulin than control cells. Furthermore, we show that normal microtubule dynamics are required for the function of microtubule-dependent processes such as the endocytic pathway.

We also show that Centrobin is required for normal mitotic progression. Centrobin-depleted cells display a range of spindle abnormalities including unfocused poles that are not associated with centrosomes, S-shaped spindles, and mini spindles. These cells undergo mitotic arrest and subsequently often die by apoptosis. Centrobin-depleted cells arrest due to an activated spindle checkpoint, which senses the lack of stable microtubule-kinetochore attachments. These results indicate that Centrobin promotes anchoring of mitotic spindle to the centrosomes and mitotic spindle stability.

Taken together, these results suggest that Centrobin is a critical regulator of microtubule function and stability throughout the cell cycle.

CELL CYCLE REGULATION OF URACIL BASE EXCISION REPAIR (BER) IN HYPERMUTATING B CELLS

Christopher J Jolly and George Sharbeen

Centenary Institute, Camperdown, NSW 2050

Central to vertebrate adaptive immunity is the rapid evolution of high-affinity antibodies in activated B-cells. This occurs by repeated rounds of antibody gene mutation ("hypermutation") and positive selection of B-cells expressing a higher affinity antigen receptor. Hypermutation is initiated by Activation-Induced cytosine Deaminase, a protein specific to activated B-cells. AID deaminates cytosine (C) in highly transcribed antibody genes (and some highly transcribed "off-target" genes) in activated B cells, generating uracil (U) opposite guanine (G). Excision by UNG or SMUG1 would normally initiate faithful reversion of such uracils to cytosine. However, in activated B cells, processing by UNG frequently converts the U:G base pair into any base pair via error-prone trans-lesion DNA synthesis. The reasons are unknown. One of the features distinguishing activated B-cells *in vivo* from other blasting cells is their cell cycle, with an unusually long G1 phase. We generated constructs which tightly restrict UNG activity to either G1 or later cell cycle phases and retrovirally expressed these constructs in activated B cells *in vivo* using a unique adoptive transfer model. We show that uracil excision by UNG is mutagenic only when it occurs in G1 phase, probably requiring recruitment of UNG to antibody genes via transcription. This contrasts with the commonly held view that uracil BER occurs primarily in S phase. We also found that UNG initiates some faithful repair of uracils in hypermutating B cells, in an unexpected phase of the cell cycle.

MITOTIC LOCALISATION AND FUNCTION OF IQGAP1 DURING GLIOBLASTOMA CELL DIVISION

Audrey T.Y. Lian, Emma K. Kettle, Phillip J. Robinson and Megan Chircop

Children's Medical Research Institute, The University of Sydney, Locked Bag 23, Wentworthville, Sydney, NSW 2145, Australia

Cytokinesis is the final stage of cell division generating two independent daughter cells. Failure of this process results in enlarged and multinucleated cells, contributing to the initiation and/or progression of tumourigenesis. These cells are histopathological hallmarks for many tumours including the primary brain tumour, glioblastoma multiforme (GBM). IQGAP1 is an actin-binding protein that is up-regulated in GBM and essential for cytokinesis in yeast. At the immunological synapse (IS) during lytic granule secretion by cytotoxic T lymphocytes, actin reorganisation and IQGAP1 clearance from the plasma membrane regulates centrosome repositioning and delivery of lytic granules. Analogously during cytokinesis, actin disassembly at the intracellular bridge (ICB) is associated with vesicle recruitment and centrosome repositioning to the ICB. Centrosome trafficking back to the cell centre is associated with membrane abscission and formation two independent daughter cells. Our hypothesis is that IQGAP1 participates in glioblastoma cell division and is involved in the regulation of actin remodelling, centrosome repositioning and abscission. In support of this idea, we demonstrate that IQGAP1 co-localises with actin at the cleavage furrow and localises to the ICB of human U-87MG glioblastoma cells that are undergoing cytokinesis. This is consistent with its actinomyosin contractile ring localisation in dividing fission yeast. Western blot analysis shows that IQGAP1 expression levels are reduced upon mitotic entry. Depletion of IQGAP1 leads to an increased number of multinucleated cells, an indication of cytokinesis failure. Our findings suggest that IQGAP1 levels are tightly regulated during mitosis and that it is associated with successful completion of cytokinesis.

SORTING NEXIN 9 IS REQUIRED FOR MITOTIC PROGRESSION

Maggie P.C. Ma, Emma Kettle, Phillip J. Robinson, Megan Chircop

Children's Medical Research Institute, The University of Sydney, Locked Bag 23, Wentworthville, Sydney, NSW 2145, Australia

Completion of the final stage of mitosis, called cytokinesis, results in two independent daughter cells. The mechanisms of membrane abscission to generate two daughter cells are unknown. However, immediately prior to abscission, vesicles derived from intracellular organelles, such as endosomes, golgi apparatus and lysosomes, are recruited to the intracellular bridge (ICB) and accumulate at the site of abscission. Here, we show that endocytosis also contributes to vesicular accumulation at the ICB. Horseradish peroxidise endocytic uptake and electron tomography microscopy revealed that endocytosis occurs at the periphery of the ICB generating tubular-vesicular structures that reside along the length of the ICB. Immunofluorescence microscopy reveals that the endocytic protein sorting nexin 9 (SNX9) locates to the mitotic centrosomes throughout mitosis and then to punctuate structures at the ingressing furrow and along the ICB. Cells depleted of SNX9 by siRNA are unable to complete cytokinesis, resulting in an accumulation of binucleated and cytokinetic cells. Time-lapse analysis reveals that SNX9 is required for efficient mitotic progression as HeLa cells depleted of SNX9 spent significantly more time in metaphase, and the ingression and abscission phases of cytokinesis. The longest delay was observed during membrane abscission. SNX9 has been proposed to tubulate membranes during endocytosis and this is further induced by its ability to recruit N-WASP and actin. Thus, we suggest that during abscission, SNX9 may co-operate with N-WASP and actin to generate tubular-vesicular structures at the ICB prior to abscission.

THE ROLE OF G1/S CHECKPOINT REGULATORS IN ENDOCRINE RESISTANCE IN ER-POSITIVE BREAST CANCER.

Liz Caldon[1], Andrew Stone[1], Marcelo Sergio[1], Catriona McNeil[1], Jane Barraclough[1], Christine Lee[1], Julia Gee[2], Rob Nicholson[2], Rob Sutherland[1], Liz Musgrove[1]

1: Cancer Research Program, Garvan Institute of Medical Research, Sydney; 2: Welsh School of Pharmacy, Cardiff University, Cardiff, UK.

Estrogen increases the expression of cyclin D1 and c-Myc, which leads to the formation of active cyclin E1 and E2 complexes that are essential for cell-cycle progression. Tamoxifen and other antiestrogens reduce the expression of these genes as part of their anti-proliferative effects. In the clinical setting, this anti-proliferative effect is often short-lived, and many patients either do not respond or experience disease relapse. Genes involved in proliferation are prominent in signatures of tamoxifen response, and overexpression of genes including c-Myc and cyclin D1 causes antiestrogen resistance in breast cancer cells in culture. Cyclin E1 overexpression led to increases in p21, cyclin D1 and cyclin E2, but cyclin E2 overexpression did not affect p21, cyclin D1 or cyclin E1 expression. However, cells overexpressing either cyclin E1 or cyclin E2 were antiestrogen resistant. In an established tamoxifen-resistant cell line (TAM-R) the expression of cyclins D1, E1 and E2 is greater than in the parental cells. Although tamoxifen reduced the expression of cyclins D1, E1 and E2 gene in the TAM-R cells, the inhibition of protein expression was less pronounced than in the parental cells and cell proliferation was sustained. Preliminary analysis has indicated changes in SCF complex components and INK4/ARF and Cip/Kip family members, suggesting possible changes in the way cyclin-CDK complexes are deactivated and degraded in the resistant cells. These studies implicate deregulation of the cell cycle machinery at multiple levels in endocrine resistance.

MCC IS A NOVEL TARGET OF THE DNA DAMAGE RESPONSE

Pangon L, Sigglekow N, Larance M, Musgrove EA, Kohonen-Corish M.

Garvan Institute of Medical Research, Sydney

The 'Mutated in Colorectal Cancer' (MCC) gene was discovered in 1991 due to its close linkage with the APC tumour suppressor gene. We have shown that the MCC defect is more common in colorectal cancer than previously thought, as this gene is frequently silenced through promoter hypermethylation. Furthermore, mutated MCC is a 'driver' of carcinogenesis in a mouse model of colorectal cancer. Using mass spectrometry we identified two highly conserved ATM/ATR consensus phosphosites in the MCC protein. This suggested that MCC may participate in the cellular process that responds to DNA damage. We then tested this response by exposing cells to various DNA damaging agents. Ultraviolet radiation, but not phleomycin, caused PI3K-dependent phosphorylation of MCC and its localization to the nucleus. Re-expression of MCC in HCT15 cells led to a G2/M arrest, and MCC knockdown in HCT116 cells impaired the induction of a G2/M arrest following UV radiation. Finally, mutation of S118/120 to alanine impaired the G2/M checkpoint activity of MCC. These results suggest that MCC activity is required in the full induction of cell cycle arrest in the G2/M phase upon UV-induced single strand DNA breaks. Single strand DNA breaks are common in all cells, including the colon, and are caused by spontaneous DNA decay or reactive oxygen species. The ability to sense and respond to DNA damage is critical to the maintenance of genomic stability and the prevention of cancer and this could be one of the mechanisms whereby loss of MCC expression promotes colorectal carcinogenesis.

GROWTH PATHWAYS CONTROLLING TRANSCRIPTION OF THE MYC ONCOGENE

N. Cranna[1], A. Lee Jue Er[1], N. Mitchell[1], Z. Nie[2] , D. Levens[2], R. Hannan[3], L. Quinn[1]

[1] Anatomy and Cell Biology, University of Melbourne, Parkville, VIC, Australia [2] Peter MacCallum Cancer Centre, Melbourne, VIC, Australia [3] National Cancer Institute, Bethesda, Maryland, United States

The mammalian Fuse Binding Protein family (FBP1-3) contains KH domains, which have traditionally associated with RNA binding. Although some studies have implicated FBP1 and 2 in RNA splicing (1), our work has demonstrated that the FBP proteins can bind the FUSE regulatory sequence in the c-myc promoter to regulate RNA polymerase II progression and, therefore, transcript elongation (2). We have shown that the interaction between FBP and the c-myc promoter is sensitive to growth signals, since serum starvation followed by re-addition results in recruitment of FBP1, which is associated with a pulse of c-myc transcription (3). Drosophila only contains a single ortholog of the FBP family, Psi, and we have evidence that Psi is also required for regulation of Drosophila myc, dmyc, transcription. In order to identify the growth signals driving Psi recruitment we have developed a Drosophila model for dmyc promoter activity and performed a candidate screen of key growth signalling pathways. Thus far we have identified two key pathways capable of modifying dmyc promoter activity, the Ras/PI3K and TGFβ/Dpp pathways. Furthermore, by carrying out RNAi ablation of Psi in the activated Ras/PI3K or TGFβ/Dpp background we have generated evidence that Psi may provide an important connection between these signals and activation of dmyc transcription. Our future studies will be directed towards understanding whether mammalian FBP might also be responsive to orthologous growth pathways, which are oncogenic in mammals.

(1) Rothe, F., Gueydan, C., Bellefroid, E., Huez, G., and Kruys, V. (2006). Identification of FUSE-binding proteins as interacting partners of TIA proteins. Biochem Biophys Res Commun. 343, 57-68.

(2) Chung, H.J., Liu, J., Dundr, M., Nie, Z., Sanford, S., and Levens, D. (2006). FBPs are calibrated molecular tools to adjust gene expression. Mol Cell Biol 26, 6584-6597.

(3) Liu J, Kouzine F, Nie Z, Chung HJ, Elisha-Feil Z, Weber A, Zhao K and Levens D (2006) The FUSE/FBP/FIR/TFIIH system is a molecular machine programming a pulse of c-myc expression. EMBO J 25: 2119-2130.

A NOVEL INHIBITOR OF RIBOSOMAL RNA GENE TRANSCRIPTION IDENTIFIES COUPLING OF POLI TRANSCRIPTION TO MULTIPLE CELL CYCLE CHECKPOINTS AND SENESCENCE.

J.E. Quin[1,2], E. Sanij[1], M. Bywater[1,2], D. Drygin[5], K. Anderes[5], G.A. McArthur[1,4], R.B. Pearson[1,2,3], and R.D. Hannan[1,2,3]

[1]Research, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia. [2]Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, VIC, Australia. [3]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia. [4]Department of Medicine, St Vincents Hospital, Melbourne, VIC, Australia. [5]Cylene Pharmaceuticals, San Diego, CA, USA

The transcription of the 45S ribosomal RNA (rRNA) genes by RNA Polymerase I (Pol I) is a major rate limiting step for ribosomal biogenesis, and consequently for cell growth and proliferation. Dysregulation of Pol I transcription of the rRNA genes is an almost universal feature of cell transformation and cancer, and work from our laboratory has shown that targeting this process is a promising new approach for cancer therapy (M.J. Bywater, Unpublished Results). A small molecule inhibitor of Pol I transcription (CX-5461) has recently been developed by Cylene Pharmaceuticals, which provides an opportunity to examine the acute response of both non-malignant and transformed cells to perturbation of rDNA transcription. We have utilized CX-5461 to examine the response of BJ (hTert) immortalized primary human fibroblasts and isogenically matched BJ cell lines, at defined stages of transformation, to inhibition of Pol I transcription. Following inhibition of Pol I transcription, BJ (hTert) cells display a proliferation defect, which is associated with both G1 and G2 cell cycle arrest, and senescence. The proliferation defect is p53-independent. Pre-transformed BJ (Large-T, hTert) cells display increased sensitivity to CX-5461 treatment, and undergo cell death. However, transformed BJ (Large-T, Small-T, hTert, Ras) cells display reduced sensitivity to CX-5461 treatment. We will present results of our ongoing studies, which include experiments to address the mechanisms underlying the observed cell cycle defects and differing responses in these cell lines.

HSSB1, THE SENSOR OF DOUBLE STRAND DNA BREAKS ?

Giuseppe Schettino[2], Kienan Savage[2], Liza Cubeddu[3], Kumkum Khanna[1], Derek J Richard[1]

[1]Queensland Institute of Medical Research, Brisbane, Australia. [2]Queens University, Belfast UK. [3]University of Sydney, Sydney Australia.

Cells have developed a vast array of surveillance, repair and signalling proteins in order to prevent the loss of valuable genetic information that results from DNA damage. It has been estimated that each cell can suffer up to 30,000 DNA damaging insults in a 24 hour period, each damage event, if not repaired correctly, could consequently result in a loss of cellular programming.

One essential family of DNA repair proteins are the single stranded DNA binding proteins (SSBs). These can be divided into the simple SSBs and the RPAs. SSBs, function in a number of cellular processes including DNA replication and DNA damage repair. Humans were believed to only contain RPA however recently we have shown that the human genome encodes two distinct, simple SSB homologues, which we have named hSSB1 and hSSB2.

Our work has now demonstrated that hSSB1 functions in the initiation of DNA signalling and repair, following the induction of double strand DNA breaks (DSB). hSSB1 is essential for ATM and ATR activated signalling following treatment with ionizing radiation. We have demonstrated that hSSB1 is a component of the MRN complex and that hSSB1 binds directly to the N-terminus of nibrin, an interaction that is perturbed in patients suffering Nijmegen breakage syndrome. This hSSB1:MRN interaction is required both for the recruitment of MRN to DSBs and for the activity of the Mre11 nuclease; explaining the signalling defect in hSSB1 deficient cells.

This data clearly demonstrates that hSSB1 is an essential component of the DNA double strand break repair pathway.

UBF CONTROLS NUCLEOLAR INTEGRITY - CHECKPOINT MECHANISM FOR CELL CYCLE PROGRESSION

Elaine Sanij[1], Gretchen Poortinga[1], Jaclyn Quin[1], Jason Ellul[1], Lee Wong[2], G. A. McArthur[1,3,4] R. B. Pearson[1,5,6], and R. D. Hannan[1,4,6]

[1]Division of Research, Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne 3002, Victoria, Australia; [2]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville 3010, Victoria, Australia; [3]Department of Medicine, St Vincent's Hospital, University of Melbourne, Fitzroy 3065, Victoria, Australia; [4]Division of Haematology /Medical Oncology, Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne 3002, Victoria, Australia; [5]Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville 3010, Victoria, Australia; [6]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia

Somatic cells have ~200 copies of ribosomal RNA genes (rDNA) per haploid genome of which only a fraction are transcribed by RNA polymerase I (Pol I). We have recently reported that depletion of the upstream binding factor (UBF) leads to stable and reversible, methylation-independent silencing of rDNA by promoting histone H1-induced assembly of transcriptionally inactive chromatin1. Further, we have shown that rDNA silencing increases during differentiation and correlates with diminished UBF expression suggesting that rDNA silencing is dynamically regulated by UBF during development 1,2.

UBF depletion in NIH3T3 and primary human fibroblasts leads to a defect in cell cycle progression. Furthermore, recent fluorescent in situ hybridization (FISH) data suggests that regulation of UBF binding to rDNA plays an essential role in regulating nucleolar structure. rDNA silencing mediated by knocking down UBF leads to nucleolar disorganization suggesting that the cell cycle arrest in response to UBF depletion is a direct consequence of nucleolar disruption. Thus, UBF role in maintaining the genetic stability of rDNA repeats has important impact in controlling nucleolar integrity and general heterochromatin to protect genome integrity and consequently influencing cellular events such as aging and senescence. We have therefore performed UBF-ChIP sequencing in order to identify different patterns of sequence variations that may distinguish the UBF-bound, active pool of rDNA from silent rDNA. Furthermore, our ChIP-sequencing analysis evaluates UBF binding on a genome-wide scale and identifies novel UBF-bound target genes. Our work implicates UBF in mediating co-regulation of Pol I and Pol II transcriptional machineries.

1. Sanij et al., J Cell Biol. 2008 Dec 29;183(7):1259-74.

2. Sanij and Hannan. Epigenetics, 2009 Aug 4(6):374-82.

MECHANISMS OF MONO- AND POLY-UBIQUITINATION: UBIQUITINATION SPECIFICITY DEPENDS ON COMPATIBILITY BETWEEN THE E2 CATALYTIC CORE AND AMINO ACID RESIDUES PROXIMAL TO THE LYSINE

Martin Sadowski, Randy Suryadinata, Xianning Lai, Jorg Heierhorst and Boris Sarcevic

St. Vincent's Institute of Medical Research and Department of Medicine, St. Vincent's Hospital, University of Melbourne

Ubiquitination involves the attachment of ubiquitin to lysine residues on substrate proteins or itself, which can result in protein monoubiquitination or polyubiquitination. Ubiquitin attachment to different lysine residues can generate diverse substrate-ubiquitin structures, targeting proteins to different fates. The mechanisms of lysine selection are not well understood. Ubiquitination is catalyzed through co-operation between ubiquitin-ligases (E3s) and the ubiquitin-conjugating enzyme (E2s). Previous studies suggest that ubiquitination sites are selected by E3-mediated positioning of the lysine toward the E2 active site. Ultimately, at a catalytic level, ubiquitination of lysine residues within the substrate or ubiquitin occurs by nucleophilic attack of the lysine residue on the thioester bond linking the E2 catalytic cysteine to ubiquitin. One of the best studied RING E3/E2 complexes is the Skp1/Cul1/F box protein complex, SCFCdc4, and its cognate E2, Cdc34, which target the CDK inhibitor Sic1 for K48-linked polyubiquitination, leading to its proteasomal degradation. Our recent studies of this model system demonstrated that residues surrounding Sic1 lysines or lysine 48 in ubiquitin are critical for ubiquitination. This sequence-dependence is linked to evolutionarily conserved key residues in the catalytic region of Cdc34 and can determine if Sic1 is mono- or poly-ubiquitinated. Our studies indicate that amino acid determinants in the Cdc34 catalytic region and their compatibility to those surrounding acceptor lysine residues play important roles in lysine selection. This may represent a general mechanism in directing the mode of ubiquitination in E2s.

REGULATION OF THE G1 PHASE OF THE CELL CYCLE BY A SPECIFIC POPULATION OF ACTIN FILAMENTS DEFINED BY TROPOMYOSIN 5NM1

Anthony J. Kee[1], Jason Coombes[2], Galina Schevzov[2], Christine A. Lucas[1], Justine R. Stehn[2], Sarah J. Creed[2], Renee C. Szokolai[1], Elizabeth A. Musgrove[3], Peter W. Gunning[2] and Edna C. Hardeman[1]

[1]Departments of Anatomy and [2]Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052; [3]Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia.

The actin cytoskeleton has long been known to be a key regulator of cell proliferation. Currently the molecular mechanisms by which actin filaments participate in cell cycle progression are not understood. We previously showed that the spatiotemporal regulation of functionally distinct populations of actin filaments is determined by their tropomyosin isoform composition. In this study we report that actin filaments containing Tm5NM1 regulate cell proliferation. In cells and in mice we demonstrate that cell proliferation is influenced by Tm5NM1 expression. An increase in proliferation was evident in Tm5NM1-overexpressing B35 cells, whereas Tm5NM1 knock-down in SHEP cells or in knock-out (KO) MEFs significantly reduced proliferation. Furthermore, we show that fat mass and adipocyte number is increased in Tm5NM1 transgenic mice and reduced in Tm5NM1 KO mice. The observed changes in cell proliferation are associated with dysregulation of the mitogen activated protein (MAP) kinase cascade, leading to altered expression of the extracellular-regulated kinase (pERK1/2), cyclin D and E2F transcription factors, key effectors of the G1/S transition of the cell cycle. In the adipose tissue there is an increase in filamentous actin suggesting that Tm5NM1 is acting via increased formation of a specific actin filament population. In summary, we have defined a specific population of Tm5NM1-containing actin filaments that promote cell proliferation via the canonical ERK/MAPK pathway.

HIGH RESOLUTION CELL CYCLE AND PLOIDY ANALYSIS IN TISSUES: A VALUABLE TOOL FOR THE *IN VIVO* ANALYSIS OF EXPERIMENTAL ANIMAL MODELS AND PATIENT BIOPSIES

Christina Heinlein[1], Wolfgang Deppert[2], Antony Braithwaite[1] and Daniel Speidel[1]

[1]Children's Medical Research Institute, Cell Transformation Unit, Westmead NSW 2145, Australia, dspeidel@cmri.org.au [2]Heinrich-Pette-Institute, Dept. Tumour Virology, Hamburg 20251, Germany

Alterations in cell cycle distribution and changes in cellular DNA content are associated with a variety of normal and pathological physiological processes. However, due to methodological limitations, the detection of subtle alterations in DNA content has proven difficult when cells are in the context of a tissue structure. Therefore, certain fundamental questions of biology that cannot be addressed in tissue culture experiments have remained unclear. We have developed a novel and optimization-free technique to obtain high resolution cell cycle profiles from nearly all tissue types derived from mouse, human and sheep. Using a standardized and non-enzymatic procedure that is universally suitable for soft, solid and epithelial tissues alike, we reproducibly obtain cell cycle profiles of highest quality with coefficients of variation below 3.0. We are able to reduce preparation-derived debris to almost zero and efficiently exclude doublets, but retain multinucleated cells and apoptotic subG1-fragments. Applying this technique, we determine DNA-indices as small as 1.09 in tumour samples containing large necrotic areas and follow ploidy changes within different sections of individual tumours. Moreover, we are able to examine tissue specific cell cycle arrest and apoptosis as an *in vivo* stress response caused by radiation of mice. This method significantly improves the quality of DNA content analysis in tissues and extends the spectrum of possible applications. It allows assessing subtle changes in ploidy, cell cycle distribution and apoptosis/necrosis *in vivo* and should be instrumental in all research that involves experimental animal models and/or patient biopsies.

ENDOCYTIC PROTEINS ARE REQUIRED FOR EFFICIENT PROGRESSION THROUGH MITOSIS

Charlotte M. Smith, Phillip J. Robinson and Megan Chircop

Children's Medical Research Institute, The University of Sydney, Locked Bag 23, Wentworthville, Sydney, NSW 2145, Australia

A role for membrane trafficking proteins in a mitotic cell division is emerging in mammalian cells. Endocytic proteins such as clathrin and epsin are associated with the metaphase to anaphase transition, whereas dynamin II (dynII) is associated with the abscission stage of cytokinesis. However the requirement and mechanism of action for the broader endocytic machinery in mitosis remains unknown. Nor is it known if these proteins participate in mitosis in an endocytic-dependent or –independent manner. We show that five other key endocytic proteins are also required for mitosis - clathrin adaptor proteins, epsin and eps15; and the BAR domain proteins, amphiphysin II (amphII), endophilin II (endII), and syndapin II (sdpnII). These proteins act at distinctly different mitotic stages. Epsin and eps15 depletion delays the onset of chromosome segregation and increased bundling of the endoplasmic reticulum during metaphase. Depletion of amphII and sdpnII caused a delay in the initial stages of the membrane ingression phase. Whilst cells depleted of epsin, amphII, sdpnII and endII spend a prolonged period of time in the abscission phase of cytokinesis (intracellular bridge connection) prior to membrane regression and multinucleate formation. Consistent with their depletion phenotype, epsin, amphII, sdpnII, and endII were observed localising to distinct punctuate structures within the intracellular bridge, reminiscent of vesicles. EndII also localised to a region abutting the central midbody ring. Thus, endocytic proteins are associated with the metaphase-anaphase transition (clathrin, epsin and eps15) and the ingression (amphII and sdpnII) and abscission (dynII, amphII, endII, sdpnII and epsin) phases of cytokinesis. Collectively, these findings suggest that endocytic proteins function in a sequential order throughout mitosis, and this appears to be in an analogous order to their order of action for endocytosis.

REGISTRANTS

Dr Jane Barraclough
Cancer Research Program
Garvan Institute of Medical Research
j.barraclough@garvan.org.au

Dr Rose Boutros
Cell Signalling Unit
Children's Medical Research Institute
rboutros@cmri.org.au

Dr Andrew Burgess
Cell Cycle Dynamics
Centre de Recherche de Biochimie Macromoléculaire
CRBM-CNRS, France
andrew.burgess@crbm.cnrs.fr

Dr Liz Caldon
Cancer Research Program
Garvan Institute of Medical Research
l.caldon@garvan.org.au

Dr Megan Chircop
Cell Signalling Unit
Children's Medical Research Institute
mchircop@cmri.org.au

Dr Nicole Cloonan
Institute for Molecular Bioscience
The University of Queensland
n.cloonan@uq.edu.au

Mr Jason Coombes
School of Medical Sciences
University of NSW
jason.coombes@gmail.com

Ms Marlene Delivuk
Cancer Research Program
Garvan Institute of Medical Research
m.delivuk@garvan.org.au

Prof Steven J. Doxsey
Program in Molecular Medicine
University of Massachusetts Medical School
Worchester, MA, USA
Stephen.Doxsey@umassmed.edu

Dr Brian Gabrielli
Diamantina Institute
University of Queensland
brianG@uq.edu.au

Dr Lakshmi Gopinathan
Cancer and Developmental Cell Biology
Institute of Molecular and Cell Biology
Singapore
lakshmi@imcb.a-star.edu.sg

A/Prof Ross Hannan
Growth Control Laboratory
Peter MacCallum Cancer Centre
ross.hannan@petermac.org

A/Prof Jörg Heierhorst
Molecular Genetics
St. Vincent's Institute of Medical Research
jheierhorst@svi.edu.au

Dr Christina Heinlein
Cell Transformation Unit
Children's Medical Research Institute
cheinlein@cmri.org.au

Mr Nicolas Hoch
Molecular Genetics
St. Vincent's Institute
nhoch@svi.edu.au

Dr Patrick Humbert
Cell Cycle and Cancer Genetics
Peter MacCallum Cancer Centre
patrick.humbert@petermac.org

Dr Jessie Jeffery
Cancer and Cell Biology
Queensland Institute of Medical Research
jessie.jeffery@qimr.edu.au

Dr Chris Jolly
DNA Repair Group
Centenary Institute
c.jolly@centenary.org.au

Dr Jian Kang
Cancer Research Program
Garvan Institute of Medical Research
j.kang@garvan.org.au

Dr Maija Kohonen-Corish
Cancer Research Program
Garvan Institute of Medical Research
m.corish@garvan.org.au

Ms Christine Lee
Cancer Research Program
Garvan Institute of Medical Research
c.lee@garvan.org.au

Miss Audrey Lian
Cell Signalling Unit
Children's Medical Research Institute
alian@cmri.org.au

Miss Maggie Ma
Cell Signalling Unit
Children's Medical Research Institute
mma@cmri.org.au

Dr Naomi Mitchell
Anatomy and Cell Biology
The University of Melbourne
n.mitchell@unimelb.edu.au

A/Prof Liz Musgrove
Cancer Research Program
Garvan Institute of Medical Research
e.musgrove@garvan.org.au

Dr Laurent Pangon
Cancer Research Program
Garvan Institute
l.pangon@garvan.org.au

Ms Jaclyn Quin
Growth Control Laboratory
Peter MacCallum Cancer Centre
jaclyn.quin@petermac.org

Dr Leonie Quinn
Anatomy and Cell Biology
The University of Melbourne
leoniequinn@me.com

Dr Derek Richard
Cancer and Cell Biology
Queensland Institute of Medical Research
derek.richard@qimr.edu.au

Dr Elaine Sanij
Growth Control Laboratory
Peter MacCallum Cancer Centre
elaine.sanij@petermac.org

Dr Boris Sarcevic
Cell Cycle and Cancer Unit
St. Vincent's Institute of Medical Research
bsarcevic@svi.edu.au

Dr Galina Schevzov
Pharmacology
University of NSW
g.schevzov@unsw.edu.au

Mr Marcelo Sergio
Cancer Research Program
Garvan Institute of Medical Research
m.sergio@garvan.org.au

Miss Charlotte Smith
Cell Signalling Unit
The Children's Medical Research Institute
csmith@cmri.org.au

Dr Daniel Speidel
Cell Transformation Unit
Children's Medical Research Institute
dspeidel@cmri.org.au

Dr Andrew Stone
Cancer Research Program
Garvan Institute of Medical Research
a.stone@garvan.org.au