ABSTRACTS: ORAL PRESENTATIONS

MADaM syndrome, a novel severe progeroid mandibuloacral dysplasia syndrome linked to primary mitochondrial and secondary nuclear defects.

Annachiara De Sandre-Giovannoli

La Timone Children's Hospital, Marseille, France

In the last two decades, several nuclear envelope-linked premature aging disorders have been discovered and the knowledge about their pathophysiological basis has greatly progressed, allowing the development of the first therapeutic trials on some patients. Our team has pursued gene-identification search on patients presenting with atypical progeroid features evocative of a laminopathy with no mutation in known genes; by these means, recessive mutations were identified in a gene encoding a mitochondrial protein in patients affected with a novel and very severe mandibuloacral dysplasia progeroid syndrome.

Patients present with growth retardation, acro-osteolyses, skin atrophy, disseminated vascular calcifications with severe hypertension and renal glomerulosclerosis. They are all issued from consanguineous unions and carry different homozygous null mutations in the gene. Functional studies on fibroblast cell lines showed absence of the encoded protein as well as secondary absence of one of its partners, altered mitochondrial network and respiratory chain composition. The patients' cell lines showed increased mitophagy, increased senescence and reduced proliferation capacities. Surprisingly, secondary nuclear morphological defects including blebbing and herniations with altered Lamin /AC staining could be observed, possibly establishing a pathophysiological link among this syndrome and known mandibuloacral dysplasia/progeroid syndromes due to *LMNA, ZMPSTE24* or *BANF1* mutations.

Progress, Lessons and the Future of Clinical Trials for Hutchinson-Gilford Progeria Syndrome

Leslie B. Gordon

The Progeria Research Foundation, Hasbro Children's Hospital and Alpert Medical School of Brown U.; Boston Children's Hospital and Harvard University

Hutchinson-Gilford progeria syndrome (HGPS) is an ultra- rare, fatal, autosomal dominant premature aging disease caused by progerin, an abnormal form of lamin A. Its prevalence is 1 in 20 million living individuals, with 123 living children and young adults currently identified through The Progeria Research Foundation (PRF) International Registry and an estimated 350-400 total population worldwide.

The first clinical treatment trial for children with HGPS was initiated in 2007, just 4 years after the discovery of its causal mutation. Since that time, The Progeria Research Foundation and Boston Children's Hospital (USA) have conducted a total of 4 serial trials, one continuing upon the next. Each trial has been based on our ever-growing understanding of the basic biology of lamins and progerin, the disease-causing protein. Many invaluable lessons have emerged from the 12 years of clinical trials for this ultra-rare disease. Twenty-one peer-reviewed publications have emerged from these trials, which help to inform the greater scientific community and foster forward progress towards new treatments and the cure.

Embedded within the trials are natural history studies that define the disease in tangible, quantitatively measurable ways. These help clinicians to understand the biology behind clinical disease, and how to best care for the children. They also give the field a foothold on new disease-relevant outcome measures that can be used in future treatment trials to better understand whether a trial medication is effective. Finally, they provide a deeper understanding of the commonalities and differences between HGPS and aging in the general population, so that each field of study can inform the other.

Trial outcomes with the farnesyltransferase inhibitor lonafarnib demonstrate some disease features that have been reversed towards health, and others that have been more difficult to influence. Most importantly, we have learned that children with HGPS can benefit from treatment at all ages and disease stages.

This presentation will discuss how we will springboard towards new treatments and ultimately the cure for HGPS using lessons provided by both natural history studies and trial outcomes.

Unexpected effects of interleukin 6 inhibition in progeroid cells

Stefano Squarzoni[1,2], Elisa Schena[1,2], Cristina Capanni[1,2], Vittoria Cenni[1,2], Elisabetta Mattioli[1,2], Catia Barboni[3], Anna Zaghini[3], Giuseppe Sarli[3], Maria Rosaria D'Apice[4], Patrizia Sabatelli[1,2], Davide Andrenacci[1,2], Giovanna Lattanzi[1,2].

[1]CNR Institute of Molecular Gentics "Luigi Luca Cavalli-Sforza", Bologna, Italy

[2]IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy

[3]Department of Veterinary Medicine, University of Bologna, Bologna, Italy

[4]Policlinico Tor Vergata, Laboratory of Medical Genetics, Rome, Italy

Interleukin 6 (IL6) is a circulating molecule mainly acting as an inflammatory cytokine. Accumulation of damaged DNA as well as occurrence of cytosolic DNA trigger an IL6-dependent inflammatory response. Since DNA damage repair is hindered in progeroid cells carrying the G608G *LMNA* mutation, we investigated IL6 effects in cells from Hutchinson-Gilford progeria patients and progeroid *Lmna*$^{G609G/G609G}$ mice.

Here, we show that inhibition of IL6 activity by a neutralizing antibody raised against the human IL6 soluble receptor, reduces DNA damage and cellular senescence in HGPS fibroblasts. Unexpectedly, we could further demonstrate that IL6 neutralization lowers progerin mRNA and protein level both in vitro and in vivo in *Lmna*$^{G609G/G609G}$ progeroid mice.

These data suggest a cross-talk between inflammation and progerin expression, which might be relevant for Hutchinson-Gilford progeria pathogenesis and therapy as well as for ageing-related pathways.

Molecular and cellular mechanisms driving cardiovascular disease in Hutchinson-Gilford progeria syndrome

Vicente Andrés

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, and CIBER de Enfermedades Cardiovasculares (CIBER-CV), Spain

Our laboratory investigates the mechanisms of cardiovascular disease (CVD) and premature aging induced by the expression of prelamin A and progerin. Using LmnaG609G knockin mice (ubiquituous progerin expression), we have identified molecular and cellular mechanisms that contribute to CVD in progeria, including excessive vascular calcification, vessel stiffening, atherosclerotic disease, and cardiac repolarization defects. We recently demonstrated that restricting progerin expression to vascular smooth muscle cells (VSMC) promotes endoplasmic reticulum stress/unfolded protein response (ER stress/UPR) and is sufficient to accelerate atherosclerosis, trigger atherosclerotic plaque vulnerability, and reduce lifespan, thus identifying progerin-induced VSMC death as a major contributor to HGPS. This ER stress/UPR pathway was also activated in cells derived from HGPS patients. Targeting the ER stress/UPR with the chemical chaperone tauroursodeoxycholic acid (TUDCA) inhibited medial VSMCs loss and atherosclerosis progression in mouse models with both ubiquitous and VSMC-specific progerin expression, and extended lifespan in the VSMC-specific model. Our results identify a mechanism underlying atherosclerosis that could be targeted in HGPS patients.

Our findings may also provide insight into ageing-related vascular damage caused by the accumulation of unprocessed toxic forms of lamin A.

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Prelamin A disrupts nuclear F-actin spatial organisation and prevents its association with sites of DNA repair

Andrew Martin Cobb and Catherine M. Shanahan

School of Cardiovascular Medicine and Sciences, King's College London, London, UK

Eukaryotic nuclei depend upon a highly ordered nucleoskeleton that begins at the nuclear envelope (NE) and spans the entire nucleoplasmic space. This framework is necessary to maintain nuclear shape and to govern the myriad of events that take place within the nucleus. Nuclear lamins are key components of this structure and their deregulation results in genomic instability and disease. During ageing, non-mature precursors of lamin A (prelamin A) can accumulate and interfere with nuclear structure, chromosomal organisation, gene expression and DNA repair. Another important structural element of the nucleus is nuclear actin which is involved in a range of processes akin to those influenced by lamins but the precise roles of actin within the nucleus remain poorly understood. It has been established that lamins interact with nuclear actin in vitro but little is known about actins dependence on a proper functioning lamina or how the accumulation of prelamin A might influence actin dynamics. Our pilot data shows that prelamin A expression dramatically alters filamentous nuclear actin architecture and that its association with PML and sites of DNA repair are diminished. These findings highlight the important interplay between different components of the nucleoskeleton and demonstrates a novel mechanism of prelamin A toxicity.

Structural defects at the nuclear periphery associated with envelopathies

Sophie Zinn-Justin

Institute of Integrative Biology of the Cell, CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

At the interface between the nuclear membrane and chromatin, the inner nuclear envelope contains both nucleoskeleton filaments (lamins) andtransmembraneproteins (NETs). Lamins tether heterochromatin to the nuclear envelope and modulate chromosome territory positions. Tissue specific expression of NETs also influencesgenome organization. Phosphorylation regulates localization and interactions of the nuclear envelope proteins during cell cycle and after a mechanical stress. We purified the complex formed by lamin A/C, the NET protein emerin and the chromatin binding protein BAF. We solved its 3D crystal structure, and we identified a new interface between lamin A/C and BAF that is disrupted in autosomal recessive progeroid syndromes[1]. We analysed the impact of cell cycle-dependent BAF phosphorylation by the kinase VRK1 on BAF structure and interaction with lamin and emerin, in order to identify how cell cycle regulates these interactions. We also showed that mutations in emerin LEM domain associated to atrial cardiac defects do not impair BAF binding, despite their localization at the interface with BAF, and we revealed a role for this domain in the cell response to a mechanical stress[2,3]. Finally, we characterized how mutations in emerin disordered region associated with muscular dystrophy modify emerin structure, self-assembly, and capacity to be phosphorylated by Src during a mechanical stress[4].

(1) Samson et al., Nucleic Acids Res. **46**,10460-10473 (2018).(2) Samson et al., FEBS J.**284**,338-352 (2017). (3) Essawy et al., Cells**8**,570-588 (2019). (4) Herrada et al., ACS Chem Biol. **10**,2733-2742 (2015).

Vulnerability of Progeroid Smooth Muscle Cells to biomechanical forces is mediated by an enzyme

Patricia R. Pitrez[1,2], Luís Estronca[1,2], Guillem Colell[3], Helena Vazão[1], Deolinda Santinha[1], Karim Harhouri4, Daniel Thornton[5], Claire Navarro[6], Anna-Laure Egesipe[7], Tânia Carvalho[8], Alessandro Ori[9], Nicolas Lévy[4], João Pedro Magalhães[1,5], Annachiara De Sandre-Giovannoli[4], Xavier Nissan[7], Anna Rosell[3], Lino Ferreira[1,2]*

1Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
2Faculty of Medicine, University of Coimbra, 3000-548, Coimbra, Portugal
3Neurovascular Research Laboratory, Neurorepair Group. Vall d'Hebron Research institute Passeig Vall d'Hebron 119-129, 08035, Barcelona, Spain
4Molecular Genetics Laboratory, Department of Medical Genetics, La Timone Children's hospital, Marseille, France
5Integrative Genomics of Ageing Group, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L7 8TX, United Kingdom
6Aix Marseille Univ, INSERM, GMGF, Marseille, France
7CECS, I-STEM, AFM, Institute for Stem Cell Therapy and Exploration of Monogenic diseases, Evry cedex, France
8IMM, Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal
9Leibniz Institute on Aging-Fritz Lipmann Institute, 07745 Jena, Germany

Hutchinson-Gilford Progeria syndrome (Progeria), a devastating premature aging disease that leads to early death. Smooth muscle cells (SMCs) are the most affected cells in Progeria patients, although the reason for such sensitivity remains poorly understood. We developed an *in vitro* mono-culture cell system to study the vulnerability of Progeria SMCs to arterial flow shear stress using a microfluidic device. Progeria SMCs derived from iPSCs could recapitulate the most important aspect of the disease, i.e., Progeria SMCs loss under flow shear stress. Microarray analysis comparing Progeria SMCs cultured in static conditions and under slow conditions reveals that Progeria SMCs have significant changes in extracellular matrix secretion, specifically an enzyme. Moreover, Progeria SMC detachment is prevented by the inhibition of this enzyme. Finally, double mutant LmnaG609G/G609GMmp13-/- mice or LmnaG609G/G609GMmp13+/+ mice treated with a MMP inhibitor showed lower SMC loss in the aortic arch than controls. Our results offer a new platform for developing treatments for HGPS patients that may complement previous pre-clinical and clinical treatments. To the best of our knowledge, this is the first study documenting part of the mechanism underlining the sensitivity of HGPS SMCs to arterial flow shear stress.

Shaping of nuclear structure during mitosis impacts stem cell survival

Tingting Duan, Rebecca Cupp and Pamela K. Geyer

University of Iowa, Department of Biochemistry, Iowa City, IA, USA

Dysfunction of nuclear lamina (NL) proteins cause age-enhanced human diseases, including premature aging syndromes. In these diseases, compromised tissue regeneration and impaired adult stem cell health are associated with progressive abnormalities of nuclear shape and heterochromatin coalescence. To understand how the NL impacts stem cell function, we focused on two Drosophila NL proteins, the LEM domain (LEM-D) protein Otefin and its partner **B**arrier-to-**A**utointegration **F**actor (BAF). Loss of either protein alters nuclear architecture in female germline stem cells (GSCs), defects that lead to GSC death. As LEM-D proteins and BAF are required for NL reformation following mitosis, we investigated how mitosis shapes GSC nuclear structure. Surprisingly, we found that wild type GSCs undergo an atypical mitosis, wherein lamin and Otefin do not disseminate and instead form a network that embeds the centrosome and surrounds mitotic spindles. This mitotic nuclear lamina differs from the interphase lamina, as several NL components are dispersed. Strikingly, this atypical mitosis is observed only in adult GSCs and not in progenitor germ cells, indicating that the developmental swith from symmetric to asymmetric cell division is accompanied by a change in the mechanism of mitosis. Retention of Otefin in the mitotic NL is essential for subsequent formation of the interphase lamina. Indeed, *otefin* mutant GSC interphase nuclei have a distorted and lobulated NL with coalesced heterochromatin, structural defects that trigger GSC death. These findings reveal new requirements for NL proteins in stem cell division and survival.

A Multistage Sequencing Strategy Pinpoints Novel Candidate Alleles for Emery-Dreifuss Muscular Dystrophy and Supports Gene Misregulation as its Pathomechanism

Peter Meinke[1,2], Alastair R. W. Kerr[1], Rafal Czapiewski[1], Jose I. de las Heras[1], Elizabeth Harris[3], Heike Kölbel[4], Francesco Muntoni[5], Ulrike Schara[4], Volker Straub[3], Benedikt Schoser[2], Manfred Wehnert[6], and Eric C. Schirmer[1]

[1]Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK; [2]Friedrich Baur Institute at the Department of Neurology, University Hospital, LMU Munich, Germany; [3]John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; [4]Department of Pediatric Neurology, Developmental Neurology and Social Pediatrics, University of Essen, Germany; [5]Dubowitz Neuromuscular Centre, University College London Great Ormond Street Institute of Child Health, London, UK; and 1 NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, London, UK; [6]Institute of Human Genetics, University of Greifswald (retired), Greifswald, Germany

Limitations of genome-wide approaches for genetically-heterogenous orphan diseases led us to develop a new approach to identify novel Emery-Dreifuss muscular dystrophy (EDMD) candidate genes.
We generated a primer library to genes (I) linked to EDMD, (II) mutated in related muscular dystrophies, (III) candidates from limited exome sequencing, (IV) encoding muscle-specific nuclear membrane proteins.
Sequencing 56 unlinked EDMD patients yielded confirmed or strong candidate alleles from all categories, accounting for most remaining unlinked patients. Known functions of newly-linked genes argue the EDMD pathomechanism is from altered gene regulation and mechanotransduction through connectivity of candidates from the nuclear envelope to the plasma membrane.

Congenital laminopathies: what have clinicians learnt from 2008

Susana Quijano-Roy

Assistance Publique des Hôpitaux de Paris, Paris, France

L-CMD was described one decade ago in a number of children with de novo mutation presenting with particular clinical features, either arrest of motor milestones or a recognisable dropped head syndrome. In contrast to EDMD phenotype, heart complications were not frequent, although one child had a sudden death. Ten years later, review of the initial series reveals that most patients have died due to cardiac arrythmias or dilated cardiomyopathy. Progressive respiratory restrictive insufficiency is also a severe complication, leading to death in the early severe children whenever no ventilatory support is offered. Most descriptions remain in children with a number of known de novo mutations but recessive or dominant mutations have been occasionally identified.
Clinically, a continuous spectrum of severity is observed with atypical cases (late onset but very progressive course; early onset with slowly progressive course). The investigator will present an update of the 2008 series and discuss the most striking findings with impact in the natural history of this devastating disease.

Impairments in contractility and cytoskeletal organisation cause nuclear defects in skeletal muscle contractile disorders

Jacob A Ross, Yotam Levy, Michela Ripolone, Justin S Kolb, Mark Turmaine, Mark Holt, Johan Lindqvist, Maurizio Moggio, Nicolas Figeac, Peter S Zammit, Heinz Jungbluth, Chiara Fiorillo, John Vissing, Nanna Witting, Henk Granzier, Edmar Zanoteli, Edna C Hardeman, Carina Wallgren-Pettersson, Julien Ochala

Julien Ochala: Centre for Human & Applied Physiological Sciences, School of Basic & Medical Biosciences, Guy's Campus, King's College London, SE1 1UL, UK; Randall Centre for Cell and Molecular Biophysics, School of Basic & Medical Biosciences, Guy's Campus, King's College London, SE1 1UL, UK.

Introduction: Nemaline myopathy is a skeletal muscle disorder caused by mutations in genes that are generally involved in muscle contraction, in particular those related to the structure and/or regulation of the sarcomeric thin filament. Many pathogenic aspects of this disease remain largely unclear.

Results: We report novel pathological defects in skeletal muscle fibres of mouse models and patients with nemaline myopathy: irregular spacing and morphology of nuclei; disrupted nuclear envelope; altered chromatin arrangement; and disorganisation of the cortical cytoskeleton. Impairments in contractility are the primary cause of these nuclear defects. We also highlight the role of the microtubule network in determining nuclear morphology, a phenomenon which is likely to contribute to nuclear alterations in this disease. Our results suggest some pathological overlap with primary laminopathies and/or diseases affecting cytoskeletal proteins.

Nuclear envelope abnormalities in myotonic dystrophy primary myoblasts

Peter Meinke, Stefan Hintze, Sarah Limmer, Benedikt Schoser

Friedrich-Baur-Institute at the Department of Neurology, University Hospital, Ludwig-Maximilians-University Munich, Munich, Germany

Myotonic dystrophies (DM) are slowly progressing multisystemic diseases with a predominant muscular dystrophy - making DM the most frequent muscular dystrophy in adulthood. DM is caused by heterozygous DNA-repeat expansions in the *DMPK* gene (DM1) or the *CNBP* gene (DM2). The repeat-containing RNA accumulates in ribonuclear foci and splicing factors are sequestered to these foci, resulting in abnormal regulation of alternative splicing.
In primary patient myoblast cell cultures we observed invaginations of the nuclear envelope (NE). These abnormalities correlate in DM1 with repeat length – which in turn correlates with disease severity. Searching for the reason of theses abnormalities we screened proteins of the nuclear lamina and NE transmembrane proteins (NETs). This revealed down-regulation of the lamins A and B1 as well as different isoforms of the NET nesprin 1.
Lamins and nesprins are having functions in the mechanical stability of the nucleus; therefore their mis-regulation is likely directly involved in the observed nuclear abnormalities. This implies possible shared pathomechanism between DM1 and nuclear envelope linked disease.

Nesprin-1-alpha2 associates with kinesin at myotube outer nuclear membranes, but is restricted to neuromuscular junction nuclei in adult muscle

Ian Holt, Heidi R. Fuller, Le Thanh Lam, Caroline A. Sewry, Sally L. Shirran, Qiuping Zhang, Catherine M. Shanahan and Glenn E. Morris

Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, UK (IH, HRF, LTL, CAS, GEM); Institute for Science and Technology in Medicine, Keele University, Keele, UK (IH, HRF, GEM); BSRC Mass Spectrometry and Proteomics Facility; University of St Andrews, St Andrews, Fife, UK (SLS); Cardiovascular Division, James Black Centre, King's College, London, UK (QZ, CMS).

Nesprins, nuclear envelope spectrin-repeat proteins encoded by the SYNE1 and SYNE2 genes, are involved in localization of nuclei. The movement and positioning of nuclei are essential steps in muscle development. The short isoform, nesprin-1-alpha2, is expressed almost exclusively in skeletal muscle and heart and is required for relocation of the microtubule organizer function from centromeres to the nuclear rim during myogenesis. Nesprin-1-alpha2 has an unstructured and highly conserved 18 amino acid sequence called the STAR domain, containing the LEWD motif which is known to bind kinesin. We have used proteomics and specific antibodies to gain further insight into the interactions of nesprin-1-alpha2.

Using recombinant preparations of nesprin-1-alpha2 (both with and without the STAR domain) as "bait" proteins, we performed "pull-down" experiments from extract of cultured myotubes, which express nesprin-1-alpha2, followed by mass spectrometry analysis.

The proteomics study revealed that kinesin heavy and light chains were the only significant proteins in myotube extracts pulled down by nesprin-1-alpha2, but not by the mutant lacking the STAR domain. The proteomic study was confirmed by western blot. Using specific antibodies, we show that nesprin-1-alpha2 and nesprin-1-giant co-localize with kinesin at the junctions of concatenated nuclei and at the outer poles of nuclear chains in human skeletal myotubes. In adult muscle, nesprin-1-alpha2 was found only on nuclei associated with neuromuscular junctions, whereas all adult cardiomyocyte nuclei expressed nesprin-1-alpha2.

The results support a function for nesprin-1-alpha2 at the outer nuclear membrane in the kinesin-mediated localization of skeletal muscle nuclei.

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Mechanically-induced nuclear damage and increased p53 signaling lead to myofiber dysfunction in skeletal muscle laminopathies

Tyler J. Kirby[1]*, Ashley J. Earle[1]*, Gregory R. Fedorchak[1]*, Philipp Isermann[1], Jineet Patel[1], Sushruta Iruvanti[1], Steven A. Moore[2], Gisèle Bonne[3], Lori L. Wallrath[4], Jan Lammerding[1]

1 Meinig School of Biomedical Engineering & Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA
2 Department of Pathology, Carver College of Medicine, University of Iowa, Iowa City, IA USA
3 Sorbonne Université, Inserm UMRS 974, Center of Research in Myology, Association Institute of Myology, Paris, France
4 Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA, USA
* These authors contributed equally

Mutations in the human LMNA gene, which encodes the nuclear envelope proteins lamins A and C, cause muscular dystrophy and other diseases collectively known as laminopathies. The molecular mechanisms responsible for these diseases remain incompletely understood, but the muscle-specific defects suggest that mutations may render nuclei more susceptible to mechanical stress. Using three mouse models of muscle laminopathies, we found that Lmna mutations caused extensive nuclear envelope damage, consisting of chromatin protrusions and transient rupture of the nuclear envelope, in skeletal muscle cells in vitro and in vivo. The nuclear envelope damage was associated with progressive DNA damage, activation of DNA damage response pathways, and reduced viability. Deletion of the key DNA damage response protein p53 in Lmna KO myofibers rescued cell viability, indicating that p53 is required for the cell death process. Intriguingly, in maturing skeletal muscle cells, nuclear envelope damage and DNA damage resulted from nuclear movement rather than actomyosin contractility, which were reversed by Kif5b depletion or LINC complex disruption. LINC complex disruption rescued myofiber function and viability, indicating that the myofiber dysfunction is the result of mechanically induced nuclear envelope damage. The extent of nuclear envelope damage and DNA damage in the different Lmna mouse models strongly correlated with the disease onset and severity in vivo, suggesting a crucial role of DNA damage in disease pathogenesis. Corroborating the mouse model data, muscle biopsies from patients with LMNA associated muscular dystrophy similarly revealed significant DNA damage compared to age-matched controls, particularly in severe cases of the disease. Taken together, these findings point to a new and important role of DNA damage as a pathogenic contributor for these skeletal muscle diseases.

Modelling skeletal muscle laminopathies using human IPS Cells and bio-engineered skeletal muscles

Luca Pinton [1,2]*, Heather Steele-Stallard[1,2]*, Daniel Moore [1], Shilpita Sarcar[1], Tanel Ozdemir [1,2], Sara M. Maffioletti [1], Jean-Marie Cuisset[3], Gisèle Bonne[4], Peter S. Zammit[2]** and Francesco Saverio Tedesco [1,5]**

(1) Department of Cell and Developmental Biology, University College London, WC1E 6DE London, UK; (2) Randall Centre for Cell and Molecular Biophysics, King's College London, SE1 1UL London, UK; (3) Centre de Référence des maladies neuromusculaires Nord/Est/Ile de France, Service de Neuropédiatrie, Hôpital Roger Salengro, CHRU Lille, Lille, France; (4) Sorbonne Université, INSERM UMRS_974, Centre de Recherche en Myologie, Institut de Myologie, G.H. Pitié-Salpêtrière, Paris, France; (5) Dubowitz Neuromuscular Centre, Great Ormond Street Institute of Child Health, University College London.

(*) Equally contributing authors; (**) Correspondence: P.S.Z. (peter.zammit@kcl.ac.uk) & F.S.T. (f.s.tedesco@ucl.ac.uk)

Laminopathies are severe heterogeneous genetic diseases caused by mutations in A-type lamins encoded by the LMNA gene. These proteins together with Lamin B1 and B2 form the nuclear lamina: a mesh-like structure located underneath the nuclear membrane which helps maintaining nuclear shape and regulating gene expression. Laminopathies affect multiple cell types and can be tissue-specific or systemic, with some subtypes affecting striated muscle. Although different mechanisms have been proposed, the precise pathophysiology of laminopathies remains unknown; additionally, their rarity and lack of easily accessible cell types for ex vivo studies negatively impact on therapy development. To bypass these hurdles, here we used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies such as LMNA-related congenital muscular dystrophy and limb-girdle muscular dystrophy 1B, to model disease-associated phenotypes in vitro.

iPS cells from three patients were differentiated into skeletal myogenic cells and myotubes. Characteristic cellular phenotypes were observed in all LMNA-mutant iPS cell lines, including nuclear shape abnormalities and mislocalisation of nuclear lamina proteins. Notably, modelling in three-dimensional (3D) artificial muscle constructs resulted in recapitulation of nuclear shape abnormalities with higher fidelity than in standard monolayer cultures and identified nuclear length as a mutation-specific reproducible phenotypic readout. Finally, we will present and discuss current efforts and future applications of this novel 3D platform for therapy development in muscle laminopathies and in other severe muscle disorders. These results demonstrate that patient-specific iPS cells can model cellular phenotypic readouts of skeletal muscle laminopathies with high fidelity upon 3D differentiation in vitro.

Defective physical coupling between the nucleus and cytoskeleton in Emery-Dreifuss muscular dystrophy

Greg R. Fedorchak [1,2], Nicholas M. Shaw [3], Margaret R. Ketterer [3], Gary H. Coombs [4], Jan Lammerding [1,2] and Lori L. Wallrath [4]

[1] Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA; [2] Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA; [3] Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA, USA; [4] Biology Department, Waldorf University, Forrest City, IA, USA

The nuclear envelope (NE) compartmentalizes the cell and defines the structure of the nucleus. In muscle cells, the NE also serves as a microtubule organizing center (MTOC). Astral microtubules surround the muscle nuclei, protecting them from mechanical forces. Force transmission through the NE is facilitated by the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, which spans the inner and outer nuclear membrane. The goal of our study was to determine the impact of muscle disease-causing mutations on the physical connection between the cytoskeleton and the nucleus. We focused on mutations in the LMNA and SYNE1/2 genes associated with Emery-Dreifuss muscular dystrophy (EDMD). We used Drosophila models of EDMD and a novel microharpoon assay to address this issue. This assay involves live imaging during unidirectional force application by a fine microneedle inserted into muscle cells at a fixed distance from the nuclear periphery. Measurements of nuclear shape and positioning before and after force application provide information on nuclear deformation and the physical coupling between the nucleus and the cytoskeleton. A subset of LMNA mutations increased nuclear deformation under force application, yet left nucleo-cytoskeletal coupling intact. In contrast, other LMNA mutations, as well as RNAi knock-down of LINC complex components, retained nuclear stability, yet uncoupled the nucleus from the cytoskeleton. These findings suggest that changes in nuclear envelope protein composition compromise the ability of the nucleus to serve as a MTOC. Lack of this function impacts muscle cell differentiation and nuclear stability, providing an explanation for muscle defects associated with EDMD.

LINC-ing the nucleus and the cytoskeleton

Patricia Davidson

Laboratoire Physicochimie Curie, UMR 168 Institut Curie-Section de Recherche, Paris, France

Mammalian cells migrate through narrow pores in tissues to perform essential duties, including wide-scale migration throughout development, immune cell migration to sites of inflammation and fibroblast migration to repair wounds. The mechanisms by which cells exert forces on their nuclei to migrate through openings smaller than the nuclear diameter remain unclear. In microfluidic devices, the hourglass shape of the nucleus and its strain patterns as it translocates through narrow constrictions suggest forward pulling forces. Here we use CRISPR/Cas9 to label nesprin-2 giant, a protein that links the cytoskeleton to the interior of the nucleus. We demonstrate that nesprin-2 giant accumulates at the front of the nucleus during nuclear deformation through narrow constrictions, independently of the nuclear lamina. We show that nesprins are more mobile than lamin A/C, and hypothesize that the accumulation is due to nesprins that are pulled forward by the cytoskeleton. Using artificial constructs, we show that the actin-binding domain of nesprin-2 is necessary and sufficient to generate this accumulation, and that microtubules are not involved. We demonstrate a barrel shape of filamentous actin around the nucleus, which colocalizes with nesprin-2, but strikingly no actin at the front surface of the nucleus. Our working hypothesis is that this barrel structure pulls the nucleus forward via nesprin-2 and is responsible for pulling the nucleus forward in these fibroblasts. These results indicate important roles for nuclear envelope proteins in force transmission during cell migration through tissues.

Response to mechanical demand: from nucleus to matrix

Joe Swift

Wellcome Centre for Cell-Matrix Research, Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, M13 9PL, UK.

Tissues are maintained by homeostatic feedback mechanisms in which cells can respond to, but also modify, the chemical and physical properties of their surroundings. These mechanisms can be misregulated in ageing, contributing to pathology. Mesenchymal stem cells (MSCs) exhibit distinct phenotypes when cultured on hydrogels with different mechanical properties. Morphological changes are known to propagate into transcriptional regulation, influencing cell behaviour and lineage specification. We have also shown that cells can utilise a number of defensive mechanisms, such as reorganization of chromatin or expression of molecular chaperone proteins, to give protection against extremes of mechanical loading. Replication-induced senescence – used as a model of ageing – can modulate how MSCs respond to substrate mechanics; extracellular matrix (ECM) secretion is also altered and differentiation potential dampened. We compared the behaviour of low passage and senescent MSCs cultured on soft and stiff substrates, and also cells under mechanical strain, using RNA-Seq to characterize changes to the transcriptome and quantitative label-free mass spectrometry (MS) to analyse intracellular and secreted proteomes. Our results, combined with an analysis of cell morphologies, suggested a central role for the linker of nucleo- and cytoskeleton (LINC) complex in coupling cell behaviour and extracellular physical stimuli. We found that effective regulation of LINC complex components was necessary for the cellular response to extreme mechanical loading – effectively decoupling the nucleus from strain – but that the same complex was broadly disrupted by senescence. This work offers insight into potential pathways by which tissue homeostasis may be abrogated in ageing.

LINC complex disruption in epithelia increases cell malleability, and cohesive cell migration in space-restrictive 3D environments

James Carthew, Katherine O'Mahony, Stefan Przyborski, Pablo Cubillas, Annette Meeson, Martin Goldberg, and Iakowos Karakesisoglou

Department of Biosciences, Durham University, Durham, UK

Cell migration requires interplay and synergy of multiple cellular compartments. Initially extracellular cues instruct plasma membrane receptors to transmit forces via the cytoskeleton to the nuclear interior through the linker of the nucleoskeleton and cytoskeleton (LINC) complex. LINC re-structures and re-positions organelles, including the nucleus, allowing directed cell motility. Despite LINC being modulated in metastatic cancers its roles in epithelial invasiveness are unknown. We show that LINC disruption compromises organelle positioning and reduces motility in non-restrictive 2D and 3D growth environments. However, in confined 3D milieus LINC disruption enhances collective cell migration, which we attribute to the decreased nuclear stiffness and pronounced cell-cell adhesions of the LINC mutants.

Our findings suggest that reconfigured nucleo-cytoskeletal coupling mechanisms operate upon LINC complex disruption, which increase nuclear malleability.Together, these results suggest that LINC deregulation in cancers may facilitate metastasis, as greater nuclear plasticity and enhanced multi-cellular migration facilitate infiltration into space-restrictive microenvironments, thus increasing the likelihood for secondary tumour formation.

Loss of cytoplasmic keratins in colonocytes downregulates nuclear lamins and compromises nuclear lamina integrity and cell cycle regulation

Joel H. Nyström, Carl-Gustaf A. Stenvall, Ciaran Butler Hallissey, Karen M. Ridge, Robert D. Goldman and Diana M. Toivola

Cell Biology, Biosciences, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland; Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA and Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; Turku Center for Disease Modeling, Turku, Finland

Keratin (K) intermediate filaments (IFs) provide epithelial cells with mechanical stability, scaffolding and protection against stress. Keratin mutations have been linked to over 60 human diseases, e.g. in skin, liver and possibly intestine (inflammatory bowel disease; IBD). K8 knockout mice (K8–/–) develop colonic epithelial hyperproliferation, diarrhoea and colitis. Lamins, nuclear IF proteins, are major components of the nuclear lamina that provide nuclear stability and regulate gene expression and cell proliferation. Lamins are connected to the cytosolic IF network through linker of nucleoskeleton and cytoskeleton (LINC) complexes. In this study, we asked whether keratin loss in colonocytes affects lamin and LINC proteins. The results show significantly decreased lamin A/C and B1/2 protein levels in K8–/– colonocytes. The decrease appears tissue-specific, as other K8–/– epithelial tissues displayed unaltered lamin levels. Additionally, the colonic K8–/– lamin phenotype is microflora- and inflammation-independent, as neither antibiotic treatment of K8–/– mice, which ameliorates the colitis, or chemically-induced colitis affected colonocyte lamin levels. A keratin level-dependent decrease in lamin A levels was also seen in vitro, as treatment of human colorectal Caco-2 cells with K8/K18 siRNA decreased lamin A protein levels. The cytolinker plectin, several LINC proteins (nesprin-3, SUN1 and SUN2) and lamin-associated proteins (emerin, LAP2α and pRb) were downregulated in K8–/– mouse colonocytes. Furthermore, lamin A and SUN2 co-immunoprecipitated with K8/K18 in Caco-2 cell lysates. In conclusion, our results indicate a colonocyte-specific association between cytoplasmic keratins and nuclear lamins, and that keratins may be important for maintaining lamin levels and nuclear function in colonic epithelial cells.

Nesprin-1a2 mediates motor protein recruitment to the nuclear envelope to control myonuclear position

Alice Haworth, Caroline Shak, Sue Shackleton

University of Leicester, Molecular and Cell Biology, Leicester, United Kingdom

Myotubes are multinucleated cells that make up muscle fibres and are formed by the fusion of myoblasts in a process termed myogenesis. In healthy muscle tissue, in the initial stages of myogenesis, the multiple nuclei of the myotube spread out to be roughly equidistant from one another and then proceed to the cell periphery. Nuclear positioning that deviates from this is observed in muscle disorders such as Emery-Dreifuss muscular dystrophy. Nesprin-1a2 is a short isoform of the ubiquitously expressed Nesprin-1 and is specifically expressed at the nuclear envelope (NE) in muscle. Nesprin-1a2 recruits microtubule organising centre (MTOC) proteins, such as PCM1, resulting in nucleation of microtubules from the NE. It is also implicated in the recruitment of motor proteins, kinesin-1 and dynein/dynactin, to the NE, which assist nuclear repositioning by facilitating nuclear movement along the microtubules. We have investigated the requirements for motor protein recruitment to the NE. Using kinesin light chains 1/2 (KLC1/2), dynein light intermediate chain 1 (LIC1) and p150Gluedas markers of kinesin, dynein and dynactin, respectively, we found that nesprin-1a2 is required for the recruitment of all 3 components. Interestingly, PCM1 is also required for the anchoring of p150Gluedbut not KLC1/2. Together with further mapping data, this supports the involvement of a Nesprin-1a2-PCM1 complex in the recruitment of motor microtubule motor proteins to the NE to control myonuclear spreading in developing myotubes.

TorsinA is a multi-tool AAA+ protein within the nuclear envelope that acts through distinct functional assembly states

Kwang Ho Hur[1,2], Jared Hennen[2], Cosmo A. Saunders[1], Amy Schoenhofen[1], Joachim D. Mueller[2], and G.W. Gant Luxton[1]

1Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455
2Department of Physics and Astronomy, University of Minnesota, Minneapolis, MN 55455
The laminopathies are a rapidly expanding collection of human diseases caused by mu

Mutations in genes that encode nuclear envelope (NE) proteins. Mutations in the TOR1Agene, which encodes the NE-localized AAA+ ATPase torsinA, cause a spectrum of poorly understood neurological diseases including DYT1 dystonia and severe arthrogryposis. While torsinA function within the NE is required for proper lipid metabolism, nuclear-cytoskeletal coupling, and nuclear pore complex (NPC) biogenesis, the molecular mechanism(s) underlying how torsinA mediates these functions and their relationship to human TOR1Adisease pathogenesis remain unclear. Here, we determined the functional assembly state of torsinA within the NE of living cells using fluorescence fluctuation spectroscopy. Because AAA+ proteins typically function as ring-shaped homo-hexameric molecular chaperones that structurally remodel their protein substrates, we expected to observe homo-hexamers of torsinA within the NE. However, the in vivohomo-oligomerization of torsinA appeared to be limited to a homo-trimer unless its membrane-associating N-terminal domain (NTD) was deleted, resulting in the assembly of homo-oligomers that were larger than expected for a homo-hexamer. Conversely, torsinA homo-oligomerization was impaired when the NTD was converted into a transmembrane domain or when a previously described proteolytic cleavage event that selectively removes the NTD was inhibited. Interestingly, the proteolytic cleavage and homo-hexamerization of torsinA were required for nuclear-cytoskeletal coupling but not for NPC biogenesis. Our results suggest that torsinA is a mutli-tool AAA+ protein that acts via distinct functional assembly states. This information will enable future studies of the role of defective torsinA-dependent NPC biogenesis during TOR1Adisease pathogenesis.

SUN-KASH complexes undergo 6:6 back-to-back assembly to mediate force transduction across branched LINC complex networks

Manickam Gurusaran and Owen R. Davies

Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom.

The LINC complex traverses the nuclear envelope to provide nucleo-cytoskeletal linkages that fulfil a myriad of functions, including nuclear structure/shape/positioning, hearing, double-strand break repair and meiotic chromosome dynamics. It consists of a complex between SUN and KASH/Nesprin proteins, through their interaction within the peri-nuclear space. The canonical model is of linear 3:3 SUN-KASH structures that provide individual rope-like nucleo-cytoskeletal linkages; however, such structures would be highly susceptible to breakage by tension forces. Instead, LINC complexes may undergo higher-order assembly into highly-branched networks that could transmit high forces through their distribution between coordinated/parallel SUN-KASH complexes in which individual breakage would have little impact on the overall system. Here, we provide the first molecular evidence in favour of this. We have solved the X-ray crystal structures of the globular domain of SUN1 in complex with KASH1, KASH4 and KASH5, revealing 6:6 complexes in which back-to-back interfaces mediate branching between SUN1 trimers. The back-to-back interface differs subtly between KASH proteins: it is mediated by the KASH-lid in SUN1-KASH1, by zinc-coordination in SUN1-KASH4 and KASH-lid/KASH5 interactions in SUN1-KASH5. Importantly, SEC-MALS and SEC-SAXS confirm that the 6:6 complex is highly-stable and apparently constitutive in solution, and its disruption through mutagenesis leads to complete LINC complex dissociation. We conclude that 6:6 back-to-back interaction is essential for LINC complex assembly and provides branch-points to enable force transduction between adjacent SUN trimers. We propose that additional branching events occur along the SUN-KASH axis to generate higher-order arrays capable of high force transduction between nuclear and cytoplasmic structures.

Lamina-associated polypeptide 1 in striated muscle and liver function

Ji-Yeon Shin,[1,2] Antonio Hernandez-Ono,[1] Tatyana Fedotova,[1] Cecilia Östlund,[1,2] Yuexia Wang,[1,2] Kurenai Tanji,[2] Michael J. Lee,[2] Sarah B. Gibeley,[1] Chun-Chi Liang,[3] William T. Dauer,[3,4*] Henry N. Ginsberg[1] and Howard J. Worman[1,2]

[1]Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA.
[2]Department of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA.
[3]Department of Neurology, University of Michigan Medical School, Ann Arbor, MI, USA.
[4]Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, USA.

*Present address: Peter O'Donnell Jr. Brain Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Lamina-associated polypeptide 1 (LAP1) is an inner nuclear transmembrane protein that interacts with lamins and emerin in the nucleoplasm and with the AAA+ ATPase torsinA in the perinuclear space. We have used conditional gene knockout mice to examine how LAP1 deletion affects striated muscle and liver. Conditional deletion of LAP1 from striated muscle at embryonic day 17 caused muscular dystrophy and the mice had a median survival of 150 days. These mice had evidence of left ventricular systolic dysfunction at approximately 70 days of age. Skeletal muscle pathology was significantly worsened and lifespan significantly shortened in the absence emerin (mice with germline deletion of emerin alone have minimal striated muscle pathology). Conditional deletion of LAP1 from hepatocytes yielded mice that were grossly indistinguishable from littermate controls for up to two years of age. However, careful analysis of their livers showed that these mice had defective VLDL secretion and steatosis, including intranuclear lipid accumulation. Deletion of torsinA from mouse hepatocytes caused even greater reductions in VLDL secretion and profound steatosis, but without evidence of intranuclear lipid accumulation. Both of these mutant mouse lines developed hepatic steatosis and subsequent steatohepatitis on a regular chow diet in the absence of whole-body insulin resistance or obesity. Mice with germline deletion of emerin had no apparent liver abnormalities. Our results suggest that LAP1 functions together with emerin in maintaining normal striated muscle physiology. In liver, LAP1 activation of torsinA appears to be required for normal lipid metabolism.

Risk stratification for sudden death in laminopathies

Karim Wahbi[1-2], Rabah Ben Yaou[3-4], Caroline Stalens[2,5], Corinne Vigouroux[6-8], Gisèle Bonne[4], and the French OPALE observatory investigators

[1]APHP, Cochin Hospital, Cardiology Department, FILNEMUS, Centre de Référence de Pathologie Neuromusculaire Nord/Est/Ile de France, Paris-Descartes, Sorbonne Paris Cité University, 75006 Paris, France
[2]INSERM Unit 970, Paris Cardiovascular Research Centre (PARCC), Paris, France
[3]APHP, Centre de référence de pathologie neuromusculaire Paris-Est, FILNEMUS, Myology Institute, Neurology Department, Pitié-Salpêtrière University Hospital, Paris, France
[4]Sorbonne Universités, INSERM UMRS 974, CNRS, UMR-7215, Center for Research in Myology, Institut de Myologie, Pitié-Salpêtrière University Hospital, Paris, France
[5]Medical Affairs Department, AFM-Telethon, Evry, France
[6]Sorbonne University, Inserm U938, Saint-Antoine Research Centre, Institute of Cardiometabolism and Nutrition, Paris, France
[7]APHP, Saint-Antoine University Hospital, Department of Molecular Biology and Genetics, Paris, France
[8]APHP, Saint-Antoine University Hospital, National Reference Center for Rare Diseases of Insulin Secretion and Insulin Sensitivity, Department of Endocrinology, Diabetology and Reproductive Endocrinology, Paris, France

Arrhythmogenic dilated cardiomyopathy is the most frequent clinical manifestation of laminopathies, alone or in combination with Emery-Dreifuss or limb girdle muscular dystrophy, lipodystrophic syndromes or peripheral neuropathy. Compared with other causes of inherited adult-onset cardiomyopathies, LMNA mutations are associated with a high risk of sudden cardiac death from ventricular tachyarrhythmias.

Several approaches have been developed to predict sudden death and guide prophylactic implantations of cardiac defibrillators. The most recent guidelines of the American College of Cardiology/American Heart Association/Heart Rhythm Society and European Society of Cardiology recommended implantable cardioverter-defibrillator therapy in patients with LMNA mutations and ≥2 of the following risk factors: male sex, non-missense mutations, non-sustained ventricular tachycardia and a left ventricular ejection fraction <45%.

We will present a new approach that we have recently developed and published using a new prediction model to estimate the absolute 5-year risk of sudden death in patients with LMNA mutations and compare its contribution with current clinical practice guidelines.

Lessons from Lamin A mutants in cardiolaminopthies: from pathophysiology to molecular targets

Andrea Gerbino, R. De Zio, S. Milano, C. Forleo, S. Favale, G. Procino, M. Svelto and Monica Carmosino

A. Gerbino - University of Bari - Department of Biosciences - Biotechnology and Biopharmaceutics - Bari- Italy;
R. De Zio - University of Bari- Department of Biosciences, Biotechnology and Biopharmaceutics- Bari- Italy;
S. Milano - University of Bari- Department of Biosciences, Biotechnology and Biopharmaceutics- Bari- Italy;
C. Forleo - University of Bari- Cardiology Unit, Department of Emergency and Organ Transplantation- Bari- Italy
S. Favale - University of Bari- Cardiology Unit, Department of Emergency and Organ Transplantation- Bari- Italy
G. Procino - University of Bari- Department of Biosciences, Biotechnology and Biopharmaceutics- Bari- Italy
M. Svelto - University of Bari - Department of Biosciences, Biotechnology and Biopharmaceutics - Bari- Italy
Monica Carmosino - University of Basilicata - Department of Sciences - Potenza - Italy

Different mutations in the Lamin A/C gene (*Lmna*) have been associated, at cellular level, to a wide spectrum of cardiac pathogenic mechanisms converging on dysfunctional processes leading to either cell death, myocardial fibrosis or conduction defects. Thus, tuning the therapeutic strategies on the pathogenic mechanisms driven by Lmna mutations requires the strict functional characterization of every Lmna mutant. Therefore, we analyzed the physio-pathological outcome of three nonsense Lmna mutations in HL-1 cardiomyocytes, with a multidisciplinary approach ranging from the analysis of Ca2+ dynamics either at subcellular level or in functional cardiac syncytia as well as the study of the membrane electric properties by whole cell patch clamp. Lamin A R321X and D243Gfs∗4 mutants mislocalized within the endoplasmic reticulum (ER) of HL-1 cardiomyocytes. However, only R321X induced ER stress, impaired intracellular Ca2+ handling and increased apoptosis. Conversely, D243Gfs∗4-expressing cardiomyocytes showed reduced expression/functionality of the gap junction protein, connexin 43, and defective electrical coupling in cardiac functional syncytia. Of note, cardiomyocytes expressing the mutant Q517X showed irregular Lamin A intra-nuclear aggregates. Also, we found a significant reduction in the action potential (AP) frequency caused by an increased duration of both action potential duration and cell cycle, indicating a possible dysfunction of one of the voltage-gated channels involve in the AP genesis and propagation. Thus, we provided the proof of concept that clinically comparable cardiolaminopathies can develop under different pathogenic contexts whose cellular mechanisms are putative pharmacological targets for drugs already used in clinical practice, paving the way for patients-specific therapeutic approaches.

Using CRISPR/Cas technology to study the role of LMNA exon 4 in the development and treatment of laminopathies

Déborah Gómez[1,2], Carolina Epifano[1,2], Borja Vilaplana[1], Halin Bareke[1], Albert García[3], Javier Ramón[3], Ignacio Perez de Castro[1]

[1] Instituto de Salud Carlos III, Institute of Rare Diseases Research; Majadahonda, Spain; [2] Fundación Andrés Marcio, niños contra la laminopatía; Madrid, Spain; [3] Institute for Bioengineering of Catalonia; Barcelona, Spain

Laminopathies are causally associated with mutations on Lamin A (LMNA). To date, more than 400 mutations in LMNA have been reported in patients. These mutations are widely distributed throughout the entire gene and are associated with a wide range of phenotypes. Unfortunately, few is known about the mechanisms underlying the effect of the majority of these mutations. This is the case of more than 10 mutations that are located at exon 4. Using CRISPR/Cas technology we have generated a collection of exon4, LMNA mutants in C2C12 myoblasts. These cell models will allow us to explore (i) the role of exon 4 on LMNA function, (ii) the effect of deleting, partially or fully, exon 4, and (iii) the pathogenic mechanisms associated with R249W, one of the most frequent mutations found in this exon. We have characterized these clones by measuring their nuclear circularity, myogenic differentiation capacity in 2D and 3D conditions, DNA damage levels and ERK activity. Our results indicate that the accumulation of DNA damage seems to be characteristic of R249W and that the lack of exon 4 might be compatible with myogenic differentiation. If confirmed by further studies, our initial conclusions would support the use of exon4-skipping and DNA damage response enhancement as new therapeutic strategies for some laminopathies.

The nuclear-cytoskeleton connection and nuclear positioning during muscle formation

Gimpel P, Lee YL, Roman W, Burke B, Gomes ER, Bruno Cadot

Institut de Myologie, INSERM U974, Sorbonne Université, Paris, France; Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR), Singapore; Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Muscle cells are characterized by the presence of multiple nuclei evenly spaced under the plasma membrane. Whether this particular arrangement is required for muscle function is still under debate. Nonetheless, several muscular diseases are characterized by abnormal nuclear positioning, such as centronuclear myopathies, titinopathies and desminopathies or due to mutations of nuclear envelope proteins known to be involved in nuclear movement in other systems. We have, since several years, investigated the mechanisms controlling three of the four different and successive nuclear movements occurring during myofiber formation through live imaging. By screening the effect of different molecular motors deletion, we have identified several microtubule-associated motors implicated at different levels on nuclear movement and positioning. We have established the connection between the nucleus and the cytoskeleton to be decisive for proper nuclear positioning. In particular, Nesprin-1, a protein mutated in congenital muscular dystrophy anchored in the nuclear envelope, is required for the reorganization of the microtubule cytoskeleton during the differentiation of muscle cells and the subsequent nuclear movements. We are now investigating its impact on nuclear deformations and cytoskeleton stabilization. Our research using in vitro systems recapitulates in vivo observations and allow the study of the impact of mutations found in muscular diseases on nuclear positioning in muscle cells.

Epigenetic inhibition of SCN5A by K219T-Lamin A/C induces myocardial conduction defects in an iPSC-based human model of cardiac laminopathy

Silvia Crasto (1,2), Nicolò Salvarani (1,2), Michele Miragoli (3), Alessandro Bertero (4), Marianna Paulis (1,2), Paolo Kunderfranco (2), Simone Serio (2), Alberto Forni (5), Carla Lucarelli (5), Matteo Dal Ferro (6), Veronica Larcher (2), Gianfranco Sinagra (6), Paolo Vezzoni (1,2), Charles E Murry (4), Giuseppe Faggian (5), Gianluigi Condorelli (1,2,7) and Elisa Di Pasquale (1,2)

1)Institute of Genetic and Biomedical Research (IRGB), UOS of Milan, National Research Council of Italy; 2) Humanitas Research Hospital, Rozzano (Milan), Italy; 3) Department of Medicine and Surgery, University of Parma, Italy; 4) Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle (WA), USA; 5) Division of Cardiac Surgery, University of Verona, Italy; 6) Cardiovascular Department, "Ospedali Riuniti" and University of Trieste, Trieste, Italy; 7) Humanitas University, Rozzano (Milan), Italy

Mutations of the LMNA gene, encoding the nuclear proteins Lamin A/C, cause dilated cardiomyopathy and typically associate with conduction defects and arrhythmias. Lamin A/C are thought to regulate various nuclear activities, including chromatin organization and gene transcription. Here, we employed induced pluripotent stem cells (iPSCs) generated from patients carrying the heterozygous K219T mutation on LMNA to develop a model of the disease. Electrophysiological analyses revealed that, compared with healthy controls, cardiomyocytes (CMs) carrying the K219T mutation (K219T-CMs) possess altered action potential properties in association with a reduced peak sodium current and diminished conduction velocity. Biochemical studies showed significant downregulation of the sodium channel Nav1.5 in K219T-CMs, which was accompanied by increased binding of Lamin A/C to the promoter on SCN5A, the channel's gene; binding of the Polycomb repressive complex 2 (PRC2) protein SUZ12 and deposition of the related repressive histone mark H3K27me3 were also increased at the SCN5A locus, which in this setting was preferentially localized to the nuclear periphery. In support to this, we found that the mutation increases the affinity of Lamin A/C for PRC2, resulting in their augmented physical interaction and consequently in the persistence of a repressive environment on SCN5A. Correction of the mutation by CRISPR/Cas9-mediated gene editing resulted in the re-establishment of sodium current density and SCN5A gene expression. In conclusion, our results demonstrate that mutated Lamin A/C cooperates with PRC2 in downregulating SCN5A, leading to decreased sodium current density and slower conduction velocity. This mechanism may underlie the conduction abnormalities associated with LMNA-cardiomyopathy.

Establishing new role(s) for the nuclear envelope in the mammalian heart

N. Arcos1, J. Ross1, T. Mohun2, L. Gerace3, Matt J. Stroud1

[1]BHF Centre of Excellence, King's College London. London. SE5 9NU. UK.; [2]Francis Crick Institute. London. UK.; [3]Scripps Research Institute, La Jolla, CA. 92037. USA.

One of the least understood areas of cardiovascular disease is the role of the nuclear envelope (NE), which is due to a lack of relevant animal models that are able to recapitulate the condition. This is remarkable considering that mutations in NE-encoding genes are the second highest cause of familial dilated cardiomyopathy. NE components play structural and mechanotransduction roles, which are crucial for heart development, physiology and pathophysiology. One such NE protein that is an excellent candidate for disease modelling is NET 25. Mutations in NET 25 lead to sudden cardiac death and cardiomyopathy in humans. However, little is known regarding the underlying molecular pathways that drive disease pathogenesis.

Here, we show for the first time that NET 25 is essential for normal heart development. Specifically, removal of NET 25 in cardiomyocytes led to late embryonic lethality after E16.5. High Resolution Episcopic Microscopy revealed cKO hearts were severely underdeveloped prior to lethality, with abnormally thin walls. Furthermore, high levels of apoptotic cardiomyocytes were observed throughout development, likely accounting for fewer cells and thinner compact myocardium observed. Because the cardiomyocyte NE is physically coupled to the cytoskeleton, it is under constant mechanical stress, and therefore requires continual maintenance. Previous work highlighted NET 25's function in NE repair in cell lines, we therefore reasoned that NET 25 might play a similar role in cardiomyocytes. Indeed, NET 25-deficient cardiomyocytes displayed increased incidences of aberrant nuclear shape, blebbing and micronuclei

Our data fit the working hypothesis whereby increased hemodynamic load during heart development or stress augments the force exerted on the NE. In the case of NET 25 mutant cardiomyocytes, this reaches a critical point at which the NE is unable to undergo proper repair, leading to rupture, apoptosis, remodeling, and death.

Altered cytoskeleton in cardiac disease caused by nuclear A-type lamins gene mutations

Antoine Muchir

Sorbonne Université, UPMC Paris 06, INSERM UMRS974, Center of Research in Myology, F-75013 Paris

Cardiomyopathy is an anatomic and pathologic condition associated with muscle and electrical dysfunction of the heart, often leading to heart failure-related disability. Dilated cardiomyopathy, the most common form, is characterized by compromised cardiac contractility ultimately resulting in poor left ventricular function. Mutations in LMNA encoding nuclear A-type lamins cause LMNA cardiomyopathy. There is no comprehensive treatment for the progressive cardiac dilatation and loss of contractility in LMNA cardiomyopathy short of heart transplantation. In addition, the prominence of electrical conduction disturbances probably contributes to the aggressive nature of LMNA cardiomyopathy. More-definitive therapies await better mechanistic understandings of the molecular basis for these electrical conduction disturbances. Recent findings of LMNA cardiomyopathy have yielded promising results for a novel pharmacological therapy. We recently reported that the cytoskeleton network plays a role in the development of cardiomyopathy caused by LMNA mutations. A main unanswered question is how mutations in genes encoding nuclear envelope proteins lead to alteration of cytoskeleton only in specific tissues. Within the next several years, we will likely have more clues to answer this question and these answers will hopefully lead to new ways to treat or prevent LMNA cardiomyopathy.

Mechanisms of Prelamin A induced Cardiovascular Ageing

Catherine M. Shanahan

BHF Centre of Research Excellence, Cardiovascular Division, King's College London, London, UK

Prelamin A has been shown to accumulate in normal ageing, particularly in aged vascular smooth muscle cells (VSMCs) in vitro and in vivo, due, in part, to downregulation of Zmpste24, the enzyme responsible for the final processing step of prelamin A into mature lamin A. We are interested in understanding how prelamin A accumulation impacts on VSMC function and in particular how it may act to accelerate vascular ageing and associated pathologies.

Using a variety of animal models that lead to the accumulation of prelamin A in VSMCs we have shown that it can impact on survival, cardiovascular physiology and vessel wall pathology, in particular extracellular matrix (ECM) remodelling and vessel wall mineralization.

Using a variety of genomics, epigenetic and proteomics approaches we have begun to dissect the mechanisms involved in driving these age-associated pathologies in response to prelamin A. These include widespread loss of heterochromatin and changes in gene expression temporally associated with the induction of DNA damage. These processes ultimately lead to the selective activation of gene networks linked to inflammatory pathways. Using these approaches, we have identified candidate pathways that may be amenable to manipulation in order to protect, delay or alleviate aspects of cardiovascular ageing.

Exploring synthetic rescue pathways in premature ageing syndromes

Delphine Larrieu

Cambridge Institute for Medical Research, Cambridge University, United Kingdom

The premature-aging disease Hutchinson-Gilford Progeria Syndrome (HGPS), results from LMNA mutations, causing the expression of a toxic protein called progerin. Progerin accumulation in cells causes misshapen nuclei, disruption of chromatin organization and increased genomic instability, resulting in premature entry of cells into senescence. Most therapeutic approaches in HGPS have aimed at targeting progerin directly, to either reduce its expression levels in cells or to enhance its solubility, thereby reducing its toxicity.

For the past few years, our lab has explored a different approach called synthetic rescue, to identify new pathways that can rescue the abnormal phenotypes in premature ageing syndromes. The synthetic rescue concept relies on genetic interactions where the abnormal phenotype associated with mutation of one gene can be corrected by mutation or depletion of a second gene. Identifying such interactions can have important clinical implications through the recognition of disease modifiers, potential biomarkers and new therapeutic targets.

Through this approach, we identified a genetic interaction where knockout or chemical inhibition of the N-acetyltransferase 10 encoded by NAT10 results in rescue of many of the characteristic phenotypes in HGPS cells and mouse models independently of progerin, therefore suggesting this target has strong therapeutic potential.

We are now working on understanding the exact mechanisms behind this synthetic rescue, by characterising NAT10 functions further, in both normal and HGPS cells. In parallel, the lab has also set up an unbiased whole genome Crispr arrayed screening approach to discover new synthetic rescue pathways in premature ageing syndromes. Some of our latest data will be presented.

Lamin B1, a new factor controlling the recruitment of 53BP1 to DNA damage

Laure Etourneaud[1,a], Angela Moussa[1,a], Emilie Rass[1a], Diane Genet[1,a], Benoit Thézé[1], Simon Willaume[1], Caroline Chabance-Okumura[1], Jordane Dépagne[2], Xavier Veaute[2], Eléa Dizet[2], Didier Busso[2], Aurélia Barascu[1], Lamya Irbah[3], Thierry Kortulewski[3], Anna Campalans[4], Catherine Le Chalony[1], Brigitte Buendia[5], Ralph Scully[6], Gaëlle Pennarun[1] and Pascale Bertrand[1]*

1. Unit 1274 CEA-INSERM-Paris Diderot University-Paris Sud University, "DNA Repair and Ageing" Team, Institute of Cellular and Molecular Radiobiology (iRCM), François Jacob Institute, DRF, CEA, Fontenay-aux-Roses, France
2. Genetic Engineering and Expression Platform (CIGEX), iRCM, DRF, CEA, 18 route du Panorama, Fontenay-aux-Roses, France
3. Imaging platform, iRCM, DRF, CEA, 18 route du Panorama, Fontenay-aux-Roses, France
4. Unit 1274 CEA- Paris Diderot University-Paris Sud University, "Genetic Instability Research" Team, iRCM, DRF, CEA, Fontenay-aux-Roses, France
5. Physiopathology of the striated muscle laboratory, Unit of Functional and Adaptive Biology, University Paris Diderot, Sorbonne Paris Cité, UMR CNRS 8251, Paris, France
6. Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA

Nuclear envelope shape alteration is one of the characteristics of cancer cells. This criteria has been used by cytologist to classify tumors in relationship of their aggressiveness. However, the causes and consequences of this nuclear shape alteration are not elucidated. Increased level of lamin B1, a major component of the nuclear envelope, are frequently found in tumors. We and other previously showed that lamin B1 overexpression leads to nuclear shape alteration. Here, we present data showing that this lamin B1 dysregulation also leads to genome instability. We show that lamin B1 interacts directly with 53BP1 protein, which plays a pivotal role in the double strand breaks (DSBs) repair pathway. This interaction is dissociated after DNA damage. Lamin B1 overexpression impedes 53BP1 recruitment to DNA damage sites and leads to a persistence of DNA damage, a defect in NHEJ and an increased sensitivity to DSBs. Expression of the interacting domain of lamin B1 is sufficient to induce a defect in 53BP1 recruitment, in contrast to the expression of lamin B1 lacking the interacting domain. These data show that lamin B1 overexpression impedes its dissociation from 53BP1 and leads to sequestration of 53BP1. It has been reported that Lamin A and 53BP1 may interact, however overexpression of lamin A does not impede its dissociation from 53BP1, suggesting that lamin A and lamin B1 dysregulation leads to defect of 53BP1 recruitment to damage by different mechanisms. This study highlights lamin B1 as a new factor controlling the recruitment of 53BP1 to DNA damage sites upon injury.

P38MAPK mediates Senescence-induced Lamin B1 Degradation

Rhys Anderson[1,2], Zhixun Dou[3], Kanad Ghosh[3], Shelley Berger[3], Sasi Conte[1], Michael Marber[2], Gian de Nicola[1].

1 Randall Division of Cell and Molecular Biophysics, King's College London, London, UK;
2 King's College London British Heart Foundation Centre of Excellence, Rayne Institute, London, UK,
3 Epigenetics Program, Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Cellular senescence is the phenomenon whereby cells permanently undergo cell cycle arrest, while also acquiring apoptosis-resistance and displaying an immmunogenic senescence-associated secretory phenotype (SASP). Senescent cells accumulate with age and have been shown to play a causal role in numerous age-related diseases. Loss of Lamin B1 has emerged as a key event in the induction of cellular senescence. It was previously shown that upon senescence-inducing insults, Lamin B1 can be degraded by nucleophagy - involving the induction of nuclear blebs which bud off to form cytoplasmic chromatin fragments (CCF), subsequently undergoing lysosomal degradation. Furthermore, CCFs have been shown to elicit an immunogenic response in senescence (and cancer). Hitherto, an upstream mechanism to explain how Lamin B1 becomes targeted for degradation has remained elusive. P38MAPK becomes activated following numerous stress stimuli, including senescence-inducing stressors. Here we show that small molecule inhibition of p38MAPK delays senescence-induced Lamin B1 degradation and is associated with an attenuation of the SASP. Furthermore, we observe a direct interaction between Lamin B1 and p38MAPK, with mass spectrometry analysis revealing a novel p38MAPK phosphorylation substrate – Serine 391 of Lamin B1. Phosphorylation of Lamin B1 on S391 appears to play a role in senescence-induced Lamin B1 degradation, evidenced by a delay in Lamin B1 degradation in phospho-null (S391A) Lamin B1 mutant cells. Our data suggest a novel mechanism for senescence-associated Lamin B1 loss, thus revealing a potential therapeutic target for alleviating the detrimental immunogenicity of the SASP.

The maintenance of nuclear envelope integrity by a new regulator of nucleo-cytoplasmic transport

Mike Schertzer, Arturo Londono-Vallejo

Instiut Curie, UMR3244, Paris, France

Altered nuclear shape is characteristic of many human diseases as well as normal aging. Hoyeraal-Hriedarsson (HHS) is a fatal and severe form of Dyskeratosis congenita that can be caused by mutations in RTELI. It is characterized by bone-marrow failure, short telomeres and microcephaly, amongst other phenotypes. RTELIHHS derived fibroblasts present misshapen nuclei that enriched in LMNA/C and depleted for LMNBI. We have discovered that RTELI play important roles in the RANGTP-dependent trafficking of ncRNAs. Furthermore, HHS cells and cells depleted or mutated for RTELI show a loss of the RANGTP gradient as suggested by a cytoplasmic accumulation or an aberrant NE localization of RAN. We explored a potential connection between the RANGTP nucleo-cytoplasmic transport system and the NE deformations. Inhibiting XPOI (with leptomycin B) rescues the NE defects, inhibiting KPNB1-based import with importazole results in identical (LMNA/C-rich LMNBI -deficient) NE deformations Strikingly, these defects were rescued by overexpression of RTELI and a cytoplasmic form of RTELI but not a mutated form of RTELI.
We propose that RTELI regulates the exchange of KPNBI import complexes and RANGTP at the nuclear pore basket (NUPI 53). Failure to do this results in an import defect and a decrease in the relative levels of nuclear RANGTP, which then leads to NE deformation and rupture through mechanisms that remain to be explored. Import defects and NE instability may thus contribute to the severity of the disease HHS.

The front-rear polarity of cell nucleus

Paulina Nastały, Divya Purushothaman, Stefano Marchesi, Tobias Lendenmann, Gururaj Rao Kidiyoor, Galina Vladimirovna Beznoussenko, Stefania Lavore, Orso Maria Romano, Dimos Poulikakos, Marco Cosentino Lagomarsino, Alexander Mironov, Aldo Ferrari and Paolo Maiuri

FIRC Institute of Molecular Oncology (IFOM), Milan, Italy; Laboratory of Thermodynamics in Emerging Technologies, Department of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland & EMPA, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland.

Cell polarity is defined as an intrinsic asymmetry observed in the structural orientation of the cytoskeleton. It is pivotal in many biological process such as development, tissue homeostasis and cell migration. While the link between cytoskeleton and nucleus is very well studied, it is still unknown whether front-rear cell polarity is also transmitted to the distribution of nuclear envelope and nucleoplasmic proteins, and subsequently to the chromatin.
To understand if polarity could be transmitted to the nucleus, we quantitatively analyzed spatial distribution of nuclear envelope and nucleoplasmic proteins in front-rear polarized cells. We generated a cell population with a front-rear polarity by plating human RPE1 cells on fibronectin-coated lines of 10μm width. Cells spreading on such substrate acquire an elongated shape, develop a spontaneous front-rear polarity and randomly migrate in 1D. Then, we quantitatively analyzed spatial protein distribution by combining images of multiple cells.
We discovered that a nuclear polarity reflects cytoskeletal polarity. Indeed, essential components of the nuclear envelope, as lamin A/C, nesprin-1 and emerin, among others, are polarized towards the direction of motion. Such asymmetric distribution of proteins at the nuclear envelope is further transmitted to the nucleoplasm and it affects spatial distribution of both chromatin and chromatin modifications, potentially affecting gene expression. Moreover, we identify emerin as a molecular determinant of nuclear polarity. In emerin-deficient primary cells, obtained from an Emery-Dreifuss muscular dystrophy patient, nuclear polarity is altered, and could be partially rescued by the ectopic expression of emerin.

Targeted Perturbation of Nuclear Envelope Integrity with Vapor Nanobubble-mediated Photoporation

Gaëlle Houthaeve[1,2], Ranhua Xiong[2,3], Joke Robijns[1], Toon Brans2,3, Coen Campsteijn[5], Sangram K. Samal[2,3], Stephan Stremersch[2,3], Stefaan C. De Smedt[3], Kevin Braeckmans[2,3] and Winnok H. De Vos[1,4]

(1) Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium;
(2) Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium;
(3) Centre for Nano- and Biophotonics, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium;
(4) Cell Systems and Imaging Research Group (CSI), Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium;
(5) Institute of Basic Medical Sciences, Department of Molecular Medicine, University of Oslo, PO Box 1112, Blindern, 0317 Oslo, Norway

The nuclear envelope (NE) has long been thought to only dismantle during mitosis. However, recent observations in cancer cells and laminopathy patient cells have revealed that the NE can also transiently rupture during interphase, thereby perturbing cellular homeostasis (1). Although NE ruptures are promoted by mechanical force and the loss of lamins, their stochastic nature and variable frequency precludes the study of their direct downstream consequences. To resolve this, we have developed a method based on vapor nanobubble-mediated photoporation that allows for deliberately inducing NE ruptures in a spatiotemporally controlled manner. Our method relies on laser illumination of perinuclear gold nanoparticles (AuNPs), resulting in the formation of vapor nanobubbles (VNBs) that inflict mechanical damage to the NE, thus creating small pores. We have demonstrated that VNB-induced nuclear ruptures are transient and recapitulate hallmarks of spontaneous NE ruptures that occur in A type lamin-depleted cells (2). However, the relative efficiency of the technique is too low for high-throughput purposes. We believe this is caused by the presence of non-perinuclear AuNPs that may cause collateral (off-target) damage upon VNB induction. We are therefore optimizing an approach to selectively enrich AuNPs at the NE. This approach is based on functionalization of AuNPs with an NPC-directed nanobody. We are currently exploring whether this approach improves the success rate of nuclear photoporation.

The R482Q Lmna mutation in a mouse model of FPLD inhibits adipogenesis

Xianrong Wong and Colin L. Stewart

Institute of Medical Biology, Immunos, 8A Biomedical Grove, Singapore 138648

We generated a R482Q *Lmna* knock-in mouse model of Dunnigan type Familial Partial Lipodystrophy to investigate the role of *LMNA* in adipose tissue. The phenotype in our FPLD2 model presents some of the clinical features associated with FPLD2, including reduced adiposity, hepatic steatosis, glucose intolerance and altered metabolism. Stromal vascular fraction (SVF) preadipocytes and embryonic fibroblasts (MEF) harvested from FPLD2 mice fail to differentiate normally into adipocytes in culture. Our data suggests that the R482Q LMNA missense mutation blocks adipogenesis in a largely cell autonomous manner and that this may contribute to the development of lipodystrophy in affected FPLD2 patients.

Using unbiased approaches to probe bona fide in vivo protein chromatin (DamID), and protein-protein (2C BioID) interactions, we find that the R482Q mutation does not affect chromatin organization at the nuclear periphery in undifferentiated preadipocytes. Conversely, several proteins interact differently between wildtype and R482Q alleles of lamin A/C in preadipocytes, including Sun1 which we subsequently show to be required for adipogenic progression, likely as a mechanosensor, via its role in the LINC complex.

LMNA-linked lipodystrophy : new insight on cardiovascular phenotypes

Corinne Vigouroux[1], R Ben Yaou[2,3], I Jeru[1], C Stalens[4], O. Lascols[1], G. Bonne[2], MC Vantyghem[5], C Vatier[1], K Wahbi[6]and the French OPALE observatory investigators.

1 Sorbonne Université, Inserm U938, Saint-Antoine Research Centre, AP-HP, Saint-Antoine University Hospital, Departments of Molecular Biology and Genetics and Endocrinology, Reference Center for Rare Diseases of Insulin Secretion and Insulin Sensitivity (PRISIS), Paris, France
2 Sorbonne Université, Inserm U974, Center for Research in Myology, Pitié-Salpêtrière University Hospital, Paris, France
3 APHP, Pitié-Salpêtrière Hospital, Centre de Référence de Pathologie Neuromusculaire Nord/Est/Ile de France, Paris, France
4 AFM-Telethon, Medical Affairs, Evry, France
5 Endocrinology, Diabetes, Metabolism and Nutrition Department, Lille University Hospital, Competence Center for Rare Diseases of Insulin Secretion and Insulin Sensitivity (PRISIS), Lille, France
6APHP, Cochin Hospital, Cardiology Department, FILNEMUS, Centre de Référence de Pathologie Neuromusculaire Nord/Est/Ile de France, Paris-Descartes, Sorbonne Paris Cité University, Paris, France

Pathogenic variants in LMNA encoding type A lamins, are responsible for different lipodystrophic syndromes ranging from typical Familial Partial LipoDystrophy Type 2 (FPLD2) to complex multisystemic diseases. Patients with LMNA-associated lipodystrophies can present with different cardiovascular comorbidities, including accelerated atherosclerosis with precocious and severe coronary artery disease, and dilated cardiomyopathy with rhythm and/or conduction disturbances. Each cardiovascular complication can lead to life-threatening risks, and each requires specific detection and management procedures, which render the clinical decision trees particularly complex.By sharing clinical cases, we will illustrate the complex and sometimes unexpected cardiovascular complications that can affect patients with lipodystrophic laminopathies.
We will then discuss recent results obtained from the French OPALE observatory cohort (Observatory of Patients with Laminopathies and Emerinopathies, coordinated by G. Bonne, Paris, France), in an attempt to evaluate the prevalence and risk factors of cardiovascular complications observed in patients referred for lipodystrophic syndromes due to LMNA pathogenic variants. Early diagnosis of cardiovascular complications is crucial for global management of laminopathies. Standardized procedures aiming to systematically detect any cardiovascular laminopathic involvement are needed in LMNA-associated lipodystrophy.

Nicotinamide riboside extends lifespan in progeroid mice

Yueh-Mei Hsu, Chen-Yu Liao, Brian K. Kennedy

Buck Institute for Research on Aging, USA

Laminopathies, diseases associated with mutations in the LMNA gene that encodes A-type nuclear lamins, range from cardiac and skeletal syndromes to Hutchinson-Gilford progeria syndrome (HGPS). The mechanisms underlying these diseases as well as the treatment approaches for preclinical studies are under intensive exploration. We have repeatedly found that suppression of mTORC1 activity, pharmaceutically (rapamycin) or genetically, extends lifespan in mice deficient in Lmna gene (Lmna-/- mice), a mouse model for skeletal muscle dystrophy and dilated cardiomyopathy. Despite reports of rapamycin-promoted autophagic clearance of progerin, a toxic form of prelamin A produced from mutant Lmna in HGPS, rapamycin failed to provide benefit to HGPS mice. However, we found that oral supplementation of NAD+ precursor nicotinamide riboside (NR) extended lifespan and improved healthspan of LmnaG609G/G609G mice. Long-term NR feeding increased adiposity in LmnaG609G/G609G mice with minimal effect on body weight. This increased adiposity could be due to NR's effect on the suppression of elevated lipolysis, indicated by ATGL protein expression, in liver and white adipose tissues. NR also increased lipid uptake and synthesis, as well as adipocyte size in white adipose tissue. Interestingly, those effects mediated by NR were not observed in Lmna-/- mice. Collectively, our study suggests rapamycin works in the dystrophic model (Lmna-/- mice) only, while NR works in the progeroid model (LmnaG609G/G609G mice) only and highlights NR as a potential therapeutic for HGPS.

The inner nuclear membrane protein Nemp1 is specifically required for fertility

Didier Hodzic[1], Yonit Tsatskis[2], Yi Qu[2], Colleen McKenzi[2], Chloe Potter[1], Andrea Jurisicova[2] and Helen McNeill[1,2]

Department of Developmental Biology, Washington University School of Medicine, St Louis, MO, 63110, USA, 2Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, Canad

Nemp1 (Nuclear envelope integral membrane protein 1, also known as Tmem194a) is a five pass transmembrane protein of the nuclear envelope (NE) that remains poorly characterized. In an effort to understand its biological function, Nemp1 orthologs were genetically inactivated in flies, fishes, worms and mice. Strikingly, these experiments revealed that Nemp1 is invariably required for fertility across all genetic models examined. In mice, whereas Nemp1-/- males remain fertile, Nemp1-/- female display a drastic loss of fertility that is associated to a drastic loss of primordial oocytes.

Here, using newly-developed antibodies, we determined the expression pattern of Nemp1. Our result show that Nemp1 is expressed at the NE of embryonic ovarian cells as early as E13.5 and becomes highly expressed in the germline within adult ovaries. Whereas its pattern remains mostly continuous at the NE during ovarian development, Nemp1 localizes to large dots after the primordial to primary transition stage of adult oogenesis. This dotted pattern gradually increases with adult oogenesis, a process that is accompanied by prominent deformations of the NE. However, the homogenous localization of Nemp1 at the NE resumes in germinal vesicles. Interestingly, no comparable dotted localization of Nemp1 is observed during spermatogenesis. Using various screening approaches, we determined that Nemp1 interacts with multiple LEM domain-containing proteins. Here, we further delineated the interacting domains that mediate the interaction between Nemp1 and emerin. In summary, our data show that Nemp1 is a NE protein that is specifically required for fertility.

Membranes regulating function and the multiple personalities of AKTIP protein.

Romina Burla, Mattia La Torre, Simona Del Giudice and Isabella Saggio

Sapienza University Department of Biology and Biotechnology, Rome, Italy

In higher eukaryotes, architectural entities establish cell and organismal properties. Through dynamic compartmentalization, the nuclear lamina organizes chromatin, the vesicles control intracellular and extracellular molecular trafficking, and microtubules and vesicles regulate cell division. We have found that AKTIP, first identified by us to be important for telomeric chromatin maintenance, is a “promiscuous” protein that participates in multiple pathways. We will describe our recent experiments that show that the function of AKTIP is intimately linked with the nuclear lamins, and that this function is altered in progeroid (HGPS) cells. Furthermore, we have found that chronic AKTIP depletion causes in vitro cell senescence and genome instability, and, in mice, progeroid traits. Interestingly, more recently, we made a further move. We discovered that AKTIP associates with the ESCRT (Endosomal Sorting Complexes Required for Transport). Using structured illumination microscopy and 3D reconstruction we show that AKTIP localizes at the lamina and at the intercellular bridge with ESCRT proteins forming a ring around microtubules. Together, our observations suggest that the phenotypes observed in AKTIP depleted cells and mice can be explained by defects in the ESCRT machinery, and open up the intriguing and novel possibility of exploring telomeres and progeria with an ESCRT perspective.

FETCH1/Tmem120A Directs Spatial Genome Organization in Adipogenesis and Knockout Mice Have a Lipodystrophic Phenotype

Rafal Czapiewski, Jose de las Heras, Dzmitry G. Batrakou, Nik Morton, Eric C. Schirmer

The University of Edinburgh; The Wellcome Trust Centre for Cell Biology, Institute of Cell Biology, Edinburgh, UK

Nuclear lamins and nuclear envelope transmembrane proteins (NETs) are involved in the regulation of development and tissue differentiation. Several tissue-specific NETs particularly direct patterns of spatial genome organization in tissues and their disruption severely inhibits tissue differentiation as tested thus far in muscle and fat cells. Mutations in lamins and several NETs have been linked to a wide range of diseases including muscular dystrophies and lipodystrophy. However, the linked proteins are widely expressed. Therefore, we postulated that tissue-specific genome-organization NETs are responsible for tissue specific manifestations of laminopathies. We recently demonstrated that Tmem120A is a fat-specific NET upregulated during adipogenesis and its knockdown disrupts normal adipogenic differentiation (Batrakou et. al., 2015 PLoS One). Here we report that NET29/Tmem120A is involved in chromosome organization changes during adipogenesis and is important for fat development in mice. Importantly, the fat specific NET29/TMEM120A knockout mouse fails to respond with fat accumulation on a high fat diet and exhibits a metabolic phenotype typical of lipodystrophy. Moreover, we show that NET29/Tmem120A binds lamin A. Together, these findings confirm that NET29/Tmem120A is crucial for normal fat development and support the hypothesis that as a partner of Lamin A it might mediate the manifestation of lipodystrophy caused by lamin mutations.

Monitoring of chromatin organization in live cells using a novel imaging tool, FRIC

Cecilia Bergqvist, Frida Niss, and Einar Hallberg

Stockholm University, Depertment of Biochemistry and Biophysics, Stockholm, Sweden

Transcriptionally inactive heterochromatin preferentially localize in the nuclear periphery and transcriptionally active euchromatin in the nuclear interior. Nuclear envelope proteins composition varies to a large extent between tissues and is believed to play an important role in spatial distribution of chromatin and gene regulation and can have pathological consequences in genetic diseases linked to these proteins, so called laminopathies. Chromatin organization has been extensively studied on a cell population level, but there is a need to understand changes in chromatin organization at the single cell level especially in live cells.

We have developed a novel quantitative image analysis tool that enables monitoring of dynamic spatiotemporal distribution of euchromatin and total chromatin in live cells, called FRIC (Fluorescence Ratiometric Imaging of Chromatin). For this we generated a vector (pTandemH) for expression of histone variant markers Histone 3.3 and Histone 2B, fused to fluorescent proteins, at stochiometrically constant levels. As expected, FRIC displayed a radial chromatin distribution with relatively more heterochromatin in the nuclear periphery as compared to the nuclear interior. Proof-of-concept experiments using agents that affect histone acetylation or expressing laminopathogenic proteins gave predictable results and also showed that FRIC accurately detects dynamic chromatin reorganization events in live cells. Interestingly, depletion of the inner nuclear membrane protein Samp1 (also called NET5 and Tmem201) resulted in formation of less heterochromatin in the nuclear periphery, an effect that was reversed by overexpression of Samp1. The results suggest that Samp1 plays an important role in promoting heterochromatin formation in the nuclear periphery.

SAMMY (Sequential Analysis of MacroMolecules accessibilitY)-seq to detect chromatin structure alterations.

Endre Sebestyén[1], Fabrizia Marullo[2], Federica Lucini[3], Cristiano Petrini[1], Valentina Rosti[3,4], Andrea Bianchi[4], Sara Valsoni[4], Francesco Ferrari[1] and Chiara Lanzuolo[3,4]

[1] IFOM, the FIRC Institute of Molecular Oncology, Milan
[2] Institute of Cell Biology and Neurobiology, National Research Council, Rome
[3] Istituto Nazionale Genetica Molecolare Romeo ed Enrica Invernizzi, Milan
[4] Institute of Biomedical Technologies, National Research Council, Milan

We developed a new high-throughput sequencing-based technique, named Sequential Analysis of MacroMolecules accessibilitY (SAMMY-seq), for genome-wide mapping of chromatin regions enriched by differential accessibility. Using SAMMY-seq in wild type fibroblasts we reliably map lamina associated heterochromatic regions. To test the sensitivity of our technology to chromatin structure alterations, we analyzed Hutchinson-Gilford progeria syndrome fibroblasts. This syndrome is characterized by the progressive accumulation of progerin, a mutated form of Lamin A, which leads to chromatin structure disruption, by interfering with lamina associated domains. In progeria fibroblasts, we were able to detect early-stage changes in chromatin accessibility. Of note, early alterations of chromatin do not modify the distribution of the heterochromatin mark H3K9me3, but are associated with transcriptional deregulation of Polycomb target genes. Our results show that SAMMY-seq is a versatile and sensitive tool to analyze heterochromatin regions. We will also present preliminary results in prostate cancer biopsies showing chromatin remodeling in patients with poor prognosis.

Lack of Active Chromosome Relocation in Senescent and HGPS Fibroblasts after an external stimulus is recapitulated in a Freshwater Molluscan Model Organism

Daniel A. Horton[1a], Kumars Riyahi[1a], Ishita S. Mehta[1a,2]; Matty Knight[3], Joanna M. Bridger[1b]

1a. Biosciences, Dept. of Life Sciences, College of Health and Life Sciences 1b. Genome Engineering and Maintenance Network, Ageing Studies Theme, Institute of Environment, Health and Societies, Brunel University London, Uxbridge, UB8 3PH, UK.
2. Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai - 400005, India.
3. Research Centre for Neglected Diseases of Poverty, School of Medicine and Health Sciences, George Washington University, Washington DC, USA and Division of Science and Mathematics, University of the District of Columbia, Washington DC, USA.

Individual chromosomes in interphase nuclei of young proliferating cells can be induced to move actively to a new non-random location. We have previously demonstrated that this movement requires nuclear motors proteins, especially nuclear myosin 1β. Chromosome movement, upon an external stimulus, in Hutchinson-Gilford Progeria Syndrome fibroblasts is thwarted until the nuclear myosin 1b distribution is reconstituted after treatment with a farnesyltransferase inhibitor, which prevents the farnesylation of the toxic mutant lamin, progerin, found in typical HGPS cells.
When investigating nuclear motor protein presence and distribution in senescent fibroblasts, many of the nuclear motor proteins were found to be differentially distributed or missing when compared to young proliferating fibroblasts. Given this, we have found it is not possible to relocate whole chromosomes or genes once a cell is senescent by two external stimuli that work in young cells. Investigations into restoring chromatin movement with drugs that reverse senescence are presently being investigated.
Interestingly, the molluscan model organism, *Biomphalaria glabrata*, also displays chromatin relocation to new non-random nuclear locations as is observed in human cells, with parasitic infection or an increase in temperature. However, when the snails are allowed to become old (1yr-18 months) these same genes cannot be relocated through either mechanism. This is coincident with an altered distribution or a dearth of nuclear myosin 1β staining. This presents *B. glabrata* as a novel model in which to study chromatin mobility over the life-span, in a whole organism, since it can be easily dosed with reagents in its aquatic environment and is amenable to siRNA and Crispr/Cas knock-down.

Interplay between chromatin and the nuclear envelope modulates spatial genome topology in health and disease

Philippe Collas

University of Oslo, Faculty of Medicine, Department of Molecular Medicine, Oslo, Norway.

Reversible changes in 3-dimensional (3D) genome topologies establish blueprints of developmental gene expression. We will first discuss recent evidence of genome reorganization in a 4-dimensional space, where the 4th dimension is time, during adipogenic differentiation. Our findings highlight a new level of higher-order chromatin topology organizing topological domains (TADs) into “TAD cliques” that segregate towards the nuclear envelope in the course of differentiation. We will then see how switches in lamin A or B interactions with chromatin affect the radial positioning of loci. We will finally address how 3D genome structural modeling reveals unexpected features of genome conformation elicited by a lipodystrophic lamin A mutation.

We propose that genome topology in pre-adipocytes is sensitive to laminopathy- causing lamin mutations that affect associations of the nuclear envelope with chromatin. We also argue that genomic investigations combining wet-lab and computational 3D modeling approaches enable the discovery of new principles of 4-dimensional genome topology.

Genomic instability in laminopathies: new mechanisms to target therapeutically

Ray Kreienkamp, Nuria Coll-Bonfill, Simona Graziano, Rafael Cancado de Faria and Susana Gonzalo

Saint Louis University School of Medicine Department of Biochemistry and Molecular Biology St Louis, MO, USA

The roles of lamins in nuclear architecture and compartmentalization of genome function, and in the maintenance of mechanical stability and genome integrity are indisputable. In recent years, we made advances uncovering molecular mechanisms contributing to the pathophysiology of laminopathies, especially Hutchinson Gilford Progeria Syndrome (HGPS). We will discuss the functions of lamins as genome caretakers, focusing on the role that lamins play in DNA replication, and how the DNA damage that builds up in lamins-deficient cells is elicited in part by replication fork instability (RFI). Lamins bind to nascent DNA and mediate the recruitment of essential factors for stalled replication fork (RF) stability, remodeling and restart. Disruption of lamins function, and especially progerin expression, results in fork deprotection and nuclease-mediated degradation, causing genomic instability. Replication stress (RS) in progeria cells is accompanied by activation of innate immune components such as the cGAS/STlNG pathway of cytosolic DNA sensing and a STATI -mediated interferon (IFN) response, which are at the crossroads of aging, cancer and tumor immunity. These data support a model whereby nuclear fragility and RS cooperate on arousing innate immunity. Moreover, we will bring awareness about the beneficial effects of the hormonal form of vitamin D (calcitriol) and high caloric/high fat diets ameliorating progeria phenotypes in cells in vitro and in mouse models of HGPS in vivo.

Downregulation of Lamin A triggers cell migration and invasion in Ewing Sarcoma

Francesca Chiarini, Antonietta Fazio, Francesca Paganelli, Tommaso Balestra, Maria Cristina Maltarello, Camilla Evangelisti, Cristina Capanni, Stefania Petrini, Alberto Maria Martelli, Katia Scotlandi and Giovanna Lattanzi.

CNR National Research Council of Italy, Institute of Molecular Genetics, Unit of Bologna, Bologna, Italy; University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy; IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy; IRCCS Bambino Gesù Children's Research Hospital, Rome, Italy.

Lamins are the main constituents of nuclear lamina and are involved in a wide range of nuclear functions, including higher order genome organization, chromatin regulation, transcription, DNA replication and repair. Alterations in lamin A/C expression may correlate with malignant transformation in several types of cancer, due to augmented nuclear deformability, which could favor the capability of cells to pass tight interstitial spaces, promoting metastasis. Moreover, considering the different expression levels of lamin A during the development, its low expression could favor cancer cells, pushing them to a more immature phenotype.

However, the role of lamin A/C has not been yet explored in Ewing sarcoma (ES), a tumour with severe prognosis affecting children and young adults. Here, we investigated lamin A/C expression and relevance in ES settings.

Our findings show that lamin A/C is expressed to very low levels in ES cells, while it is upregulated upon induction of neural differentiation. Moreover, a significant inverse correlation between lamin A expression and invasiveness was found in ES, with lamin A overexpression leading to reduced cellular migration.

Based on these findings, we tested the effect of drugs known to interfere either with gene expression or lamin A processing. Intriguingly, while 5-azacytidine treatment was able to re-induce the expression of lamin A/C, thereby leading to ES neural differentiation, the prelamin A processing inhibitor mevinolin induced lamin A/C expression, increased neural differentiation and reduced cellular migration. These findings pave the way to new therapeutic strategies for ES.

Increased lamin B1 levels promote cancer cell migration by altering perinuclear actin organization

Andrea Fracchia and Gabi Gerlitz

Department of Molecular Biology, Faculty of Life Sciences, Ariel University, Ariel 40700, Israel

Cell migration requires reposition and reshaping of the cell nucleus. The nuclear lamina is highly important for migration of both primary and cancer cells. B-type lamins are important for proper migration of epicardial cells and neurons and increased lamin B to lamin A ratio accelerates cancer cell migration through confined spaces. Moreover, positive association between lamin B1 levels and tumor formation and progression is found in various cancer types. Still, the molecular mechanism by which B-type lamins promote tumor cell migration is not understood. To better understand this mechanism we tested the effects of lamin B1 on perinuclear actin organization.

Here we show that induction of melanoma cell migration leads to the formation of a cytosolic perinuclear actin rim, which has not been detected in migrating cells, yet. Significantly, increasing the levels of lamin B1 but not the levels of lamin A prevents perinuclear actin rim formation while accelerates the cellular migration rate. To verify if the perinuclear actin rim attenuates cell migration, we generated a chimeric protein that is localized to the outer nuclear membrane and cleaves the perinuclear actin filaments in a specific manner without disrupting other cytosolic actin filaments. Using this tool we found that disruption of the perinuclear actin rim accelerates the cellular migration rate in a similar manner to lamin B1 over-expression.

Taken together, our results suggest that increased lamin B1 levels accelerates cancer cell migration by inhibiting the association of the nuclear envelope with actin filaments that may reduce nuclear movement and deformability.

ABSTRACTS: POSTERS

Role of PARylation on cardiovascular disease in progeroid syndromes

Deborah Cardoso[1], Catherine Bartoli[2], Nicolas Levy[2], Antoine Muchir[1]

[1]Sorbonne Université, Inserm, Center of Research in Myology, U974 – Paris (France)

[2]Aix Marseille Univ, Inserm, Marseille Medical Genetics, U1251 – Marseille (France)

Introduction: Progeroid syndromes are rare genetic disorders mimicking clinical and molecular features of aging. Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder associated with a characteristic aged appearance very early in life. Children with HGPS appear normal at birth but within a year begin to display the effects of accelerated aging. HGPS is a sporadic autosomal dominant disease caused in nearly all cases by a de novo single-base substitution of LMNA, encoding nuclear A-type lamins. The LMNA mutation creates a cryptic splice donor site, resulting in a lamin A mutant called ‘‘progerin’’, which remains farnesylated. Among all of the clinical features associated with premature aging, the cardiovascular disease is the cause of death in almost all patients. Despite its great importance, investigation of the arterial pathology has been extremely limited mainly due to the low incidence of progeroid syndromes.
Vascular smooth muscle cells (SMCs) are important in maintaining aortic integrity. It is known that the abundance and functionality of SMCs decline with aging in animal models of progeroid syndromes. However, the mechanisms of how ‘‘progerin’’ leads to massive SMC loss are unknown.
Results: Using aorta from a mouse model of HGPS, LmnaG609G/G609G mice, we showed an increase of SMCs cell death, caused by the inability to correctly repair DNA damages. Poly(ADP-Ribose) (PAR) is now recognized as a central post-translational protein modification that coordinates the building of repair complexes at damage sites with the timely controlled dissociation of the repair factors. Our results demonstrate for the first time that aberrant PARylation occurs in SMCs of premature aging mouse model, and participates to the pathogenesis. The chemical stability of the genome is permanently challenged by toxic stresses. If not repaired or incorrectly repaired, the lesions can result in mutations and chromosomal aberrations, diseases and cell death. The extent of the PARylation response to DNA damage largely depends on the nature and amount of DNA breaks produced. In response to low levels of DNA lesions, PARP-1 activity favors repair and survival. In the presence of extensive DNA injury, the massive production of PARylation ultimately causes cell-death.
Conclusion: These preliminary results support the fact that PARylation can be involved in genomic alterations, which causes VSMC loss in patients with HGPS. However, the understanding of molecular and cellular mechanisms underlying the modulation of PARylation signaling in the SMCs remains to be uncovered. Positive results will break new ground for future work in developing novel treatments for cardiovascular involvement in progeroid syndromes and aging in general.

MDPL Syndrome: the role of POLD1 gene in the DNA repair process

P. Spitalieri1, M. Murdocca1, R.V. Talarico1, M. Sanchez2, C. De Masi1, G. Novelli1,3,4, Maria Rosaria D'Apice and F. Sangiuolo1,3.

1 Dept of Biomedicine and Prevention, Division of Genetic, University of Rome Tor Vergata, Rome, Italy
2 Core Facilities Center - Section of Cytometry, Istituto Superiore di Sanità - Rome, Italy.
3 Laboratory of Medical Genetics, Policlinico Tor Vergata, Rome, Italy
4 Neuromed IRCCS Institute, Pozzilli, IS, Italy

Segmental progeroid syndromes constitute a group of disorders characterized by clinical features mimicking physiological aging at an early age. They are clinically and genetically heterogeneous and can be separated in subcategories corresponding to (i) genes encoding DNA repair factors and (ii) genes affecting the nuclear structure and function. In recent years, mutations in POLD1 gene, encoding DNA polymerase delta, has been associated to mandibular hypoplasia, deafness, progeroid signs and lipodystrophy, MDPL syndrome. The enzyme plays an important role in genome maintenance through its involvement in synthetic repair processes.
In this study we investigated the functional link between DNA repair process and POLD1-associated syndrome, through the characterization of MDPL fibroblasts. Cells harboring the recurrent heterozygous in-frame p.Ser605del deletion revealed abnormalities of nuclear envelope morphology, presence of micronuclei, and significant accumulation of prelamin A, associated with altered cellular proliferation and senescence markers, strongly linked to genomic instability. Moreover, DNA damage-induced treatment in MDPL fibroblasts revealed a poor capacity of DNA damage repair, the increase of micronuclei, an arrest in the phase G0/G1 transition, and a lower number of cells in the phase S.
Understanding the mechanistic basis for the association of DNA damage and DNA repair with aging would give insight into contravening age-related diseases and promoting a healthy life span.

Pathological modelling of vascular phenotypes associated to Hutchinson-Gilford Progeria Syndrome (HGPS) using induced pluripotent stem cells (IPS)-derived Vascular Smooth Muscles Cells (VSMCs)

Solenn M. Guilbert, Déborah Cardoso, Patricia Pitrez, Lino Ferreira, Annachiara De Sandre-Giovannoli, Nicolas Lévy, Antoine Muchir, Xavier Nissan

CECS, I-Stem, 91100, Corbeil-Essonnes, France; Sorbonne Université, UPMC Paris 06, INSERM UMRS974, Center of Research in Myology, Institut de Myologie, Paris, France; CNC-Centre for Neuroscience and Cell Biology, University of Coimbra, 3030-789, Coimbra, Portugal; CNC-Centre for Neuroscience and Cell Biology, University of Coimbra, 3030-789, Coimbra, Portugal; Department of medical genetics, La Timone Children's hospital, Marseille, France; Department of Medical Genetics, Children's Hospital La Timone, Marseille, France; Sorbonne Université, UPMC Paris 06, INSERM UMRS974, Center of Research in Myology, Institut de Myologie, Paris, France; CECS, I-Stem, 91100, Corbeil-Essonnes, France

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder caused by a de novo single-base substitution c.1824C>T in the LMNA gene. This mutation activates a cryptic splicing donor site, resulting in the expression of a truncated form of lamin A mutant called progerin. HGPS is associated with a premature aging and death typically from cardiovascular complications. Although the mechanisms underlying VSMCs loss in animal progeroid models were nicely reported in several studies, this process remains unclear in human cells. Here, we took advantage of the self-renewal and differentiation capacities of induced-pluripotent stem cells to study the impact of progerin on vascular smooth muscles cells. To do so, healthy and HGPS iPS-cells were differentiated into VSMCs showing the expression of the main hallmarks of the disease as nuclear abnormalities, premature senescence and calcification in cells carrying the classical HGPS mutation. In this study, we have also investigated the role of several molecular pathways dysregulated in HGPS-VSMCs and are currently developing new pharmacological approaches by high throughput screening.

PCAF involvement in lamin A/C-HDAC2 interplay and its recruitment at the nuclear lamina by lamin A/C

Spartaco Santi [1, 2], Vittoria Cenni[1, 2], Cristina Capanni[1, 2], Giovanna Lattanzi[1, 2], Elisabetta Mattioli[1,2]

1CNR Institute of Molecular Genetics, Unit of Bologna, Bologna, Ita

2Rizzoli Orthopedic Institute, IRCCS, Bologna, Italy

Recent findings demonstrated that lamin A/C regulates histone deacetylase 2 (HDAC2) activity, influencing histone acetylation and p21 gene expression. We observed that lamin A/C-HDAC2 interaction preferentially occurs in cells subjected to deacetylase inhibitors. In those conditions, not only global histone acetylation is increased, but also HDAC2 undergoes acetylation, a condition required for enzyme activation. In this study, we present evidence that HDAC2 binding to lamina A/C is related to acetyltransferase p300-CBP associated factor (PCAF) expression, an acetyltransferase known to acetylate HDAC2. Our data show that, upon lamin A or farnesylated prelamin A accumulation, PCAF is recruited to the nuclear lamina and binds lamin A/C. However, accumulation of non-farnesylated prelamin A fully abolishes PCAF recruitment and, as previously shown, weakens lamin A/C-HDAC2 interaction. Interestingly, increase of PCAF acetylation by sirtinol treatment, a pCAF activator, strengthens lamin A/C-HDAC2 interplay. These results support the view that PCAF recruitment and possibly PCAF-mediated HDAC2 acetylation contribute to stabilization of HDAC2-containing lamin A/C platforms.

Interrogation of Acto-myosin Mediated Nuclear Force Coupling in HGPS

Thomas Waring, Daniel Newman, Luning Liu, Iakowos Karakesioglou, Tobias Zech

University of Liverpool, Cellular and Molecular Physiology/ITM, Liverpool, UK; University of Durham, School of Biological and Biomedical Sciences, Durham, UK

The nuclei of cells are directly linked to their extracellular environment via a bridge which encompasses focal adhesions, the actin cytoskeleton and the LINC complex. This bridge can act as a conduit via which extracellular forces are channelled directly to the nuclear interior, triggering changes in cellular behaviour. Hutchinson-Gilford Progeria Syndrome (HGPS) is a degenerative ageing disease resulting from de novo point mutations within the LMNA gene. These mutations are known to affect nuclear morphology, as well as disrupt localisation of nesprin-2, the LINC complex component that interacts with actin filaments. This project looks to assess force transduction between the environment and the nucleus using a FRET-based tension sensor (TS) formed from the mini-Nesprin-2 protein, and how HGPS mutations affect this transduction. Comparison of cells within a 2- and 3-D context demonstrates that the nuclei of cells within a 3-D environment experience greater tension than those within a 2-D environment. Analysis of time-lapse images of cells migrating through a 3-D environment indicates that nuclei are pulled forwards during migration. Cloning of mRuby2 tagged WT and progerin mutant Lamin A has allowed for initial characterisation of the effect of this mutant on nuclear tension, as well as tension at the focal adhesions (via a Talin-TS). Changes in Lamin A/C expression are shown to change focal adhesion dynamics and nuclear force coupling.

A case of LMNA-limb girdle muscular dystrophy with dilated cardiomyopathy and muscle biopsy's changes suggestive of calpain3-related myopathy

Giulia Ricci[1], Andrea Barison[2], Alberto Giannoni[2], Valentina Spini[2], Fulvia Baldinotti[3], Greta Alì[4], Michele Emdin[2], Gabriele Siciliano[1]

1Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
2Fondazione Toscana G. Monasterio, Pisa, Italy
3Laboratory of Medical Genetics, Azienda Ospedaliero-Universitaria Pisana, S. Chiara Hospital, Pisa, Italy
4Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy

The patient, a 67-year-old female, was hospitalized at our Cardiological Unit for dilatative cardiomyopathy with moderate dysfunction of left ventricular function (ejection fraction, EF, 40%). Since the age of 45, she has complained progressive muscle weakness at pelvic girdle, with difficulty in climbing stairs and anserine gait. Her family history was apparently negative for muscle disease, although her father died at the age of 53 years for not well-defined cardiac problems. A muscle biopsy, performed at the age of 55, showed degenerative changes with lobulated fibers; at western blot a deficit of calpain was detected, therefore the diagnosis of calpain3-related LGMD was supposed, but, at that time, DNA analysis was not performed. At age of 58, she presented the first episode of paroxysmal atrial fibrillation and started oral anticoagulant therapy. In the following cardiological follow-up, a dilatative cardiomyopathy with progressive dysfunction of left ventricular function has been detected. When she came to our attention during the last hospitalization, an Holter EKG showed predominantly nocturnal ventricular ectopics, short runs of no sustained ventricular tachycardia, together with first-degree atroventricular block and left bundle branch block. A coronary angiography ruled out an ischaemic aetiology. A magnetic resonance scan confirmed the moderate left ventricular systolic dysfunction, with several areas of myocardial fibrosis. Based on these data, an implantation of cardioverter defibrillator (ICD) was performed.

The previous diagnosis of calpain3-related LGMD was therefore reconsidered: DNA sequencing analysis did not show CAPN1gene variants but detected a heterozygous mutation in LMNAgene (c.1130G>A, p.Arg377His), previously reported as pathogenetic associated with AD-EDMD.

Schwann cells pericentromeric heterochromatin is involved in neuromuscular junction morphological remodelling during development

Paloma Alvarez-Suarez, Gawor, M., Prószyński, T.J.

Institution, Department, City, and Country Nencki Institute of Experimental Biology, Laboratory of Synaptogenesis, Warsaw, Poland

INTRODUCTION: The neuromuscular junction (NMJ) is a specialized synapse formed between motor neuron, skeletal muscle fiber, and associated perisynaptic Schwann cells. NMJs undergo intense structural remodelling during early postnatal development, where initially oval-shaped clusters of neurotransmitter receptors (AChRs) transform into complex pretzel-like structures at the postsynaptic membrane. Abnormalities in this process are often observed in several muscle diseases, however the molecular and cellular mechanisms underlying NMJ maturation are unknown. We performed a systematic localization analysis of the main three NMJ components to get more insight into the coordinated process of synapse remodelling.

METHODS: Immunohistochemical studies were performed on P9 tibialis anterior muscle of transgenic mice expressing fluorescent protein (dTomato) in motor neurons and analysed by confocal microscopy.

RESULTS: We found that the early remodelling of the postsynaptic machinery (perforations in oval-shaped clusters) follows the pattern of motor neuron net-like processes, strongly suggesting a presynaptic specification of postsynaptic rearrangements. Unexpectedly, we observed that Schwann cell nuclei structures of pericentromeric heterochromatin (chromocenters) precisely localize with the synaptic area unoccupied by the motor axon terminal. Terminal Schwann cells' chromocenters seem to be sites where the nuclear envelope is in close contact with the synaptic cleft, determining specific subsynaptic microdomains. As previously shown, local Ca2+ nuclear flux in such domains could regulate gene expression through its effects on chromatin organization. Our results support the tripartite synapse model while providing a new perspective on intercellular signalling driving synapse remodelling.

The Missing ‘LINC’ to Human Healthy Ageing: associations between ageing, physical activity status and myonuclear architecture and mechanics in human skeletal muscle

Ed Battey, Dr Matthew Stroud, Dr Julien Ochala

Centre for Human & Applied Physiological Sciences, School of Basic & Biomedical Sciences, Faculty of Life Sciences and Medicine, Guy's Campus, Shepherd's House, King’s College London; British Heart Foundation Centre of Excellence, Cardiovascular division, James Black Centre, King's College London

Ageing is associated with aberrant nuclear morphology and mechanics; this project focuses on whether these changes can be offset by physical activity in skeletal muscle. Nuclear architecture and the distribution of nuclear envelope proteins SUN1 and LEM2 were studied in isolated human myofibres from individuals of different ages and physical activity statuses. So far, the data indicate that changes in myonuclear morphology occur in response to exercise regardless of chronological age, indicated by a 25-30% reduction in myonuclear aspect ratio in young and older exercise-trained individuals compared to inactive young and older counterparts. No differences in the distribution of proteins SUN1 or LEM2 were revealed through super-resolution microscopy. Exercise therefore appears to influence nuclear morphology in a manner unaffected by chronological age; alterations to nuclear envelope proteins other than SUN1 and LEM2 may be involved in this process. Future research will be focused on investigating 3D myonuclear morphology, the distribution and concentration of Nesprin-1 and Lamin A and how alterations to myonuclear mechanics influence gene transcription.

Elucidating the role of nuclear envelope proteins in the regulation of pro-fibrotic gene expression

Emma J. Carley, Rachel M. Stewart, Xueyan Peng, Erica L. Herzog, Megan C. King

Institution, Department, City, and Country Yale School of Medicine, Department of Cell Biology, New Haven, CT, USA; Yale University Department of Molecular Cell and Developmental Biology, New Haven, CT, USA; Yale School of Medicine, Section of Pulmonary, Critical Care, and Sleep Medicine, Department of Internal Medicine, New Haven, CT, USA

Mechanotransduction is critical for cells to adapt to changes in their environment. The linker of nucleoskeleton and cytoskeleton (LINC) complex physically connects the cytoskeleton to the nuclear lamina. If and how force-transduction through this complex directly influences gene expression is unknown. Recently, our group demonstrated that loss of the LINC complex component SUN2 in mice leads to cardiac hypertrophy that is surprisingly uncoupled from fibrosis. Moreover, we find that SUN2-null mice are also completely protected from fibrosis in a well-established bleomycin-induced lung injury model. Accordingly, in both LINC complex-null mouse keratinocytes and the myocardium of SUN2-null mice we observe significant down-regulation of pro-fibrotic genes, suggesting that the LINC complex regulates pro-fibrotic gene expression, a process controlled by TFGb signaling. Despite repression of TGFb gene targets we find a surprising increase in nuclear pSmad2/3 in both isolated cells and tissue lacking SUN proteins, suggesting that the LINC complex regulates TGFb signaling at the level of the nucleus. A mechanism for uncoupling Smad targeting and activity is suggested by the observation that both SUN2-null cardiac tissue and LINC complex-null mouse keratinocytes display a dramatic increase in MAN1, a known negative regulator of TGFb signaling, at the nuclear envelope. We propose a model in which the LINC complex regulates TGFb signaling through MAN1 in a pathway that communicates mechanical signals to influence the production of extracellular matrix.

LINC complexes are mechanotransducers that fine-tune b-catenin signaling along the Epithelial-Mesenchymal Transition spectrum through a-catenin nuclear translocation

T. Déjardin1, Pietro Salvatore Carollo1, P. Davidson2, C. Seiler1, D. Cuvelier3, B. Cadot4, C. Sykes2, E.Gomes5, N. Borghi1

1 Institut Jacques Monod, CNRS UMR7592, Université Paris-Diderot, 75013 Paris, France;
2 Institut Curie, CNRS UMR168, Université Pierre et Marie Curie, PSL Research University, 75006 Paris, France;
3 Institut Pierre-Gilles de Gennes, PSL Research University, 75005 Paris, France;
4 Center for Research in Myology, INSERM U974, CNRS FRE3617, Université Pierre et Marie Curie, Sorbonne Universités, GH Pitié Salpêtrière, 75013 Paris, France;
5 Instituto de Medecina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisbon, Portugal.

LINC complexes are transmembrane protein assemblies that physically connect the nucleo- and cytoskeletons through the nuclear envelope. Dysfunctions of LINC complexes impact on cell polarity, migration, division, or differentiation in a variety of contexts and are associated with pathologies such as cancer and muscular dystrophies. The mechanical roles of LINC complexes in these contexts are poorly understood. To address this, we combined in fibroblastic and epithelial cells in culture genetically encoded FRET biosensors of molecular tension in LINC complex proteins with mechanical, genetic and pharmacological perturbations aimed at mimicking a range of physiological and pathological situations. We showed that LINC complex proteins experience tensions almost exclusively mediated by the cytoskeleton and act as mechanosensors of multicellular packing that discriminate between inductions of partial and complete epithelial-mesenchymal transitions (EMT). This function involves the tension-dependent recruitment at the nuclear envelope of a-catenin, which acts as a nuclear retention, but also transcription-limiting factor for b-catenin. Our data thus support that LINC complexes are mechanotransducers that fine-tune b-catenin signaling along the EMT spectrum.

Nesprin-1-alpha2 at the outer nuclear membrane

Ian Holt and Glenn E Morris

Wolfson Centre for Inherited Neuromuscular Disease, Keele University and RJAH Orthopaedic Hospital, Oswestry, SY10 7AG, UK.

It was once a reasonable and popular hypothesis that giant forms of nesprins have functions at the outer nuclear membrane (ONM) while the shortest forms, such as nesprin-1-alpha2, are at the inner nuclear membrane (INM). This was partly based on evidence that nesprins interact directly with lamin A/C and emerin, both of which are associated with the INM. The hypothesis was encouraged by the presence of a possible interrupted LEM domain in nesprin-1-alpha for interaction with chromatin via BAF, although BAF binding was not confirmed. Two electron microscopy studies were ambiguous in that they detected both full-length and short nesprin isoforms at both INM and ONM. The discovery of SUN proteins which span the lumen of the nuclear envelope (NE) created a whole new ballgame. The interaction of the SUN protein trimer with BOTH lamin A/C at the INM AND with the nesprin KASH domain inside the lumen (the “LINC” complex) is clearly oriented for placing all nesprins with a KASH domain at the ONM. Nesprin-1-alpha2 has recently been shown to have an ONM role in movement of nuclei by linking microtubules to the ONM via its LEWD-motif-mediated interaction with kinesin. We discuss the evidence for and against an additional role for nesprin-1-alpha2 at the INM and conclude that the experimental data can be explained without necessarily invoking an INM role.

An investigation into variations in the LINC complex in breast cancer

Kleopatra Papa[1]; Tom Allen[2]; Bogusdlaw Obara[2]; Eric Schirmer[3]; Adam Benham[1]; Martin Goldberg1; Iakowos Karakesisoglou[1]

1 Durham University, Department of Biosciences, Durham, UK

2 Durham University, Department of Computer Science, Durham, UK

3 University of Edinburgh, School of Biological Sciences, Edinburgh, UK

Cell motility is an important feature for many cell types both during physiological conditions and in cancer metastasis. Signal and force transmission as a response to stimuli is mediated through the mechanical tethering of the nucleoskeleton to the cytoskeleton through the LInker of the Nucleoskeleton and Cytoskeleton (LINC) complex. The quaternary structure and interactions of LINC complex components and LINC-associated proteins are a crucial determining factor of nuclear envelope architecture and cell stiffness, restricting cell migration through confinement. 3D Structure Illumination Microscopy (SIM) has been used to study variations in LINC complex components in MCF10A breast epithelia and MDA-MB-231 breast cancer epithelia. Additionally, we have assessed disulphide-dependent interactions in SUN-domain proteins and the effect of Protein Disulphide Isomerase inhibition on cell viability and cell migration. Our findings suggest that the composition and structure of the LINC complex is different in the MDA-MB-231 breast cancer cell line compared to healthy MCF10A breast epithelia. Hence, shedding more light onto the regulation of the LINC complex could contribute to a novel understanding of the mechanisms underlying cellular migration. More specifically, it will allow an investigation into the relationship between LINC complex deregulation in breast cancer cells, nuclear stiffness and breast cancer metastasis.

Investigating a novel role for the nuclear envelope LINC complex in cardiomyocyte mechanotransduction

Zhou C, Wilson D, De Silva Shanelle Amelia, Hodzic D, Shanahan CM, Zhang QP

King's College London British Heart Foundation Centre of Research Excellence, Cardiovascular Division, London, UK; Department of Developmental Biology, Washington University School of Medicine, St. Louis, USA.

Cardiomyopathies are an important cause of heart failure and sudden cardiac death. Nesprin-1/-2 are highly expressed in skeletal and cardiac muscle and together with SUN (Sad1p/UNC84)-domain containing proteins form the LInker of Nucleoskeleton and Cytoskeleton (LINC) complex at the nuclear envelope (NE), which, in association with NE proteins lamin A/C and emerin, mechanically couples the nucleus to actin cytoskeleton. Mutations in genes encoding these components are associated with dilated cardiomyopathy (DCM), however, understanding of the mechanisms through which these mutations lead to DCM is limited. To gain further insight, we have generated a cardiac-specific nesprin-2 KASH transgenic mouse model of LINC complex disruption. These mice exhibit a hypertrophic response with re-activated fetal and pro-fibrotic gene expression at baseline. Under pathological haemodynamic stress cardiac function was severely impaired, and at a cellular level cardiomyocytes (CMs) displayed altered nuclear morphology and abnormal expression of mechanosensitive genes. This suggests hearts with LINC complex disruption are more susceptible to strain potentially via aberrant mechanical signalling. Therefore, we hypothesise LINC complex disruption leads to defective mechanotransduction, manifesting as defects in force transmission and Ca2+ handling in CMs. We propose to investigate the role of the LINC complex in CMs during mechanical stress by determining how CMs derived from this transgenic mouse model respond to matrix rigidity using 2D polyacrylamide hydrogels with variable stiffness that mimic the environment experienced by cells in cardiac tissue.

Identification and characterization of novel proteins involved in nuclear envelope repair

Aurélie Bellanger, Sissel Eikvar, Romy Timmer, Annemijn Arns, Coen Campsteijn

Department of Molecular Medicine, Institute for Basic Medical Sciences, University of Oslo, Oslo, Norway

Restoration of the nuclear envelope integrity during mitotic exit and interphase nuclear envelope ruptures is essential for the maintenance of genome integrity and overall cell fitness, as illustrated in the context of cancer and nuclear envelopathies. Recent studies have emphasized the central role of the Endosomal Sorting Complex Required for Transport-III (ESCRT-III) membrane fission machinery in the reformation and repair of the nuclear envelope. Despite this insight, the orchestration of nuclear envelope repair is poorly understood.

Our work focuses on the identification and characterization of novel proteins involved in nuclear envelope repair. We use a unique proteomics setup exploiting proximity ligation to identify novel proteins associated with nuclear envelope repair events. The validity of this approach is underscored by the identification of the core nuclear envelope repair machinery, including ESCRT-III proteins and LEM domain proteins, as well as factors associated with DNA damage response. In addition, we identified a list of additional proteins that are enriched during nuclear envelope ruptures. Among these proteins we extracted several novel nuclear envelope repair candidates, including a number of proteins known to be deregulated in cancers.

Our current work focuses on assessing the biological significance and mechanistic relevance of these candidates during nuclear envelope repair in normal and breast cancer contexts. This work will provide crucial new comprehension of the events associated with nuclear envelope ruptures and could provide new prospects regarding the role of nuclear envelope integrity in diseases such as cancer and nuclear envelopathies.

Emerin post-translational modifications are affected during early phase of oxidative stress response

Vittoria Cenni, Manuela Loi, Joke Robijn, Winnok H. De Vos, Stefano Squarzoni, Elisabetta Mattioli, Giovanna Lattanzi, Cristina Capanni

Vittoria Cenni, CNR, Institute of Molecular Genetics “Luigi Luca Cavalli Sforza”, Unit of Bologna, Bologna, Italy and IRCCS Istituto OrtopedicoRizzoli, Bologna,Italy.
Manuela Loi, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy.
Joke Robijns, Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium.
Winnok H. De Vos, Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium.
Stefano Squarzoni, CNR, Institute of Molecular Genetics “Luigi Luca Cavalli Sforza”, Unit of Bologna, Bologna, Italy and IRCCS Istituto OrtopedicoRizzoli, Bologna,Italy.
Elisabetta Mattioli, CNR, Institute of Molecular Genetics “Luigi Luca Cavalli Sforza”, Unit of Bologna, Bologna, Italy and IRCCS Istituto OrtopedicoRizzoli, Bologna,Italy.
Giovanna Lattanzi, CNR, Institute of Molecular Genetics “Luigi Luca Cavalli Sforza”, Unit of Bologna, Bologna, Italy and IRCCS Istituto OrtopedicoRizzoli, Bologna,Italy.
Cristina Capanni, CNR, Institute of Molecular Genetics “Luigi Luca Cavalli Sforza”, Unit of Bologna, Bologna, Italy and IRCCS Istituto OrtopedicoRizzoli, Bologna,Italy.

Emerin is a nuclear envelope protein that contributes to nuclear architecture, chromatin structure and gene expression through its interaction with various nuclear proteins including lamin A/C, BAF etc. Emerin post-translational modifications including phosphorylation and O-GlcNAcylation, affect emerin molecular weight and emerin interaction with its protein-partners. We observed that the emerin molecular weight is modified in several models with altered lamin A/C. Western blot analysis performed in HGPS cells, mevinolin treated cells, prelamin A constructs-expressing cells and lamin A or ZMPSTE24 null cells shows an additional emerin-upper band. Since prelamin A accumulation is linked to ROS increase, we wondered if the oxidative stress can affect emerin post-translational modifications in cultured cells. We found that ionizing treatments (H2O2, ubiquinone or U.V. radiations) are able to modify emerin: an emerin double band appears during the early phase of oxidative stress response, characterized by P21 protein degradation and gamma-H2AX increase. The opposite happens during the recovery-phase which follows. The involvement of both phosphorylation and O-GlcNAcylation mechanisms in such phenomenon is confirmed using inhibitors of phosphorylation, dephosphorylation and O-GlcNAcylation (staurosporine, okadaic acid, OSMI-1). Interestingly, BAF nuclear localization changes when the emerin upper band appears. In this condition, BAF loses its nuclear periphery distribution and becomes exclusively detectable at the intranuclear region suggesting that emerin-BAF interaction could be regulated during oxidative stress response. Finally, we observed that in EDMD1 cells P21 and gamma-H2AX protein level are not properly modulated during the oxidative stress response suggesting a potential emerin/BAF role in DNA damage response.

A nuclear envelope function for STING in mediating innate immunity to RNA viruses?

Charles R. Dixon[1], Martin Goldberg[2], Paul Digard[3], and Eric C. Schirmer[1]

1Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK;
2School of Biological and Biomedical Sciences, Durham University, Durham, UK;
3Roslin Institute, University of Edinburgh, Edinburgh, UK;

The innate immune response (IIR) is the cell's first line of defence against pathogens. STING is a key host adaptor protein for IIR signalling triggered by cytosolic DNA, whereby STING localised throughout the ER becomes activated upon host cell recognition of cytosolic DNA and instigates signalling cascades which induce the expression of type-I interferon. STING also plays a role in IIRs stimulated by pathogen RNA, during RNA virus infection, although the mechanisms through which STING antagonises RNA viruses are incompletely resolved.

STING is also present in the nuclear envelope (NE) and given STING's early identification as a NE transmembrane protein (NET23) by our lab, we set out to investigate NE-specific roles for STING. Isolation of STING from NE fractions revealed several putative NE-specific STING partners which, are all known RNA or DNA binding proteins, and according to interactome datasets, form indirect interactions with key immune transcription factors. We hypothesise that they could provide a novel mechanism to activate STING signalling from the nuclear envelope upon detection of nuclear localised pathogen DNA or RNA.

Knockdown of some NE-STING partners reduces the activation of immune reporter constructs during immune stimulation with dsDNA and reduces expression of endogenous IFN-β following immune stimulation with synthetic dsRNA. Knockdown of one partner, SYNCRIP, enhances the replication of the RNA virus influenza A. Finally, immunogold-EM shows that STING is present in both the inner and outer nuclear membranes and we are now following the dynamics of STING at the nuclear envelope following immune stimulation.

Unrestrained ESCRT-III drives chromosome fragmentation and micronuclear catastrophe

Marina Vietri, Sebastian W. Schultz, Aurélie Bellanger, Carl M. Jones, Louise I. Petersen, Camilla Raiborg, Ellen Skarpen, Christeen Ramane J. Pedurupillay, Eline Kip, Romy Timmer, Ashish Jain, Philippe Collas, Roland L. Knorr, Sushma N. Grellscheid, Halim Kusumaatmaja, Andreas Brech, Francesca Micci, Harald Stenmark and Coen Campsteijn

Centre for Cancer Cell Reprogramming, Faculty of Medicine, University of Oslo, Oslo, Norway: Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway: Department of Molecular Medicine, Institute of Basic Medicine, University of Oslo, Oslo, Norway: Department of Biological Sciences, Durham University, Durham, UK: Computational Biology Unit, Department of Biological Sciences, University of Bergen, Bergen, Norway: Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, Oslo University Hospital, Oslo, Norway: Max Plank Institute of Colloids and Interfaces, Department of Theory & Bio-Systems, Potsdam, Germany: Dept. Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, University of Tokyo, Tokyo, Japan: Department of Physics, Durham University, Durham, UK

Loss of nuclear integrity due to nuclear envelope ruptures results in DNA damage and innate immunity signaling, and is associated with poor cell fitness and pathologies such as cancer and nuclear envelopathies. The ESCRT-III is a membrane fission machinery that maintains nuclear envelope integrity, resealing nuclei within minutes upon rupture. In contrast, micronuclear envelope ruptures appear to be irreversible and are associated with catastrophic membrane collapse and chromothripsis, the complex rearrangement of chromosomes thought to be a major driving force in cancer development. Why micronuclei are not repaired has remains unknown. Here we show that the CHMP7/LEMD2 complex act as a compartmentalization sensor that detects loss of nuclear envelope integrity. We find that the influx of CHMP7 into the nucleus is the determinant factor in the formation of stable CHMP7/LEMD2 complexes that allow for polymerization of the ESCRT-III protein CHMP4B required for membrane repair. Furthermore, we show that micronuclei inherently lack the capacity to restrict accumulation of the CHMP7/LEMD2 complex to the site of rupture, resulting in unrestrained ESCRT-III accumulation that instead drives extensive membrane deformation and DNA damage. Our results indicate that the ESCRT-III machinery act as a double-edged sword, as its sensitivity ensures rapid repair at primary nuclei while causing unrestrained activity at ruptured micronuclei, with catastrophic consequence for genome stability.

A-type lamin status determines recovery from compression-induced nuclear envelope rupture

Ana Leal, Joke Robijns, Guilherme Nader, Juan Manuel Garcia, Matthieu Piel, Winnok H. De Vos

Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium; Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium; Institut Curie, Institut Pierre-Gilles de Gennes pour la Microfluidique (IPGG), Paris, France; Institut Curie, Institut Pierre-Gilles de Gennes pour la Microfluidique (IPGG), Paris, France; Institut Curie, Institut Pierre-Gilles de Gennes pour la Microfluidique (IPGG), Paris, France; Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium

The cell nucleus is supported by a network of nuclear lamins. Mutations in the LMNA gene, which encodes A-type lamins, cause a variety of diseases called laminopathies. We have discovered that nuclei from laminopathy patient cells undergo repetitive, non-lethal ruptures of the nuclear envelope (NERs). Migrating cancer cells experience a similar process, suggesting it represents a broad-spectrum pathogenic mechanism. To understand the contribution of A-type lamin defects to the susceptibility to NERs, we have previously developed cell lines that do not produce A-type lamins (LMNA KO), or that produce a mutant prelamin A isoform (ZMPSTE24 KO). Since A-type lamins contribute to the structural integrity of the nucleus and one of the major elicitors of NERs is compressive force, we asked whether the A-type lamin status would render cells more susceptible to compression-induced NER. To this end, we made use of a multi-well confinement paradigm that squeezes cells in suspension between two surfaces down to a well-defined height of 4 µm. NERs were automatically detected by the transient translocation of a nuclear-localized fluorescent protein (mCherry-NLS) to the cytoplasm by means of an in-house developed image analysis pipeline. Our results showed that there is not a significant difference in the compression-induced NER frequency of LMNA KO and ZMPSTE24 KO compared to control cells. However, LMNA KO cells experienced elongated recovery times after NER, whereas ZMPSTE24 KO cells inclined towards a faster recovery. Thus, A type lamin status determines the repair efficiency after compression-induced NER. Currently, we are investigating the underlying molecular mechanisms of NERs that can uncover new pathways involved in laminopathies diseases.

Genome wide CRISPR/Cas9 screen identifies genetic modifiers of tau aggregation that contribute to nuclear envelope integrity

Marine Prissette, Matthew Koss, Georgia Clarke, Ella Dragileva, Daria Fedorova, Lynn Lee, Michael Glasser, Gregg Warshaw, Jose Rojas, Charleen Hunt, Joyce Chiu, Diana Ahrens, Vitaliy Rogulin, Michael Podgorski, Mathieu Desclaux, Wen Fury, David Frendewey and Brian Zambrowicz

REGENERON Pharmaceuticals; Velocigene; Tarrytown, NY, USA

The CRISPR-Cas9 system enables site-directed mutagenesis (CRISPRn) in cells and organisms. This powerful tool can be coupled with sgRNA libraries to perform genome-wide genetic screens. We used CRISPR libraries to perform genome-wide screens and identify genetic modifiers of pathogenic protein aggregation, a common mechanism in neurodegenerative diseases. We used a biosensor human cell line, consisting of HEK293T cells in which transgenes express pathogenic tau protein fused to either CFP or YFP (Holmes et al. PNAS 2014). Protein aggregation produces a FRET signal, the result of Förster Resonance Energy Transfer from donor CFP to acceptor YFP. FRET-positive cells, FRET(+), which contain tau aggregates, can be sorted and isolated by fluorescence activated cell sorting (FACS). We further modified these biosensor cells by introducing a Cas9-expressing transgene.

We conducted five replicate experiments of a genome-wide gain-of-FRET screen to identify modifier genes that promote tau aggregation when disrupted in our biosensor cell line. We developed a screening assay in which conditioned medium (CM) collected from a tau-YFP cell line that contains stably propagating tau aggregates is applied to the tau-CFP/tau-YFP biosensor cell line to trigger~0.1% FRET(+) cells after 3 Days in culture. We isolated FRET(+) cells by FACS and sequenced the sgRNA sequence to reveal those enriched in the FRET(+) cells, thereby identifying genes that promote tau aggregation when disrupted. We performed secondary screens with individual sgRNAs to validate the hits from the replicate screens. High-confidence hits emerged for two genes that contribute to the processes that maintain nuclear envelope integrity. From an examination of other proteins that participate in this biological process, we identified one additional gene that, when mutated by CRISPR/Cas9, also enhanced tau aggregation. Functional assessment of these three genes in human iPS-derived neurons is ongoing.

Lamin B1, a new factor controlling the recruitment of 53BP1 to DNA damage

Laure Etourneaud1,a, Angela Moussa1,a, Emilie Rass1a, Diane Genet1,a, Benoit Thézé1, Simon Willaume1, Caroline Chabance-Okumura1, Jordane Dépagne2, Xavier Veaute2, Eléa Dizet2, Didier Busso2, Aurélia Barascu1, Lamya Irbah3, Thierry Kortulewski3, Anna Campalans4, Catherine Le Chalony1, Brigitte Buendia5, Ralph Scully6, Gaëlle Pennarun1 and Pascale Bertrand1,*

1. Unit 1274 CEA-INSERM- Paris Diderot University, Paris Sud University, “DNA Repair and Ageing” Team, Institute of Cellular and Molecular Radiobiology (iRCM), François Jacob Institute, DRF, CEA, Fontenay-aux-Roses, France ;
2. Genetic Engineering and Expression Platform (CIGEX), iRCM, DRF, CEA, 18 route du Panorama, Fontenay-aux-Roses, France ;
3. Imaging platform, iRCM, DRF, CEA, 18 route du Panorama, Fontenay-aux-Roses, France ;
4. Unit 1274 CEA-Paris Diderot University, Paris Sud University, “Genetic Instability Research” Team, iRCM, DRF, CEA, Fontenay-aux-Roses, France ;
5. Physiopathology of the striated muscle laboratory, Unit of Functional and Adaptive Biology, University Paris Diderot, Sorbonne Paris Cité, UMR CNRS 8251, Paris, France ;
6. Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA

Nuclear envelope shape alteration is one of the characteristics of cancer cells. This criteria has been used by cytologist to classify tumors in relationship of their aggressiveness. However, the causes and consequences of this nuclear shape alteration are not elucidated. Increased level of lamin B1, a major component of the nuclear envelope, are frequently found in tumors. We and other previously showed that lamin B1 overexpression leads to nuclear shape alteration. Here, we present data showing that this lamin B1 dysregulation also leads to genome instability. We show that lamin B1 interacts directly with 53BP1 protein, which plays a pivotal role in the double strand breaks (DSBs) repair pathway. This interaction is dissociated after DNA damage. Lamin B1 overexpression impedes 53BP1 recruitment to DNA damage sites and leads to a persistence of DNA damage, a defect in NHEJ and an increased sensitivity to DSBs. Expression of the interacting domain of lamin B1 is sufficient to induce a defect in 53BP1 recruitment, in contrast to the expression of lamin B1 lacking the interacting domain. These data show that lamin B1 overexpression impedes its dissociation from 53BP1 and leads to sequestration of 53BP1. It has been reported that Lamin A and 53BP1 may interact, however overexpression of lamin A does not impede its dissociation from 53BP1, suggesting that lamin A and lamin B1 dysregulation leads to defect of 53BP1 recruitment to damage by different mechanisms. This study highlights lamin B1 as a new factor controlling the recruitment of 53BP1 to DNA damage sites upon injury.

Regulation of ESCRT-III-dependent nuclear envelope regeneration during mitotic exit

Caroline Stoten, Yolanda Olmos, Alberto Gatta, Jeremy Carlton

Institution, Department, City, and Country Division of Cancer Studies, King's College London, London SE1 1UL, UK.

The Francis Crick Institute, London, NW1 1AT, UK

Through the process of annular fusion and disassembly of spindle microtubules, the Endosomal Sorting Complex Required for Transport-III (ESCRT-III) machinery has emerged as a key player in the regeneration of a sealed nuclear envelope during mitotic exit, and in the repair of this organelle during interphase rupture. ESCRT-III polymerisation at the nuclear envelope occurs transiently during mitotic exit and CHMP7, an ER-localised ESCRT-II/ESCRT-III hybrid protein, initiates this assembly in a manner dependent upon the inner nuclear membrane protein LEM2. However, how this is regulated is unclear. Here, we show that CHMP7 contains a nuclear export sequence which acts to separate CHMP7 from LEM2 during interphase. However, this mechanism of regulation is lost when cells enter mitosis and NE and ER identities are mixed. We further show that CDK1 phosphorylation of CHMP7 occurs upon mitotic entry with dephosphorylation during mitotic exit accompanying assembly at this organelle, suggesting that phosphorylation may act to inhibit CHMP7 assembly during early mitosis until it is required. These data suggest that a key cell-cycle control programme allows ESCRT-III-dependent nuclear regeneration.

Role of LncRNA HOTAIR in nuclear architecture remodeling during adipose differentiation

Evdokiia Potolitsyna, Anita Sørensen, Coen Campsteijn, Philippe Collas, Nolwenn Briand

University of Oslo, Faculty of Medicine, Department of Molecular Medicine, Oslo, Norway

Familial partial lipodystrophy of Dunnigan type (FPLD2) is caused by mutations in LMNA gene encoding nuclear Lamin A/C, and is characterized by the selective loss of adipose tissue in the extremities and redistribution of fat to central areas. Proposed mechanisms for FPLD2 involves a deregulation of nuclear architecture, impaired interaction of mutated Lamin A with transcription factors, and altered recruitment of the epigenetic modifier PRC2 leading to defective adipocyte differentiation. However, how LMNA mutations affect adipogenesis in a depot-specific manner has not been investigated. LncRNA HOTAIR is the most differentially expressed gene between lower and upper adipose tissues and has been shown to serve as a scaffold for PRC2. Therefore, we hypothesize that lncRNA HOTAIR could mediate FPLD2 lower body specific defects in adipogenesis. Preliminary results show that lncRNA HOTAIR is expressed in lower body adipose stem cells (ASCs), but not in mature adipocytes, and that distinct HOTAIR isoforms are induced during early adipose differentiation. In addition, we show that HOTAIR localizes in the nucleolus throughout adipose differentiation, and that HOTAIR knock-down remodels nucleoli in ASCs. These results suggest a role of HOTAIR in organizing nucleoli during adipose differentiation and add to the growing body of evidence that nucleolar dysfunction might play a key role in lipodystrophic syndromes.

Transposable Element Dysregulation as a Consequence of the Nuclear Lamina Impairment

Valeria Cavaliere[1], Giuseppe Gargiulo[1], Giovanna Lattanzi[2,3] and Davide Andrenacci[2,3]

1Dipartimento di Farmacia e Biotecnologie, Alma Mater Studiorum Università di Bologna, Bologna, Italy
2 CNR Institute of Molecular Genetics “Luigi-Luca Cavalli-Sforza”, Unit of Bologna, Bologna, Italy
3 IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy

Genome of eukaryotic cells contain active transposable elements that can move into new locations and produce mutations, chromosomal rearrangements and genomic instability. Therefore, transposable elements are silenced by heterochromatin-mediated repression and post-transcriptional RNA silencing mechanisms. It has been demonstrated that the Lamin protein of Drosophila is involved in the repression of transposable elements, suggesting the involvement of the nuclear lamina in the regulation of mobile elements. Since not all transposable elements appear upregulated in Lamin depleted flies, we have analyzed the effect of the genetic background on the expression of a number of transposable elements. We also looked for a possible correlation between the genomic localization of transposable elements insertions and the effect of Lamin depletion on transposable element expression. Finally, we tried to understand whether the phenotypes induced by Lamin depletion could be influenced by the presence of specific transposable elements in the genom

Effects of ageing on nuclear organisation and induced genome reorganisation in a molluscan model organism

Daniel Adam Horton[1]; Halime D. Arican-Goktas[1]; Gabriel Rinaldi[4]; Matty Knight [2,3]; Joanna M. Bridger[1]

1 Department of Life Sciences, Division of Biosciences, College of Health and Life Sciences, Brunel University London, UK; 2 Department of Microbiology, Immunology & Tropical Medicine, and Research Centre for Neglected Diseases of Poverty, School of Medicine & Health Sciences, The George Washington University, USA; 3 Division of Science & Mathematics, University of the District of Columbia, USA; 4 Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK

The nucleus is a highly organised organelle with chromosomes, and even genes, occupying distinct and reproducible locations. However, this spatial organisation is not fixed and different events such as differentiation, environmental stimuli, stress and replicative senescence can trigger genome reorganisation within nuclei. Replicative senescence is a state whereby cells are unable to divide and it is hypothesised that the accumulation of these cells in organs is responsible for the general decline during ageing. It is hallmarked by changes in genome organisation and our lab has generated data revealing that senescent cells can no longer relocate their genomes.

Biomphalaria glabrata is a freshwater snail employed in our laboratory as an in vivo whole organism model to study gene dynamics. We have previously shown that gene movement and relocation to a new non-random location is possible within a short time period following a heat shock or an infection. *B.glabrata* interphase genome organisation exhibits similarity to mammalian cells, making it an excellent model organism for investigating the effects of genome reorganisation, and with a comparatively short lifespan of 12 months, ideal for exploring, in vivo, ageing related changes to genome organisation.

Using fluorescent imaging approaches, alterations to histone markers, protein distribution and gene loci positioning within nuclei have been investigated at varying ages within B.glabrata. This recapitulates work done in human senescent cells in vitro replicating changes to genome organisation and chromobility. As such we would present B.glabrata as a new potential model for investigating the Nuclear structure, functional modulation of transcription and maintenance of genomic integrity are important aspects that are tightly regulated when cells face extraneous stresses. Hyperthermia is one such stress wherein the induction of heat shock responsive genes is initiated, many of which are molecular chaperones that prevent protein misfolding and preserve nucleoplasmic and ribosomal DNA integrity and quality. Proteins that maintain the nuclear architecture like the nuclear lamins are known to show changes in expression and localization in response to heat shock. However, the molecular mechanisms through which heat shock response may be regulated and fine-tuned by the nuclear lamins are not well understood. Here we show that expression of Lamin A and B1 is upregulated when DLD-1 cells are exposed to heat shock at 42ºC. Functionally, presence of Lamin A/C, B1 and B2 is required for the transcriptional induction of the heat shock gene HSPA1A. Remarkably, Lamin A/C depletion abrogates the heat shock mediated movement of the Hsp70 gene locus towards the nuclear interior and possibly nuclear speckles, which is a pre-requisite for transcriptional upregulation. Taken together, our study highlights a novel role for both A and B-type lamins at two different stages of transcriptional regulation of the Hsp70 gene locus.

Lamin A/C regulates the heat shock induced expression of Hsp70 gene locus by assisting its movement away from nuclear periphery

Dr. Roopali Pradhan, Muhunden Jayakrishnan, Dr. Kundan Sengupta

Dr. Roopali Pradhan - Indian Institute of Science Education and Research (IISER) Pune , India

Muhunden Jayakrishnan - IISER Pune, India / University of Edinburgh, UK

Dr. Kundan Sengupta - IISER Pune, India

Nuclear structure, functional modulation of transcription and maintenance of genomic integrity are important aspects that are tightly regulated when cells face extraneous stresses. Hyperthermia is one such stress wherein the induction of heat shock responsive genes is initiated, many of which are molecular chaperones that prevent protein misfolding and preserve nucleoplasmic and ribosomal DNA integrity and quality. Proteins that maintain the nuclear architecture like the nuclear lamins are known to show changes in expression and localization in response to heat shock. However, the molecular mechanisms through which heat shock response may be regulated and fine-tuned by the nuclear lamins are not well understood. Here we show that expression of Lamin A and B1 is upregulated when DLD-1 cells are exposed to heat shock at 42ºC. Functionally, presence of Lamin A/C, B1 and B2 is required for the transcriptional induction of the heat shock gene HSPA1A. Remarkably, Lamin A/C depletion abrogates the heat shock mediated movement of the Hsp70 gene locus towards the nuclear interior and possibly nuclear speckles, which is a pre-requisite for transcriptional upregulation. Taken together, our study highlights a novel role for both A and B-type lamins at two different stages of transcriptional regulation of the Hsp70 gene locus.

Nuclear envelope dynamics during cell migration in melanoma

Jung Garcia Yaiza Mariela (1, 2, 3), Carlton Jeremy (2, 3)*, Sanz Moreno Victoria (1)*

(1) Barts Cancer Institute, Queen Mary University of London; (2) The Francis Crick Institute; (3) Division of Cancer Studies, King's College London.

* Equal contribution.

The ability of the cell to move its nucleus through tight spaces largely relies on the capacity of the nuclear envelope (NE) to bend while minimising damage to the precious genome. Here, the nuclear lamina provides support and the endosomal sorting complex required for transport (ESCRT) III and associated proteins mediate NE resealing if the NE ruptures. Cells with exigent migratory demands, such as tumour cells, could benefit from a right balance between deformation and nuclear integrity maintenance to disseminate in dense tissue. To test if this is true for migrating melanoma cells, we challenged melanoma cells of different metastatic potential to get through constrictions using transwells and a microfluidic device. In parallel, we interrogated microarray and patient data to select target genes related to NE organisation and function and carried out validation by expression analyses. We showed that metastatic melanoma cells have an advantage over primary melanoma cells to translocate their nuclei through constrictions. Metastatic cells can pass repeatedly through constrictions and repair their NE. Furthermore, we found that metastatic melanoma cells have more of the lamina-associated polypeptide 1 (LAP1) and the ESCRT-III subunit CHMP2B, which correlates with the upregulation observed in metastasis samples from melanoma patient datasets. Altogether, our data indicate that metastatic melanoma cells have a NE that is better suited for constricted migration. The reconstruction of the pathways that make the NE more resilient in metastatic cells could reveal novel vulnerabilities in tumour cell migration that could be exploited therapeutically.

Lamin A overexpression in murine embryonic stem cells blocks neural lineage specification

George Wylde(1,2), Mehdi S Hamouda(1,2,3), Céline Labouesse(1), Kevin J. Chalut(1,2)

(1) Wellcome-Trust-MRC Stem Cell Institute, University of Cambridge, UK; (2) Cavendish Laboratory, Department of Physics, University of Cambridge, UK; (3) Cell Biology and Physiology Center, NHLBI, NIH, Bethesda MD, USA

Embryonic Stem (ES) cells are defined by their competence to contribute to every germ layer. Differentiation and lineage commitment are encoded into gene regulatory networks and determined by a precise sequence of signalling events and epigenetic changes. Activation of lineage markers is also dependent on their nuclear localisation and tethering to the nuclear lamina1,2. Notably, ES cells do not express A-type lamins, which contribute to nuclear stiffness and to chromatin tethering. To better understand the role of the nuclear envelope in gene regulation during differentiation, we ectopically expressed Lamin A (LmnA) in ES cells, and used qPCR, ChIP and staining methods to look at expression, histone methylation and locus localisation of early neural lineage marker Sox1.

We found that LmnA overexpression during a critical time window of early differentiation (first 20hrs) impairs downstream neural lineage commitment at a later stage (days 3-4). We also observed an enrichment of repressive histone mark H3K27me3 at the promoter of Sox1, suggesting some interaction between the nuclear lamina and the Polycomb machinery. Finally, 3D DNA-FISH assays showed that LmnA overexpression results in mislocalisation of Sox1 locus closer to the nuclear periphery. Together, these results suggest that ectopic expression of LmnA hinders the chromatin reorganisation of early differentiation3, resulting in defects in lineage commitment. To test whether this is only due to chromatin tethering or also to changes in nuclear mechanics, we are using perturbations of nuclear envelope proteins to abolish force transmission to the nucleus in differentiating ES cells.

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Characterisation of lamin A/C interaction with phosphoinositides

Rositsa Maystorova, Sara Lopes, Lenka Jarolímová, Margarita Sobol, Vlada Filimonenko, Pavel Hozák

Laboratory of Biology of the Cell Nucleus, Institute of Molecular Genetics of the ASCR, Prague, Czech Republic

Lamins are intermediate filaments found in the nuclei of eukaryotes where they are essential for a range of molecular events. While the role of lamins at the nuclear periphery is well studied, less is known about their assembly, dynamics and interactions in the interior. Recent studies suggest a role of the nucleoplasmic pool in chromatin organization, motion and in its accessibility by regulating epigenetic modifier complexes. The localization and interactions of lamins is proposed to be regulated by their complex post-translational modifications. Importantly, depletion of the nucleoplasmic lamin A is correlated with disease phenotypes like Hutchinson-Gilford progeria.

Preliminary data from the Laboratory of Biology of the Cell Nucleus show lamin A in a complex with nuclear myosin I and a phosphoinositide phosphatidylinositol 4,5-bisphosphate, PIP2. Phosphoinositides have recently been reported in the nuclear interior and implicated in transcriptional regulation (Sobol et al). Many nuclear proteins have been shown to associate with phosphoinositides via lysine/arginine-rich areas in their sequences (K/R-rich motifs) (Jungmichel et al 2014).

We aim to elucidate which domain of lamin A directly binds to PIP2. The lamin A gene was first screened for putative K/R-rich motifs, and four lamin A truncation mutants bearing clusters of those regions were purified from bacteria. The mutants will be tested for direct binding to PIP2 and other phosphoinositides in vitro.

Elucidating the binding domain of lamin A to PIP2 would allow us to disrupt the interaction and address the question of its biological relevance in the context of the lamin A/NMI/PIP2 complex.

Ovarian Cancer Cytogenomics and Nuclear Motors

Aakila Sammy and Joanna Bridger

Brunel University London, Life Sciences, Uxbridge, United Kingdom

In 2012, worldwide there were 152,000 deaths due to ovarian cancer and by 2035 this is projected to increase by 67%. Contributing factors vary by region, however, the two main challenges currently faced are late diagnosis and drug resistance. This study represents a step forward against the disease by probing into the depths of the nucleus, an organelle that codes and arranges essential information by processes not fully understood yet.

To do this we have started by measuring the extent of reorganisation of specific ovarian cancer-related chromosomes and genes whilst comparing them to the cytogenetics of healthy ovarian cells using Fluorescence in-situ Hybridisation (FISH). Also, under examination is chromosome reorganisation in drug-resistant cells to likely elucidate the poorly understood dilemma. If drug resistance is recognised as a cytogenetic issue, investigations would carry onto the nuclear motors such as nuclear myosins that chromosomes have been found to use as an adaptation under different physiological conditions. Using RNA interference (RNAi) in conjunction with FISH, the nuclear motor proteins would be temporarily knocked down thus, starving the chromosomes of their adaptive motor facilitator, where exploration of their cytogenetic stagnancy or changes, as a result, can present therapeutic breakthroughs.

Analytic cytogenomics is a new avenue for ovarian cancer that can possibly replace late diagnosis with early onset diagnosis, to progression and prognosis. Also, understanding drug therapy on the same level can present the potential to refine and personalise treatments to effectively reduce toxic drug dose and resistance.