INVITED SPEAKERS ABSTRACTS

I. Molecular Machines

Resolving proteins structure and interactions to understand molecular machines

John CHRISTODOULOU *(University College London)*
"Protein Folding on the Ribosome"

The ability for proteins to successful acquire their 3D structures is an essential requisite for biological activity within all cells. Our understanding of the fundamental principles underlying the chemical kinetics of protein folding has been derived almost exclusively from the study of renatured isolated proteins using both experimental and theoretical approaches. However, in vivo, protein folding can begin at the earliest stages of biosynthesis, which is carried out by the ribosome. Co-translational folding of nascent polypeptides is a fundamental process that is now beginning to be explored at atomic resolution through improvements in preparative biochemistry and physical and computational techniques. In particular the ability of NMR spectroscopy to provide a simultaneous description of the structure and dynamics of the fledgling nascent polypeptide will be described.

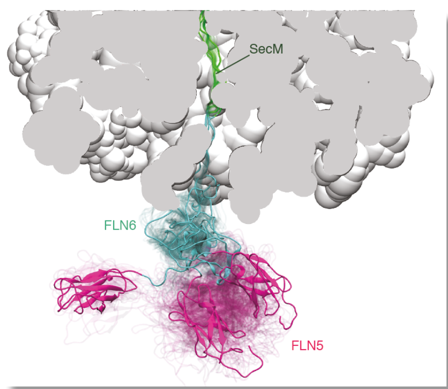


Figure: An NMR and cryoEM restrained structural ensemble of a folded Ig-like domain (FLN5)tethered to the ribosome by its partially translated subsequent domain (FLN6). A number of key interactions between the nascent chain and the ribosome at the ribosomal exit are observed.

Charlie BOONE *(University of Toronto)*
"The Genetic Landscape of a Cell "

We generated a global genetic interaction network for Saccharomyces cerevisiae, testing most of the 18 million possible gene pairs for genetic interactions, identifying ~550,000 negative and ~350,000 positive genetic interactions. This comprehensive network maps genetic interactions for essential gene pairs, highlighting essential genes as densely connected hubs. Genetic interaction profiles enabled assembly of a hierarchical model of cell function, including modules corresponding to protein complexes and pathways, biological processes, and cellular compartments. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. The global network illustrates how coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. We are also exploring more complex interactions involving three different genes. Like their digenic counterparts, trigenic interactions often occurred among functionally related genes and essential genes were hubs on the trigenic network. Despite their functional enrichment, trigenic interactions tended to link genes in distant bioprocesses. Nevertheless, the digenic network underlies the trigenic network because trigenic interactions often overlap a digenic interactions.

Astoundingly, we estimate that the global trigenic interaction network is ~100-fold larger than the global digenic network, highlighting the potential for complex genetic interactions to impact the biology of inheritance, including the genotype to phenotype relationship.

Tom KERPPOLA *(University of Michigan, Ann Arbor)*

"Visualization of transcription regulatory complexes in animals and Development of drugs for rare cancers"

Investigation of molecular mechanisms under physiological conditions requires new methods for the detection of macromolecular complexes in tissues and organs. Our laboratory has developed methods to visualize protein interactions in cells and tissues that are based on complementation between fragments of fluorescent proteins (bimolecular fluorescence complementation analysis). We present an adaptation of these methods to study protein complex binding at specific genetic loci on Drosophila polytene chromosomes. These methods facilitate genome-wide visualization of protein complexes on chromatin in single cells.

One area where the investigation of molecular mechanisms in animals is important is the study of responses to drugs and synthetic compounds. Animals can recognize and respond to xenobiotic compounds, including drugs, and can protect cells from the effects of such compounds. These mechanisms mediate the resistance to drug treatment that often develops in response to cancer chemotherapy.

We investigated the mechanisms of transcription regulation by the xenobiotic response regulators dKeap1 and CncC (homologues of mammalian Keap1 and Nrf2) in Drosophila larvae. Visualization of chromatin binding by dKeap1 and CncC separately and in complexes on polytene chromosomes revealed that they bind and regulate genes that are transcribed in response to endocrine hormones (ecdysteroids). The mechanisms whereby dKeap1 and CncC regulate these genes differ from the mechanisms whereby they regulate transcription in response to xenobiotic compounds.

The limited understanding of molecular mechanisms that determine drug pharmacodynamics and pharmacokinetics is a major cause of the low success rate of drug development. We present the initial results of our efforts to repurpose compounds for the treatment of rare endocrine cancers. We focus on compounds that have undergone advanced pre-clinical characterization to reduce the likelihood of unfavorable outcomes during late stage development and in clinical trials. The initial results suggest that consideration of bioavailability and bioactivity at an early stage of drug development can improve the likelihood that the prospective drugs benefit patients.

Mark HOCHSTRASSER *(Yale University, New Haven)*

"Distinct Toxin-Antidote Modules Underlie Reproductive Manipulation of Insect Hosts by Intracellular Bacteria *Wolbachia*"

Wolbachia are obligate intracellular bacteria that infect many species, including nearly two-thirds of all insect species. These symbionts often manipulate host reproduction to enhance their inheritance through the female germline. The most common mechanism of reproductive alteration is called cytoplasmic incompatibility (CI), wherein eggs from uninfected females fail to develop when fertilized by sperm from Wolbachia-infected males. By contrast, if infected females mate with either infected or uninfected males, the resulting embryos are fully viable. CI is a potent gene-drive mechanism that impacts population structure and evolution of new species, but its molecular mechanisms have remained uncertain. Here we show that Wolbachia operons encoding either a deubiquitylating enzyme (DUB) called CidB or a nuclease called CinB can induce CI. In transgenic fruit flies, the DUB or nuclease appears to act as a toxin when sperm introduce either into eggs. The Wolbachia DUB and nuclease proteins are encoded by genes that are each downstream of factors that bind in a cognate-specific fashion to these enzymes. When expressed in yeast, the DUB or nuclease proteins are also toxic, and their enzyme activity is required for toxicity. Toxicity is suppressed by coexpression of the cognate upstream factor. Our data suggest several distinct mechanisms for CI involving toxin and antidote-like proteins secreted into germline cells by resident bacteria. These results have potential practical applications in limiting the populations of disease vectors, such as the mosquitoes that carry Zika or dengue fever viruses, or insect crop pests.

Tomas KIRCHHAUSEN *(Harvard University, Boston)*

"Imaging subcellular dynamics from molecules to multicellular organisms"

Frontier optical-imaging modalities exemplified by the lattice light-sheet microscope invented by Eric Betzig sets new visualization standards for analyzing and understanding sub-cellular processes in the complex and dynamic three-dimensional environment of living-cells in isolation and within tissues of an organism. By using ultra-thin sheets of light to rapidly illuminate biological samples with extremely low photon doses, 3D experiments previously limited to seconds or minutes by photo-bleaching or by photo-toxicity, can now be done at diffraction limited resolution and high-temporal precision with unprecedented duration of minutes or hours. We believe this ability to image with minimal perturbations is ideally suited to support hypothesis-generating research geared towards new discoveries.

The talk will illustrate how we use lattice light-sheet microscopy to 'see' in three dimensions the intracellular delivery of RNAi and antisense oligonucleotides in cells maintained in tissue culture conditions and also will describe our most recent efforts using lattice light sheet microscopy with adaptive optics to link processes that mediate and regulate the movement of vesicular carriers throughout cells and the biogenesis of organelles in both, isolated cells maintained in tissue culture conditions and cells within tissues of a living zebrafish embryo.

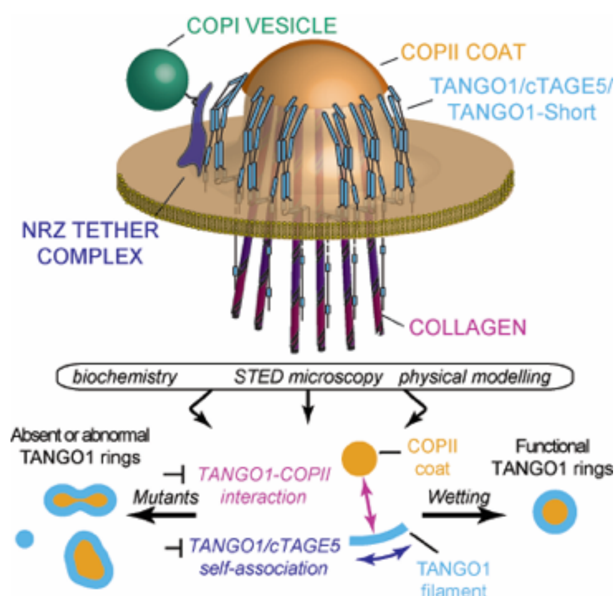
II. Cellular Pathways and Mechanisms

Cellular Pathways and Mechanisms

Vivek MALHOTRA *(Centre for Genomic Regulation, Barcelona)*

"Building a machine at endoplasmic reticulum for collagen export"

Secreted collagens compose 25% of our dry body weight and necessary for tissue organization, and skin and bone formation. But how are these bulky cargoes that are too big to fit into a conventional COPII vesicle exported from the ER? Our discovery of TANGO1 (Bard, Nature 2006; Saito, Cell 2009; Saito, Mol Biol Cell 2011; Santos, J Cell Biol 2016; Malhotra, Ann Rev Cell Dev Biol 2019), a ubiquitously expressed, ER-exit-site-resident, transmembrane protein has made the pathway of collagen secretion amenable to molecular analysis. TANGO1 acts as a scaffold to connect collagens in the lumen to COPII coats on the cytoplasmic side of ER. However, the growth of the collagen containing mega transport carrier is not simply by accretion of a larger COPII coated patch of ER membrane, but instead by rapid addition of premade small vesicles. This mode of transport carrier formation is fundamentally different from that used to produce small COPII vesicles. We have seen that TANGO1 rings the ER exit site and thus organizies a sub compartment within the ER (Nogueira, eLife 2014; Santos, eLife 2015; Raote, J Cell Biol 2017). We have now mapped all the components that work in concert along with the cargo to assemble TANGO1 into a ring (Raote et al., 2018. In review). Mathematical modelling, biochemistry and super resolution microscopy based analyses of this process will be discussed.



TANGO1-family proteins (cyan) assembly into a ring at an ERES is mediated by interactions 1. with COPII (orange) 2. with triple helical collagen (purple), 3. amongst the TANGO1 family proteins 4. with the NRZ tether (dark blue) which links TANGO1 to ERGIC membranes. TANGO1 acts as a lineactant, delaying the binding of the outer COPII coat and allowing for the formation of a mega-carrier.

Alberto LUINI *(Istituto Biochimica Proteine, Roma)*

"Auto-regulatory mechanisms help the membrane transport apparatus to perform in a safe, stable and optimal fashion"

Maintaining the homeostasis and optimal performance of cell processes and organelles is the task of auto-regulatory systems. Some such systems have proven to be of great relevance in human physiology and pathology. We have embarked on a plan to uncover the auto-regulatory systems operating in the transport pathways. Here we describe an auto-regulatory device that helps to maintain the homeostasis of the Endoplasmic Reticulum (ER). The cargo-recruiting subunit of the COP-II coat complex, sec24, doubles as a sensor of folded cargo and, upon cargo binding, acts as a guanine nucleotide exchange factor to activate the signalling protein Gα12. Gα12 in turn activates a complex signalling network that accelerates ER export and attenuates proteins synthesis, preventing large fluctuations of folded and potentially active cargo that could be harmful to the cell or the organism. We term this mechanism as AREX (for AutoRegulation of ER eXport). The impairment of AREX function results in inefficient secretion and accumulation of cargo in the ER. Genetic defects in AREX proteins correlate with problems in secretion and with disease in humans.

Jingshi SHEN *(University of Colorado-Boulder)*

"Genome-scale dissection of mammalian membrane trafficking – new players and unexpected mechanisms"

Cargo proteins moving between organelles are transported by membrane-enclosed vesicles. The core engines mediating vesicle trafficking are now well established. However, we are only beginning to understand the regulatory networks superimposed upon the core engines to adjust the rate and direction of membrane transport according to physiological demands. The advent of the revolutionary CRISPR-Cas9 genome editing system enabled us to systematically identify new components of the regulatory networks. We developed new screening platforms and performed unbiased genome-wide CRISPR genetic screens to dissect the exocytosis and endocytosis of cell surface transporters, fundamental processes in cell physiology. Our screens identified known regulators but most of the hits were not previously known to regulate the pathways. I will focus on the unexpected mechanisms of RABIF/MSS4 in exocytosis and AAGAB in endocytosis. I will also discuss how the principles uncovered in our studies shed light on vesicle trafficking in general.

David DRUBIN *(UC Berkeley)*

"Harnessing actin dynamics for endocytic trafficking"

Clathrin-mediated endocytosis (CME) is the best-studied pathway by which cells selectively internalize molecules from the plasma membrane and surrounding environment. We study this process by live-cell microscopy in yeast and mammalian cells. The yeast studies have revealed a regular sequence of events necessary for endocytic vesicle formation involving some 60 proteins, which induce a highly choreographed series of changes in membrane geometry, ultimately resulting in scission and vesicle release. To analyze endocytic dynamics in mammalian cells in which endogenous protein stoichiometry is preserved, we have used genome editing for the clathrin light chain A and dynamin-2 genomic loci and generated stem cells expressing fluorescent protein fusions from each locus. These cell lines are being used to study actin assembly at endocytic sites and to make 3D organoids in culture. Use nano-patterned substrates, we are actively studying roles for membrane curvature in endocytic dynamics. At the same time, studies in yeast cells have recently focused on discovery of regulatory mechanisms for insuring the proper order and timing of events in the endocytic pathway and how actin assembly at endocytic sites is regulated. Studying the yeast and mammalian systems in parallel is allowing us to translate what is learned from one system to the other.

Judith KLUMPERMAN *(University Medical Center Utrecht)*

"Multitasking CORVET/HOPS complex components mediate multiple pathways in the endo-lysosomal system"

Membrane fusion is a tightly regulate process depending on Rab-GTPases, SNARE proteins and tethering proteins. Multisubunit tethering complexes (MTCs) regulate various aspects of membrane fusion. They establish the first contact between opposing membranes, promote SNARE assembly, regulate movement along microtubules and are involved in membrane bending. Moreover, individual MTC subunits may act in multiple complexes and vesicular transport pathways. Hence, these versatile complexes are emerging as 'multitasking' complexes forming a link between membrane fusion, organelle motility and signalling.

CORVET and HOPS are MTCs required for fusion in the early and late stages of endocytosis, respectively. They share a core of four proteins, which for CORVET is extended with Vps3 and Vps8 and for HOPS with Vps39 and Vps41. Previously it was shown that the CORVET complex is required for fusion between early endosomes, an important step for endosomal maturation and conversion to lysosomes, the main degradative compartments of the cell. Our present studies show that Vps3 and Vps8 are also required for a specialized recycling pathway from early to recycling endosomes important for integrin trafficking (Jonker et al., Nature Comm., 2018). Depletion of Vps3 or Vps8 resulted in defects in integrin-dependent cell adhesion and spreading, focal adhesion formation, and cell migration. Of note, this pathway was independent of other CORVET subunits and did not affect the canonical recycling pathway marked by transferrin receptor. Thus, these data unexpectedly reveal a CORVET-independent role for Vps3 and Vps8 in a specialized recycling pathway emitting from early endosomes.

As part of the HOPS complex, Vps41 is involved in fusion between late endosomes, lysosomes and autophagosomes. Independently of HOPS, Vps41 is required for direct trans-Golgi network (TGN) to lysosome transport of lysosomal membrane proteins (Pols et al., Nature Comm. 2013; Swetha et al., Traffic, 2011). And in secretory cells, Vps41 self-assembly at the TGN promotes formation of secretory granules (Asensio et al., Dev. Cell. 2013). Recently, two brothers were identified bearing compound heterozygous mutations in Vps41 (S285P and R662*), presenting cerebellar atrophy accompanied with ataxia and dystonia. VPS41-R662* is unable to bind to the HOPS complex leading to decreased fusion between late endosomes and lysosomes. VPS41-S285P does bind HOPS but is no longer capable of self-assembly or association with TGN-resembling liposomes. Only when both mutations are expressed the defects lead to a visible phenotype. Based on these data we hypothesize that Vps41 acts in both biosynthetic and endocytotic transport pathways to lysosomes and that these pathways are partially redundant.

A major challenge in studying intracellular transport is to relate the dynamic characteristics of single organelles to their 3D ultrastructure Recently we developed live-cell correlative light-electron microscopy (live-cell-CLEM), which integrates live movies with the corresponding EM image using focused ion beam scanning electron microscopy (FIB-SEM) (Fermie et al., Traffic, 2018). Our method presents a novel way to integrate multiple parameters of subcellular dynamics and architecture onto a single organelle. We will use this methodology to further address the role of MTCs in membrane trafficking, organelle biogenesis and positioning.

III. Intra-and Extra-Cellular Coordination and Communication

Regulation, Coordination and Communication

Ana-Maria LENNON-DUMENIL *(Institut Curie, Paris)*
"Dendritic Cell migration : from microfluidics to in vivo imaging"

The migration of immune cells is guided by specific chemical signals, such as chemokine gradients. Their trajectories can also be diverted by physical cues and obstacles imposed by the cellular environment, such as topography, rigidity, adhesion, or hydraulic resistance. On the example of hydraulic resistance, it was shown that neutrophil preferentially follow paths of least resistance, a phenomenon referred to as barotaxis. We here combined quantitative imaging and physical modeling to show that barotaxis results from a force imbalance at the scale of the cell, which is amplified by the acto-myosin intrinsic polarization capacity. Strikingly, we found that macropinocytosis specifically confers to immature dendritic cells a unique capacity to overcome this physical bias by facilitating external fluid transport across the cell, thereby enhancing their space exploration capacity in vivo and promoting their tissue-patrolling function. Conversely, mature dendritic cells, which down-regulate macropinocytosis, were found to be sensitive to hydraulic resistance. Theoretical modeling suggested that barotaxis, which helps them avoid dead-ends, may accelerate their migration to lymph nodes, where they initiate adaptive immune responses. We conclude that the physical properties of the microenvironment of moving cells can introduce biases in their migratory behaviors but that specific active mechanisms such as macropinocytosis have emerged to diminish the influence of these biases, allowing motile cells to reach their final destination and efficiently fulfill their functions

Bruno GOUD *(Institut Curie, Paris)*
"The RAB6 GTPase, a master regulator of post-Golgi trafficking pathways"

The members of the RAB GTPase family (>60 proteins in man) are master regulators of intracellular transport and membrane trafficking in eukaryotic cells. RAB6 is one of the five ancestral RAB genes conserved from yeast to human. The RAB6 family comprises four proteins, named RAB6A, RAB6A', RAB6B and RAB6C. The two ubiquitously expressed isoforms, RAB6A and A, are generated by alternative splicing of the same gene and localize to the Golgi complex. We and others have established that RAB6 regulates several transport pathways at the level of this organelle.

Our recent work has focused on several aspects of RAB6 function:

- The mechanisms involved in the fission of RAB6-positive transport carriers from Golgi/TGN (Trans-Golgi Network) membranes. We have previously shown that RAB6-Myosin IIA interaction is critical for this process [1]. Recently, we showed that the kinesin protein KIF20A is also involved in the fission process and serves to anchor RAB6 on Golgi/TGN membranes near microtubule (MT) nucleating sites. Our results suggest that the coupling between actin and MT cytoskeletons driven by Myosin II and KIF20A ensures the spatial coordination between the fission of RAB6-positive vesicles from Golgi/TGN membranes and their exit along microtubules [2].
- The role of RAB6 in post-Golgi transport. Using the RUSH system [3] and a variety of secretory cargos, including GPI-anchored proteins, TNF-alpha and Collagen-X, we found that RAB6 associates with post-Golgi vesicles containing all of these cargos. Depletion of RAB6 inhibits their arrival at the plasma membrane. These results suggest that RAB6 could be a general regulator of post-Golgi vesicles, possibly targeting secretory vesicles to defined exocytic sites on the cell surface [4].
- The role of RAB6 in cell lineages and tissues. We have generated mice with a conditional null allele of RAB6A [5]. The mice, which do not express RAB6A and RAB6A', die at an early stage of embryonic development (day 5.5), indicating that RAB6A is an essential gene. The phenotype of RAB6A-/-embryos is very similar to that of beta-1 integrin null embryos. This result is consistent with the finding that a pool of inactive integrins follows the retrograde pathway regulated by RAB6 and that this pathway is critical for adhesion and persistent cell migration [6]. The RAB6 k/o mice were crossed with several mouse lines to deplete RAB6 in various tissues or cell lineages. We will present data illustrating that RAB6 fulfills different functions depending on the cell or tissue context.

References
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2. Miserey-Lenkei et al. Nat Commun. 8, 1254 (2017).
3. Boncompain et al. Nat. Methods 9, 493 (2012).
4. Miserey-Lenkei et al. submitted for publication.

5. Bardin et al. Biol. Cell 107, 427 (2015).
6. Shafaq-Zadah, Gomes-Santos, et al. Nat. Cell Biol. 18, 54 (2015).

Nava SEGEV *(University of Illinois, Chicago)*
"Regulation and coordination of intra-cellular trafficking pathways"

Our research is aimed at understanding how a basic cellular process, trafficking inside cells, is regulated. In the multiple intracellular trafficking pathways, proteins and membranes are transported between intracellular compartments. Individual pathway steps are regulated by molecular switches termed Ypt/Rab GTPases, whose switching depends on upstream activators.

Our goal is to elucidate how Ypt/Rab GTPases and their cognate activators regulate individual transport steps and coordinate them in the same pathway and in different pathways. Landmark discoveries about the mechanisms and machinery that underlie intracellular trafficking were made in yeast and shown to pertain to humans. Therefore, we are using yeast as a model system to address these complicated issues, because it allows easy utilization of sophisticated genetic approaches in combination with molecular and cellular methods. Furthermore, the relatively small number of players (e.g., 11 Ypts in yeast versus ~70 Rabs in humans) and the resultant simplified interaction networks make yeast an excellent model for studying the coordination of transport steps. Our recent findings show that during cell growth, Ypt1 and Ypt31 GTPases together with their activators coordinate transport through the Golgi, the sorting compartment of the secretory pathway. In contrast, under stress, Ypt1 with different activators regulate shuttling of cargo destined for recycling through the autophagy pathway.

This study is highly relevant to human health because multiple essential processes, such as secretion of hormones, presentation of receptors on the outer cell membrane, internalization of ligands and receptors, and response to stress, depend on efficient and well-coordinated intracellular trafficking. Therefore, impairment of trafficking affects every system in the human body, including the development and functioning of the brain, heart, and immune system. Human homologs of the yeast trafficking regulators we study were implicated in multiple human illnesses, including cancer and neurodegenerative diseases.

Keith MOSTOV *(UC San Francisco)*
"Control of length of epithelial tubes in mammals"

Most internal organs consist of tubes lined by a single layer epithelial cells; these tubes usually have a characteristic length. For most organs, little is known about how the length of these tubes is controlled. For example, the small intestine of mammals has a defined length, though very little is known of about the mechanisms that control this length. If a portion of the small intestine is damaged due to disease or injury, either embryonically or postnatally, the length of the small intestine never regenerates. We have uncovered a portion of pathway that controls the length of the small intestine during embryonic development.

Yves BARRAL *(ETH Zürich)*
"Yeast as a model for studying the biology of ageing"

Ageing is defined as the process that causes the decline of organismal fitness and increase of mortality with time that is observed through many organisms throughout the tree of life. However, the underlying biology is poorly understood. The strongest current hypothesis poses that ageing evolves through the selection of mutations that have selective advantages through their effects early in life at the cost of fitness later in time. Interestingly, not all organisms undergo ageing, indicating that it is the result of very specific evolutionary strategy or strategies. We study the process of replicative ageing in the budding yeast Saccharomyces cerevisiae, investigating the nature of the processes that cause ageing of yeast mother cells and promote the rejuvenation of their daughters. In my presentation, I will focus on the role of protein aggregates and DNA circles in replicative ageing and the mechanisms ensuring their retention in the mother cell. I will discuss the possible selective advantages of these ageing factors and their retention in yeast mother cells and the consequence that these advantages could have in the context of neurodegenerative diseases, cancer and innate immunity.

IV. Genomes and Cell fate

Genome expression and manipulation

Ruedi AEBERSOLD *(ETH Zürich)*
"The proteome in context"

The question how genetic variability is translated into phenotypes is fundamental in biology and medicine. Powerful genomic technologies now determine genetic variability at a genomic level and at unprecedented speed, accuracy and (low) cost. Concurrently, life style monitoring devices and improved clinical diagnostic and imaging technologies generate an even larger amount of phenotypic information. However, the molecular mechanisms that translate genotypic variability into phenotypes are poorly understood and it has been challenging to generally make phenotypic predictions from genomic information alone.

The generation of a general model or theory that makes accurate predictions of the effects of genotypic variability on a cell or organism seems out of reach, at least for the intermediate future. We therefore propose that the precise measurement of molecular patterns that best reflect the functional response of cells to (genomic) perturbations would have great scientific significance. We define the term "proteotype" as a particular instance of a proteome in terms of its protein composition and organization of proteins into functional modules.

In the presentation we will discuss recent advances in SWATH/DIA mass spectrometry that support the fast, accurate and reproducible measurement of proteotypes. We will show with specific case studies that i) the proteotype is highly modular, ii) genotypic changes cause complex proteotype changes and iii) that altered proteotypes affect phenotypes.

Overall, the presentation will introduce the proteotype as a close indicator of the biochemical state of a cell that reflects the response of the cell to (genomic) perturbation and is strong determinant of phenotypes.

Romain KOSZUL *(Institut Pasteur, Paris)*
"Probing the dynamics of complex microbial communities using DNA tridimensional contacts"

A fundamental question in biology is the extent to which physiological and environmentally-acquired information can be transmitted from an animal to its descendants. I will present an example of trans-generational epigenetic inheritance where a temperature-induced change in gene expression lasts for >10 generations. I will also present an example of an inter-generational effect whereby the physiological state of an animal (it's age) has a large influence on the characteristics of the next generation.

Ben LEHNER *(Centre for Genomic Regulation, Barcelona)*
"Inter- and trans-generational epigenetic inheritance"

A fundamental question in biology is the extent to which physiological and environmentally-acquired information can be transmitted from an animal to its descendants. I will present an example of trans-generational epigenetic inheritance where a temperature-induced change in gene expression lasts for >10 generations. I will also present an example of an inter-generational effect whereby the physiological state of an animal (it's age) has a large influence on the characteristics of the next generation.

Joel BADER *(Johns Hopkins University, Baltimore)*
"Mistakes in genome design"

Computational design of DNA sequences with defined function is a goal of synthetic biology, but we still lack crucial information required to construct objective functions reflecting reality. Thus, much work in synthetic biology still relies on synthesize-and-screen rather than design-and-build. Because of known unknowns and unknown unknowns, design challenges at the genome level require tradeoffs between the benefits of more ambitious designs and the risks of fatal flaws. The

complete synthesis of the yeast genome by the international Saccharomyces cerevisiae (Sc2.0) consortium has reached a milestone of 3.5 MB out of 12 MB finished in the form of entire synthetic chromosomes replacing their wild-type cognates, which in turn provides a first opportunity to characterize design flaws. We provide an overview of our mistakes, including both systematic errors and random failures, and discuss how we would have revised the design had we known then what we know now. A general conclusion is that our bug rate was very low, about 5x10^-5 in fitness defects per bp changed in protein-coding regions, and that our design was much more robust than we had anticipated.

Miguel SEABRA *(Centro de Estudos de Doenças Crónicas, Lisboa)*
"From gene identification to gene therapy trial in 20 years: the Choroideremia example"

The pace of medical progress since the mid-twentieth century has been astonishing. Here we will provide a personal overview to one such achievement. The case in question is a rare X-linked inherited retinal degeneration called Choroideremia (CHM), whose gene was among the first genes cloned using positional cloning techniques in 1990. The function of this gene in cell physiology was discovered only two years later, and this was due to the power of the (then nascent) gene/protein databases. The sequence of the CHM gene matched that of a protein called Rab Escort Protein because it functions on the lipid modification and activation of an important class of cellular GTPases, the Rab family, regulators of membrane traffic.
Much work since led to the idea that Choroideremia is a disease of intracellular membrane traffic. The loss of the CHM protein REP1 is partially compensated by REP2, a related gene. However selected Rab GTPases are not efficiently acted upon by REP2 and therefore their function in membrane traffic events is missing. The combined deficiencies of multiple Rabs lead over time to significant defects in cellular physiology (premature ageing?) and eventually cell death. Surprisingly, the molecular phenotype of CHM is expressed in all cells but the phenotype is restricted to the retina. A convincing explanation for this fact remains to be had. Development of murine models of CHM using conditional knock-out techniques allowed for the generation of mice with CHM disease restricted to one layer of the retina and therefore allowed study the importance of the two critical cell layers in the retina, the photoreceptors and the retinal pigment epithelium. The disease manifests itself in each diseased layer but pigment epithelium disease accelerates photoreceptor degeneration. The availability of mouse models and a very good understanding of the function of the gene allowed for preclinical gene therapy studies to proceed, using various vectors. Studies with AAV2 eventually provided proof-of-concept evidence to support the design of a phase 1 clinical trial, whose first encouraging results were published in early 2014. That study was recently completed encompassing a 2-year follow-up of 12 patients. Several phase II/III are now underway. It is not too farfetched to predict that gene therapy could be a general treatment for CHM in the next 2-3 years.

V. Disease, Cancer and Aging

From sequencing genomes to cracking human disease and aging

Jean-Philippe VERT *(ENS Paris - MINES ParisTech - Institut Curie - INSERM)*
"Machine learning for patient stratification from genomic data"

The possibility to collect genomic profiles (gene expression, mutations, …) from cancer patients paves the way to automatic patient stratification from genomic data to predict survival, risk of relapse or response to a therapy, for example. The stratification rule itself is usually estimated automatically on retrospective cohorts of patients with both genomic information and output, using a regression or classification algorithm. This estimation problem is however challenging from a statistical point of view, since candidate genomic markers (expression, mutations…) usually outnumber the number of patients in the cohorts. In this talk I will illustrate the difficulty of estimating such genomic signatures, and present a few methods we have developed in recent years to improve the estimation of genomic signatures, in particular the use of gene networks and of permutations to learn signatures from gene expression or somatic mutations.

Nahum SONENBERG *(McGill University, Montréal)*
"Translational control of Autism Spectrum Disorder and Fragile-X syndrome"

Translational control plays a critical role in essential cellular processes including cell growth, proliferation, development, and learning and memory. Under most circumstances, translational control is exerted at the initiation step in which the eukaryotic translation initiation factor 4E (eIF4E) interacts with the mRNA 5'cap structure to facilitate the recruitment of ribosomes and promote translation. Importantly, eIF4E preferentially stimulates the translation of a subset of mRNAs. The activity of eIF4E is regulated chiefly by two major signalling pathways: PI3K/Akt/mTOR and Ras/MAPK/Mnk. mTOR directly phosphorylates the 4E-BPs (eIF4E-binding proteins), which are inhibitors of eIF4E, to relieve translational suppression, while Mnk phosphorylates eIF4E to stimulate translation. Aberrations in these pathways result in dysregulated eIF4E activity, which engenders tumorigenesis and neurological disorders such as autism and Fragile-X syndrome.

Dafna BAR SAGI *(NYU School of Medicine)*
"Oncogenic Ras-dependent Determinants of Tumor Fitness"

Mutations in ras genes are highly prevalent in human cancers and are universally predictive of resistance to virtually all anti-cancer therapeutic modalities. Mutant Ras proteins are endowed with a diverse set of biological capabilities that impinge on homeostatic mechanisms that control cell growth and survival. We have been interested in defining the molecular basis of these capabilities and delineating their contribution to the execution of the oncogenic program. Specifically, our recent efforts have focused on the impact of Ras-dependent oncogenic output on key elements of tumor evolution – metabolic reprogramming, immune evasion and stress resistance. The presentation will provide updates pertaining to these efforts highlighting newly identified Ras-dependent vulnerabilities that could potentially be exploited in the design of therapeutic interventions.

Michael KARIN *(UC San Diego)*
"Metabolic and Immune Control of Liver Tumorigenesis"

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths and develops as a result of chronic liver injury and inflammation. The major causes of chronic liver injury are hepatitis B and C virus infections, but in the US and Europe their pathogenic importance has been eclipsed by non-alcoholic (NASH) and alcoholic (ASH) steatohepatitis. Using a novel mouse model developed in our lab, we have shown that NASH pathogenesis and HCC development depend on endoplasmic reticulum (ER) stress, elevated de novo lipogenesis, ballooning degeneration of hepatocytes, induction of TNF expression and hepatic accumulation of p62. NASH and ASH are also associated with upregulation of immunoglobulin A (IgA), first identified in human patients and confirmed in our mouse model. We found that in both human and mouse livers, elevation of circulating IgA is due to accumulation of IgA-producing plasmablasts and plasma cells. These IgA-producing cells express high amounts of PD-ligand 1 (PD-L1) and IL-10 and possess the ability to

inhibit activation of HCC-directed cytotoxic $CD8^+$ T lymphocytes (CTL). The inhibition of CTL activation prevents liver protective immunosurveillance, thereby resulting in growth and malignant progression of neoplastic nodules that develop as a result of chronic liver inflammation. Importantly, depletion or ablation of liver IgA producing cells results in re-invigoration of HCC-directed CTLs, thereby leading to tumor regression. Thus, treatments that inhibit accumulation of liver IgA-producing cells should be effective in preventing progression from NASH and ASH to HCC and inducing regression of established tumors.

Ludo Van Den BOSCH *(VIB, Flanders, Belgium)*

"From whole genome sequencing to therapeutic strategies in neurodegeneration"

The increasing number of patients suffering from neurodegenerative diseases in our aging Western society becomes a major burden to our social care system. There is currently no cure for any of these disorders. This lack of effective therapies is in a large part due to an insufficient understanding of the etiology and pathogenesis of neurodegenerative disorders. One of the most dramatic neurodegenerative disorders is amyotrophic lateral sclerosis (ALS). It is a fatal neurodegenerative disorder primarily affecting the motor system. The disease presents with progressive muscle weakness and median survival is limited to 36 months after the disease onset. No effective therapies exist. In about 10% of the patients, ALS is a familial disorder. Mutations in 4 different genes (C9orf72, SOD1, TARDBP and FUS) explain a large proportion of these, but the cause of the disease remains enigmatic for the majority of patients. Over the last 2 decades, multiple clinical trials had only negative results, in part because our understanding of the disease mechanisms is insufficient to define clear therapeutic targets. Even in the light of an unknown disease cause, a better understanding of the molecular mechanisms that affect the severity of the disease would greatly advance the ALS field. A considerable degree of heterogeneity exists between patients in terms of both the age of onset and the disease progression rate. This suggest that important genetic modifiers exist. Modifiers of disease progression in ALS are candidate targets for therapeutic interventions. Within the Project MinE consortium, whole genome sequencing (WGS) is already performed on almost 5000 ALS patients and more than 2000 controls. This international collaborative effort is unprecedented and uses the state of the art technology for WGS. The analysis of modifiers of age of onset and of survival of the whole consortium will provide information on genetic modifiers. The hits are prioritized based on the likelihood of being detrimental on protein and regulatory level using various bio-informatical prediction tools. Subsequently, validation of these hits is performed in vitro and in vivo. In vitro models consist of different cell types derived from induced pluripotent cells (iPSCs) derived from ALS patients. In vivo models range from Drosophila, zebrafish and rodents. By performing all these experiments, we will get a better understanding of the molecular mechanisms underlying the disease that could also be responsible for the wide range in age of disease onset and survival after disease onset. Altogether, this could pave the way for the development of novel therapeutic strategies to treat ALS.