[PL01]

THE UBIQUITIN PROTEOLYTIC SYSTEM FROM BASIC MECHANISMS THROUGH HUMAN DISEASES AND ONTO DRUG DEVELOPMENT

Aaron Ciechanover

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Between the 50s and 80s, most studies in biomedicine focused on translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded at certain time points, or when they are not needed, or following denaturation/misfolding whereas their normal counterparts are spared - was not appreciated. The discovery of the lysosome did not significantly change this view, as it was clear that this organelle is involved in degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin pathway solved the enigma. It is clear now that degradation of cellular proteins is a temporally controlled and tightly regulated process that plays major roles in numerous basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

[PL02]

EXPLOITING THE METABOLIC DEPENDENCIES OF CANCER CELLS

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Tumorigenesis is dependent on the reprogramming of cellular metabolism as both direct and indirect consequence of oncogenic mutations. An emerging feature of cancer cell metabolism is the ability to acquire necessary nutrients from a frequently nutrient-poor environment and utilize these nutrients to both maintain viability and build new biomass. The alterations in intracellular metabolites that can accompany cancer-associated metabolic reprogramming have profound effects on gene expression, cellular differentiation and the tumor microenvironment. Cancer-associated metabolic changes can be grouped into several distinct hallmark features: (1) deregulated uptake of glucose and amino acids, (2) use of opportunistic modes of nutrient acquisition, (3) use of glycolysis/TCA cycle intermediates for biosynthesis and NADPH production, (4) increased demand for nitrogen, (5) alterations in metabolite-driven gene regulation, and (6) metabolic interactions with the microenvironment. While few tumors display all six features, most display several. The specific hallmarks exhibited by an individual tumor are improving tumor classification while ultimately directing more individualized approaches to cancer therapy.

[PL03]

GENOMIC ANALYSIS OF LATE ONSET NEURODEGENERATIVE DISEASES

John Hardy

UCL Institute of Neurology, UK

In my lecture I will cover two topics. First, I will review the genetics of Alzheimer's disease, discuss the evidence for the amyloid hypothesis for the disorder and discuss the shortcomings of the hypothesis, why clinical trials have failed so far and what we need to do to achieve some therapy. I will also discuss more generally the genetics of neurodegenerative disease Including AD, PD, FTD and ALS) and suggest that what the genetics of all these disorders has in common is that each disease appears to reflect a genetic failure in damage response pathways. In each disease the damage is different and thus the damage response pathways involved also appear to be different.

[PL04]

PYROPTOSIS: FROM INNATE IMMUNITY TO CANCER

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Pyroptosis, originally known to be activated by caspase-1/4/5/11, is critical for immune defenses and development of many immunological diseases. While caspase-1 is downstream of the inflammasome complex that senses infections, caspase-11 and its human counterparts caspase-4/5 serve as cytosolic receptors for bacterial lipopolysaccharide (LPS) to activate pyroptosis-mediated immune defenses. These caspases cleave Gasdermin D (GSDMD) to release the autoinhibition on its Gasdermin-N domain that executes pyroptosis via an intrinsic membrane pore-forming activity. Gsdmd-/- mice are susceptible to various bacterial infections but also resistant to LPS-induced septic shock. GSDMD belongs to a large Gasdermin family sharing the autoinhibited pore-forming domain. Another family member GSDME harbors a caspase-3-recognition motif also in the middle linker region and can switch caspase-3-induced apoptosis to pyroptosis. Similarly, caspase-3 cleavage releases the pore-forming domain of GSDME, and the resulting pyroptosis also occurs in cells treated with DNA-damaging chemotherapy drugs. GSDME is silenced in most cancer cells but expressed in normal tissues. GSDME-positive cells from normal human tissues undergo caspase-3-dependent pyroptosis in response to chemotherapy drugs. Knockdown of GSDME expression in these primary cells converts the death from pyroptosis to apoptosis. Importantly, Gsdme-/- mice are protected from chemotherapy drug-induced tissue damage and weight loss. These findings define pyroptosis as Gasdermin-mediated programmed necrotic cell death that shall have important functions in a wide spectrum of biological and pathological processes.

[PL05]

MICRORNA REGULATION

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MicroRNA (miRNA) are ~22 nt noncoding RNAs that bind to and suppress mRNAs through post-transcriptional mechanisms. Biogenesis of miRNA is mediated by multiple endoribonucleases and terminal nucleotidyl transferases. The maturation process is initiated by Microprocessor composed of RNase III DROSHA and its cofactor DGCR8, which cleaves the stem-loop of primary microRNA (pri-miRNA) to release pre-miRNA. Pre-miRNA is subsequently processed by DICER to yield a miRNA duplex, which is then loaded onto AGO to form an effector complex. Recent studies have revealed that uridylation and adenylation machineries control miRNA maturation. Uridylation suppresses or stimulates miRNA biogenesis depending on the molecular and cellular contexts whereas adenylation is associated with miRNA decay. In this presentation, I will summarize and discuss our recent progress on the mechanisms underlying the single-nucleotide precision of miRNA processing and its regulation that allows alternative production of isomiRs.

[PL06]

POSING A CONTORTIONIST E3 LIGASE (ANAPHASE-PROMOTING COMPLEX/CYCLOSOME) FOR STEPWISE REGULATION OF CELL DIVISION

Brenda A. Schulman

Max Planck Institute of Biochemistry, USA

The massive, multiprotein E3 ligase Anaphase-Promoting Complex/Cyclosome (APC/C) is a dazzling molecular machine that regulates mitosis, meiosis, and numerous facets of neurobiology by targeting key regulatory proteins for ubiquitin-mediated degradation. APC/C initiates chromosome segregation by promoting ubiquitin-mediated proteolysis of anaphase inhibitors such as Securin, and of B-type cyclins to terminate CDK1 activity. The timing of their degradation is crucial for faithful cell division, which is ensured by extensive regulation of APC/C E3 ligase activity. This involves appropriately timed APC/C phosphorylation, inhibition, activation, and unprecedented mechanisms of ubiquitylation. I will present our current understanding of the structural and molecular mechanisms explaining how this fascinating molecular machine elegantly orchestrates accurate, stepwise progression through the cell cycle.

[PL07]

NUCLEAR TO MITOCHONDRIAL DNA DAMAGE SIGNALING IN NEURODEGENERATION AND AGING

Vilhelm A. Bohr

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We find that some DNA repair defective diseases with severe neurodegeneration have mitochondrial defects. Our studies involve cell lines, the worm (c.elegans), and mouse models and include the conditions Xeroderma pigmentosum group A, Cockaynes syndrome and Ataxia telangiectasia. We find a pattern of hyperparylation, deficiency in the NAD+and Sirtuin signaling and mitochondrial stress. We are pursuing mechanistic studies of this signaling and interventions at different steps to improve mitochondrial health and the neurodegeneration. I will discuss intervention studies in these diseases models including a new Alzheimer mouse model with NAD supplementation. NAD supplementation stimulates mitochondrial functions including mitophagy and stimulates DNA repair pathways. In AT cells, mice and nematode worms, base excision repair is stimulated. This also happens in an Alzheimers mouse deficient in DNA polymerase ß, and this will be discussed. DNA Polß affects mitochondrial functions via its nuclear role, and via a newly identified role in mitochondrial DNA repair.

[PL08]

MITOCHONDRIA, METABOLISM AND AGING

Toren Finkel

Aging Institute, University of Pittsburgh/UPMC, USA

I plan to discuss our interest in mitochondria function and quality control and how these properties might contribute to aging. Briefly, I will discuss our model of increased lifespan with reduced mTOR expression. This model led us to devise new strategies to measure and manipulate mitophagy and autophagy in animal models. I will discuss our approach to measure in vivo mitophagy and ways we are attempting to manipulate mitophagy genetically and pharmacologically. I will also discuss ongoing efforts to understand the role that decreased autophagy might play in vascular aging. Finally, I will discuss how mitochondrial substrate utilization effects cell fate decisions.

[PL09]

CRISPR GENOME EDITING

Jin-Soo Kim

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Genome editing with CRISPR systems that allows targeted mutagenesis in cells and organisms is broadly useful in biology, biotechnology, and medicine. Despite broad interest in CRISPR RNA-guided genome editing, Cas9, Cpf1, and Cas9-fused deaminases (a.k.a., Base Editors) are limited by off-target mutations. We developed nuclease-digested whole genome sequencing (Digenome-seq) to profile genome-wide specificities of Cas9 and Cpf1 nucleases and Cas9-fused deaminases in an unbiased manner. Digenome-seq captured in vitro cleavage sites at single nucleotide resolution and identified off-target sites at which indels or base conversions were induced with frequencies below 0.1%. We also showed that these off-target effects could be avoided by using preassembled ribonucleoproteins (RNPs) and modified guide RNAs. Digenome-seq is a robust, sensitive, unbiased, and cost-effective (< USD 1,500) method for profiling genome-wide off-target effects of programmable nucleases and deaminases.

[PL10]

STRUCTURAL INVESTIGATION OF NAV CHANNELS

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The voltage-gated sodium (Nav) channels are responsible for the initiation and propagation of action potentials. Being associated with a variety of channelopathies, they are targeted by multiple pharmaceutical drugs and natural toxins. We determined the crystal structure of a bacterial Nav channel NavRh in a potentially inactivated state a few years ago, which is a homotetramer in primary sequence but exhibits structural asymmetry. Employing the modern methods of cryo-EM, we recently determined the near atomic resolution structures of a Nav channel from American cockroach (designated NavPaS) and from electric eel (designated EeNav1.4). These structures reveal the folding principle and structural details of the single-chain eukaryotic Nav channels that are distinct from homotetrameric voltage-gated ion channels. Unexpectedly, the two structures were captured in drastically different states. Whereas the structure of NavPaS has a closed pore and the four VSDs in distinct conformations, that of EeNav1.4 is open at the intracelluar gate with VSDs exhibiting similar "up"states. The most striking conformational difference occurs to the III-IV linker, which is essential for fast inactivation. The III-IV undergoes a pronounced repositioning from NavPaS to EeNav1.4, resulting in the insertion of the IFM fast inactivation motif on the III-IV linker into the corner enclosed by the S4-S5 and S6 segments in repeats III and IV of EeNav1.4. Based on the structural features, we suggest an allosteric blocking mechanism for fast inactivation of Nav channels by the IFM motif. Structural comparison of the conformationally distinct EeNav1.4 and NavPaS provides important insights into the electromechanical coupling mechanism of Nav channels and offers the 3D template to map hundredes of disease mutations.

Keyword: Voltage-gated sodium channels, Nav, Cryo-EM, Fast inactivation, Electromechanical coupling

[PL11]

CANCER IMMUNOTHERAPY BY PD-1 BLOCKADE

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PD-1, a negative coreceptor expressed on antigen-stimulated T cells and B cells, seems to serve as a 'rheostat' of the immune response. The molecular mechanisms of the functions of PD-1, in conjunction with the mild, chronic and strain-specific autoimmune phenotypes of PD-1-deficient mice, suggest that immunoregulation by PD-1 is rather antigen specific and is mainly cell intrinsic. Such unique properties make PD-1 a powerful target for immunological therapy, with highly effective clinical applications for cancer treatment.

In fact, immune checkpoint blockade with anti-PD-1 has revolutionalized cancer therapy as it has many advantages over the other treatments; (a) applicable to almost all types of cancer at any stages; (b)long duration; and (c) weak side-effects. It is most likely that anti-PD-1 will be the first choice of cancer treatment in a near future. The striking effects of anti-PD-1 depend on three basic principles; (a) the immune system can recognize mutated cancer antigens (b) the diversity of the immune repertoire is much larger than variations generated by mutations in tumor cells, and (c) the immune system is tolerized in tumor patients by excessive negative regulations of the immune system. I will provide historical perspective how we reached the new innovation of cancer treatment and discuss future perspective.

[PL12]

CELL PLASTICITY IN DEVELOPMENT AND DISEASE

Angela Nieto

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Epithelial homeostasis is crucial to maintain tissue architecture, and therefore, it needs to be tightly regulated in the adult. By contrast, embryonic cells show a high degree of epithelial plasticity required for proper morphogenesis and, in particular, for the implementation of massive cell movements that occur during gastrulation and neural crest delamination among other processes. We have been interested in the analysis of cell movements, plasticity and epithelial to mesenchymal transitions (EMT) for many years, and found that the reactivation of developmental EMT-like programs in adult cells leads to several pathologies including tumor progression and organ degeneration. While the epithelial and mesenchymal cells are usually considered as extreme phenotypes, intermediate EMT states also exist. Under those circumstances cells depict a hybrid phenotype expressing both epithelial and mesenchymal markers and from which they can reverse to the original state or move towards a more mesenchymal phenotype.

Hybrid transitory states can favor coordinated cell migration or wound healing but they can also enable the formation of clusters of migratory cancer cells with increased metastatic potential. However, in contrast to the situation in cancer, the intermediate phenotype holds promise for new antifibrotic therapeutic approaches, as inhibiting EMT can attenuate established fibrosis. I will discuss

different scenarios in which this intermediate phenotype is observed both in development and in disease, and will also refer to a new developmental EMT that we have found to be crucial for heart laterality and morphogenesis in vertebrates.

References:

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[PL13]

FOLDING, UNFOLDING AND REFOLDING OF GENOMES

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In order to understand how the genome operates, we need to understand not only the linear encoding of information along chromosomes, but also its 3-dimensional organization. The spatial organization of the genome is critical for gene regulation, genome stability and faithful transmission of chromosomes to daughter cells. Chromosome Conformation Capture-based technologies and live cell and high-resolution imaging approaches are now widely used to determine how cells fold their chromosomes, to discover the processes that drive the spatial organization of genomes and to identify the mechanisms by which this organization contributes to genome regulation and activity.

At the nuclear level, chromosomes are compartmentalized into large multi-Mb compartments that are either active and open or inactive and closed. At the scale of hundreds of Kb chromosomes form Topologically Associating domains (TADs). Gene regulation occurs mostly within TADs through long-range looping interactions between genes and regulatory elements. In mitotic cells chromosome conformation is completely different: inside compact metaphase chromosomes the genome folds as longitudinally compressed randomly positioned loop arrays, consistent with classical models proposed by the Laemmli lab.

I will present recent insights into the detailed spatial arrangement of the genome at different stages of the cell cycle, and the folding pathways by which these states interconvert. I will describe new folding intermediates and the roles of key protein complexes such as CTCF, cohesin and condensins. Finally I will discuss a common mechanism by which the genome folds, unfolds and refolds to facilitate gene regulation and chromosome transmission.

Keyword: Chromosome folding, Mitosis, Chromatin loops, Epigenetics