Plenary Lectures

PL.1

Structural studies of medically-interesting protease inhibitors and lectins

Alexander Wlodawer[1], Jacek Lubkowski[1], Alla Gustchina[1], Dongwen Zhou[1], Michal Jakob[1,2], Barry R. O'Keefe[2], Rodrigo da Silva Ferreira[3], Yara A. Lobo[3], Daiane Hansen[3], Maria L. V. Oliva[3]

[1]Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA; [2]Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA; [3]Departamento de Bioquímica, Universidade Federal de São Paulo, 04044-020 São Paulo, SP, Brazil

Alexander Wlodawer <wlodawer@nih.gov>

Several protease inhibitors and lectins with anti-cancer properties have been investigated by X-ray crystallography as well as by biochemical and biophysical techniques. Two of them are potent inhibitors of trypsin-related enzymes. EcTI, isolated from the seeds of *Enterolobium contortisiliquum*, inhibits the invasion of gastric cancer cells through alterations in integrin-dependent cell-signaling pathway. BbKI, found in *Bauhinia bauhinioides* seeds, is a kallikrein inhibitor with a reactive site sequence similar to that of kinins, the vasoactive peptides inserted in kininogen moieties. A much weaker protease inhibitor isolated from the bark of *Crataeva tapia* tree (CrataBL) also functions as a lectin. BfL, a GalNAc-specific lectin from Brazilian orchid tree *Bauhinia forficata* was shown to inhibit growth of several cancer lines. CGL, a lectin isolated from the sea mussel *Crenomytilus grayanus*, was investigated based mainly on the similarity of its sequence to another lectin, MytiLec, which was resistant to crystallization.

We determined high-resolution crystal structures of free EcTI and in complex with bovine trypsin, in the process re-determining the amino acid sequence. Modeling of the putative complexes of EcTI with several serine proteases and a comparison with equivalent models for other Kunitz inhibitors elucidated the structural basis for the fine differences in their specificity. The structure of free BbKI indicated that the presence of disulfide bonds is not necessary for stabilization of the fold of the members of this family. A model of a complex of BbKI with plasma kallikrein indicates the need for mutual rearrangement of the interacting molecules.

We have also determined the high-resolution crystal structure of glycosylated CrataBL. We have shown that, as a lectin, CrataBL binds only sulfated oligosaccharides, most likely heparin and its derivatives.

CGL displays antibacterial, antifungal, and antiviral activities, and displays high affinity for mucin-type receptors, abundant on some cancer cells. We determined its crystal structure and modeled the glycan-binding pockets, based on the location of the glycerol molecules bound in the three sites exhibiting quasi-threefold symmetry.

A number of structures of BfL elucidated the mode of binding of its primary ligand GalNAc, as well of a number of cancer-related Tn-antigens and blood group antigens, explaining the basis of its very strict specificity, similar to the specificity of CGL despite a completely different three-dimensional structure.

Keywords: protease inhibitors, lectins, crystal structures

PL.2

Chemistry that converts light into vision

Krzysztof Palczewski

Department of Pharmacology and Cleveland Center for Membrane and Structural Biology School of Medicine, Case Western Reserve University, USA

Krzysztof Palczewski <kxp65@case.edu>

The retina converts light into an electrical signal through a series of biochemical steps collectively referred to as phototransduction. This signal is eventually relayed to the visual cortex of the brain, where visual perception occurs. Photoreceptor cells are able to respond to light throughout our lives because they have the ability to regenerate proteins as well as a light-sensitive chromophore. The long-term objective of our research is to elucidate the molecular reactions involved in phototransduction, including those directly involved in the regenerative capability of photoreceptor cells. Phototransduction serves as a prototype for a multitude of G protein-mediated signal transduction events initiated by activation of G protein-coupled receptors (GPCRs) and thus understanding of this process is broadly applicable to other signal transduction cascades. Although outnumbered more than 20:1 by rod photoreceptors, cone cells in the human retina mediate daylight vision and are critical for visual acuity and color discrimination. Originating in the early 1900s, past research has begun to provide insights into cone ultrastructure but has yet to afford an overall perspective of cone cell organization. Mutations in the rod and cone genes encoding are among the main causes of blinding diseases in humans. We ultimate goal to advance our understanding of the molecular basis of vision and to develop strategies to stop progression of human retinal diseases using animal models. Pharmacologic interventions to save vision are now within reach due to a significantly improved understanding of these chemical transformations.

PL.3

Linear and circular RNAs

Nikolaus Rajewsky

Systems Biology of Gene Regulatory Elements, Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany
Nikolaus Rajewsky <rajewsky@mdc-berlin.de>

I will summarize our current understanding of the biogenesis and function of circular RNAs. I will then present recent insights into splicing mechanisms that guide differentiation from totipotent stem cells into differentiated cells (*in vivo*). Finally I will present single cell sequencing methods that allow us to quantify transcriptomes in thousands of cells at a very low cost. We use these methods for identifying novel cell types and markers in neural tissues or in the embryo.

PL.4

Next generation antibiotics

Ada Yonath

Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel
Ada Yonath <ada.yonath@weizmann.ac.il>

Resistance to antibiotics and the spread of their metabolites are severe problems in contemporary medicine and ecology. Structures of complexes of eubacterial-ribosomes with antibiotics paralyzing them illuminated common pathways in inhibitory-actions, synergism, differentiation and resistance. Recent studies on ribosomes from a multi-resistant pathogens identified features that can account for species-specific diversity in infectious-diseases susceptibility. These may be exploited for the design of environmental-friendly degradable antibiotics, which may also be species-specific, namely a revolution in the antibiotics field which will reduce resistance while protecting the environment and preserving the microbiome.