Monday 22nd July

MACROMOLECULAR COMPLEXES

O-063

Recent insights into the peptide-loading complex machinery

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Abstract
Identifying and eliminating infected or malignantly transformed cells are fundamental tasks of the adaptive immune system. For immune surveillance, the cell's metastable proteome is displayed as short peptides on major histocompatibility complex class I (MHC I) molecules to cytotoxic T-lymphocytes. Our knowledge about the track from the proteome to the presentation of peptides has greatly expanded, leading to a quite comprehensive understanding of the antigen-processing pathway, which comprises many transient and dynamic macromolecular machineries. I will report on the mechanisms of antigen translocation, chaperoning, and editing, as well as on quality control mechanisms for peptide-MHC I complexes that are key for the understanding of autoimmune diseases. Based on an integrative approach, we have elucidated the contribution of individual proteins to the architecture of the MHC I peptide-loading complex (PLC) and other MHC I editing complexes. Consequences of viral immune evasion strategies will be discussed. The data provide a framework for a mechanistic understanding of quality control steps during antigen selection and unveil the molecular details underlying the onset of an adaptive immune response.

O-064

LUBAC and linear ubiquitin chains: novel tools to study immune signalling

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Linear ubiquitin chains linked through Met1 are important players of immune and inflammatory signalling and apoptotic cell death. They are generated by a multi-subunit E3 ligase complex called linear ubiquitin assembly complex (LUBAC) that is thus far the only E3 ligase capable of forming linear ubiquitin chains. The complex consists of three subunits, HOIP, HOIL-1L and SHARPIN, with the HOIP subunit providing the E3 ligase activity of the complex. While the HOIL-1L and SHARPIN subunits are clearly required for the overall activity of the complex, their precise contribution to the observed biological functions of LUBAC remains unclear. I will discuss recent work on the design of novel tools to study LUBAC function and interfere with activity in a cellular context.

O-065

Analysing cryoEM data quality in the post resolution revolution era: Validity criteria

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Cryo Electron Microscopy (cryoEM) is possibly the fastest growing area in Structural Biology. Many specimens are amenable to their study by Electron Microscopy, and a logical question inside and outside of the cryoEM field is to ask for information about the quality and possible "peculiarities" of this new boom of data. Logically, we are all interested in validity criteria. In this talk I will start reviewing current practices in cryoEM to help addressing this issue, to then introduce a new quality/validation criterion that has the capacity to work only on the cryoEM maps and to derive an impressive amount of new quantitative information about map quality. We refer to the new Directional Local Resolution

O-066 (P-185)

Histone tails in nucleosome: fuzzy interaction with DNA

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New evidence from NMR spectroscopy suggests that histone tails remain highly dynamic even in the condensed state of chromatin. To probe the dynamic behavior of H4 histone N-terminal tail in greater detail, we prepared a sample of mononucleosome containing ^{15}N,^{13}C-labeled H4 histone. The HSQC spectrum of this sample features observable signals from the first fifteen residues in H4; half of these signals have been successfully assigned and used for site-specific ^{15}N relaxation measurements. The experimentally obtained chemical shifts and relaxation rates paint the picture of moderately mobile H4 tail with random-coil-like conformational properties. We have also recorded a µs-long MD trajectory of mononucleosome in the explicit TIP4P-D solvent, which has been designed specifically for (partially) disordered protein systems. This trajectory successfully reproduced the experimentally measured chemical shifts and relaxation rate constants. According to the MD data, the positively charged H4 tail hovers over the negatively charged ds-DNA, making transient contacts with both DNA backbone and major/minor grooves. This type of behavior, underpinned by electrostatic attraction and characterized by substantial mobility of H4 tail relative to the DNA chain, can be classified as "fuzzy interaction". The research was supported by RSF grant 15-14-20038 (modeling component) and NIH grant GM118664 (experimental component).

O-067 (P-186)

New protein-protein interaction modulators for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration

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The protein complex formed by the Ca^{2+} sensor neuronal calcium sensor 1 (NCS-1) and the guanine exchange factor protein Ric8a co-regulates synapse number and probability of neurotransmitter release, emerging as a potential therapeutic target for diseases affecting synapses [1]. In neurodevelopmental disorders, such as Fragile X syndrome (FXS) or Autism, neurons show an abnormally high synapse number. On the contrary, in neurodegeneration, such as Alzheimer´s, Huntington´s or Parkinson´s diseases, patients show a low synapse number. In the recent years, we have been investigating the structural basis of the NCS-1/Ric8a interaction and found out that the formation of this complex is essential to increase synapse number [1,2]. Therefore, an inhibition of the NCS-1/Ric8a complex would constitute a potential strategy to regulate synapse function in FXS and related disorders. Conversely, the stabilization of this protein-protein interaction could be key to regulate synapses in neurodegeneration. With this aim, virtual screenings and dynamic combinatorial chemistry approaches have been used to find out regulatory molecules of this protein-protein interaction. Further, a multidisciplinary approach including, biochemical, biophysical, crystallographic, cellular and *in vivo* studies have been performed to demonstrate the activity of the compounds, their therapeutic potential and molecular mechanism of action [3,4].

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[2] Baños-Mateos, S. *et al.* (2014) *Acta Crystallographica* A**70**, 530-534.
[3] Mansilla, A. et al. (2017) *PNAS* **114(6)**, E999-E1008.
[4] Canal-Martín, A. et al. (2019) *Under review*.