
Session 3: EMBO Session

Lectures

L3.1

Dynamic rearrangements of the spliceosomal catalytic center

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All current cryo-EM spliceosome structures show the catalytic center in a conformation that does not change between the catalytic and non-catalytic phases of splicing. This structure includes the U6 intramolecular stem-loop (ISL), the adjacent triple stranded U2-U6 structure, and the U2-Branch Site (U2-BS) duplex, and is conserved between the spliceosome and group II introns. Using yeast genetic approaches, we show that the catalytic center undergoes specific conformational changes that facilitate transitions between the first and second steps of splicing. We conclude that two competing conformations of the catalytic center form transiently during the transition between the first and second steps of splicing.

L3.2

Mysteries of entanglement – from a single protein to a pair of chromosomes

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Topology plays an ever-increasing role in modern life sciences since the discovery and artificial creation of knots in DNA and proteins. Nowadays, at least 6% of proteins are known to be knotted, slipknotted, linked [1] or to contain lassos [2], even though for a long time it was believed that proteins should not be entangled. Knotted proteins are believed to be functionally advantageous and to provide extra stability to protein chains. During the talk, I will discuss available methods to analyze entanglement in the open chains and present a new type of entanglement in proteins. It is well-known that the 3D structure of the genome plays a critical role in regulating gene expression. Recent developments have for the first time enabled the determination of three-dimensional structures of individual chromosomes and genomes based on Hi-C chromosome conformation contact data. While some model structures are highly knotted, other models based also on Hi-C data predict only few knots. Moreover recently we found that maybe some chromosomes could be linked. Even though the abundance of entanglements in chromosomes is still controversial, there is a clear need to check model structures for entanglements, in particular when higher resolution data becomes available in the near future. During the talk, I will present the KnotGenome server – the first server that detects and characterizes knots in single chromosomes, as well as links between chromosomes [3].

References:

1. Dabrowski-Tumanski P, Sulkowska JI (2017) Topological knots and links in proteins. *PNAS* **114**: 3415–3420.
2. Niemyska W *et al.* (2016) Complex lasso: new entangled motifs in proteins. *Scientific Reports* doi: 10.1038/srep36895.
3. Sulkowska JI *et al.* (2018) KnotGenom: server to detect entanglement in and between chromosomes, NAR.

L3.3

Control of meiotic recombination: from chromosomal distribution to crossover frequency

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During meiosis homologous chromosomes pair and reciprocally exchange their fragments in a process called crossover. This is required for proper chromosome segregation and for reshuffling of genetic information from both parents. For this reason, crossover significantly contributes to variation between individuals in natural populations. Due to its significance, crossover number and spatial distribution along the chromosome are tightly controlled. In my talk I will discuss current progress on our understanding of the crossover control in eukaryotes with a special focus on plants. Specifically, I will show our recent data on the sensitivity of different crossover pathways towards sequence polymorphisms between the chromosomes and the effects on crossover distribution. Next, I will present our attempts to identify crossover modifiers – genes, which can affect the crossover frequency in a genome-wide scale. Finally, I will discuss the perspective of application of this knowledge for developing of new plant breeding strategies.

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L3.4

Inflammatory signaling from the endocytic pathway

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Endocytosis was initially viewed as a mechanism of signal termination through the downregulation and degradation of surface receptors. However, more recent data argue that endosomal compartments contribute to intracellular signaling by transporting ligand-receptor complexes and affecting their activity inside the cell.

Cytokine receptors such as TNFR1 (tumor necrosis factor receptor) or lymphotoxin β receptor (LT β R) induce inflammatory NF- κ B signaling but their endocytic trafficking remains understudied. We have recently uncovered a role of Endosomal Sorting Complexes Required for Transport (ESCRT) in trafficking of cytokine receptors, preventing their signaling. Upon ESCRT depletion, defects in transport of these receptors can induce their local accumulation, oligomerization and intracellular signaling from endosomes in a ligand-independent manner.

We further found that accumulation of ligand-free LT β R on endosomes occurred also upon depletion of two regulators of late endosome function, namely the HOPS complex and Rab7A but in this case without inducing NF- κ B signaling. We demonstrated that LT β R accumulated on the outer membrane of endosomes after ESCRT depletion. In contrast, HOPS or Rab7A knockdown caused LT β R accumulation inside endosomes in intraluminal vesicles.

We propose that impairment of endocytic sorting of LT β R can cause its perturbed localization and accumulation. However, NF- κ B signaling is induced only when LT β R accumulates on the outer membrane of endosomes.

L3.5

How to end a viral RNA – lessons from the IFIT proteins

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Detection of viral infection in the cell often occurs by recognition of unusual features in the RNA present in the cytosol that differ from the properties of the host transcripts. The interferon-induced proteins with tetratricopeptide repeats (IFITs) are potent innate immune effectors that bind non-self RNA, which results in the inhibition of translation of viral transcripts. Among human IFIT proteins, the monomeric IFIT5 recognizes the 5' triphosphate (PPP) group on RNA, the dimeric IFIT1 preferentially binds cap 0 groups, IFIT2 prefers dsRNA and IFIT3 has no known RNA-binding capability. We study the role of the higher-order IFIT complexes, in particular the interaction of IFIT1 with IFIT2 and IFIT3. In cells, IFIT1 and IFIT3 re-distributed and co-localized together with PPP-RNA, and associated together even in the absence of viral infection, whereas IFIT2 was recruited to IFIT1 only in infected cells. The IFIT1-IFIT3 assembly is mediated by the last tetratricopeptide repeat motifs in both proteins, and results in reorganization of the RNA-binding site in IFIT1, so that the IFIT1-IFIT3 complex binds RNA with a higher affinity than IFIT1 alone. We propose a role for IFIT3 as a constitutive partner and an enhancer of IFIT1 activity. Regulation of the IFIT1-IFIT3 complex may provide additional possibility for signal integration in the antiviral response.

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L3.6

Chaperone-directed ubiquitylation maintains proteostasis at the expense of longevity

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The integrity of the cellular proteome is supported by quality control networks, which govern protein synthesis, folding, and degradation. It is generally accepted that an age-related decline in protein homeostasis (proteostasis) contributes to protein aggregation diseases. However, the mechanistic principles underlying proteostasis imbalance and the impact on life expectancy are not well understood. We recently demonstrated that this interrelation is affected by chaperone-directed ubiquitylation, shifting the amount of the conserved DAF-2/insulin receptor both in *Caenorhabditis elegans* and *Drosophila melanogaster*. The ubiquitin ligase CHIP either targets the membrane-bound insulin receptor or misfolded proteins for degradation, which depends on the cellular proteostasis status. Increased proteotoxicity triggers chaperone-assisted redirection of CHIP toward protein aggregates, limiting its capacity to degrade the insulin receptor and prevent premature aging. In light of these findings, I will discuss a new concept for understanding the impact of proteome imbalance on longevity risk.

L3.7

Undercover translation. Hidden secrets of the ribosome

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Ribosomes are a centre of every living cell. For decades, they were perceived as homogeneous macromolecules carrying a constant set of ribosomal RNAs and proteins. Consequently, they were not considered to actively participate in the regulatory role of gene expression. The hypothesis of specialized ribosomes assumes the existence of a sub-population of ribosomes carrying unique structural properties allowing fast and precise response to environmental stimuli throughout selectivity for distinct mRNAs.

We use sporulation in *Bacillus subtilis* bacteria as a model to study regulation of gene expression on translational level. Using combination of ribosome profiling, genetics, biochemistry and microscopy, we aim to identify factors modulating translation and accounting for ribosomal selectivity towards mRNAs. Initial data shows massive global rearrangements in proteins synthesis profile and unveils interesting events, like expression of previously unannotated genes, occurrence of paralogues of ribosomal proteins or rearrangements in the ribosomal structure – implying a presence of distinct sub-sets of ribosomes.

This work will shed more light on how translation contributes to the gene expression regulation during sporulation. Finding specialised ribosomes will add a new level of regulation of gene expression with a ribosome as an active element.