Thursday, March 13, Session I

L5

APPLICATION OF DIFFERENCE 3D ELECTRON MICROSCOPY TO IDENTIFY DSS1 SUBUNITS AND INDUCED CONFORMATIONAL CHANGES IN THE COMPLEX WITH RAD52

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The Rad52 protein is a recombination enzyme conserved among eukaryotic organisms that plays an important role in the initial steps of the homology-directed DNA repair by loading Rad51 recombinase on ssDNA as well as by catalyzing annealing of two complementary ssDNA strands [1]. However in human cells, the recombination mediator function is carried out primarily by BRCA2, while Rad52 constitutes a secondary pathway [2]. The BRCA2 protein is stabilized by a small highly acidic protein DSS1 [3]. We discovered that DSS1 is also able to directly interact with human Rad52 and might regulate homologous recombination repair in mammalian cells.

Previous biophysical and structural studies have shown that the full-length human Rad52 protein assembles into a heptameric ring with a funnel-like shape [4]. The conserved N-terminal part of human Rad52 was crystallized as an undecameric ring and provided insight into the interaction of Rad52 with DNA [5, 6]. However, the molecular mechanism and structural basis of the full-length Rad52 function as well as the role of the DSS1 protein remain unknown.

We used electron microscopy and 3-D image processing to determine the structure of the full-length Rad52 heptamer and its complex with DSS1. Initial reference-free class averages of Rad52 appeared as tilted top-side views of rings with a diameter 110-130 Å, consistently with previous studies [4]. Refined 3-D structure of the ring using C7 symmetry showed a funnel-like structure with a central channel wide ~40 Å. The top and bottom rims of the central channel are connected by strong densities that form the body of the channel and likely correspond to the compact fold of the Rad52 N-terminal part. The top of the channel is covered by a weak density.

The 3D reconstruction of the Rad52-DSS1 complex revealed significant conformational changes induced by DSS1. The Rad52 ring is wider and flatter, while the bottom of the central channel is sealed with solid density. The lobes at the ring circumference are larger and likely include additional density of the 8-kDa DSS1 subunits. The DSS1 subunits could this way act as a molecular switch by modulating Rad52 affinity towards DNA.

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[3] Li, J. et al. (2006) *Oncogene*, 25:1186-94.

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L6

INITIAL BRIDGES BETWEEN THE TWO RIBOSOMAL SUBUNITS ARE FORMED WITHIN 9.4 MILLISECONDS: A TIME-RESOLVED CRYO-EM STUDY

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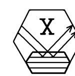
The bacterial 70S ribosome are held together by 13 dynamic bridges between its two subunits (30S and 50S) involving RNA-RNA, RNA-protein, and protein-protein interactions. We have developed and implemented a class of microfluidic devices that mixes two components to completion within 0.4 milliseconds (ms) and sprays the mixture



in the form of microdroplets onto a cryo-electron microscopy grid, yielding a minimum reaction time of 9.4 ms before cryo-fixation. We have used such devices to study association between the two *E. coli* ribosomal subunits, by collecting cryo-EM data corresponding to reaction times of 9.4 and 43 ms. According to our image analysis, about 25 % of ribosomal subunits are already engaged in the formation of 70S ribosome particles within 9.4 ms, and at 43 ms, 49 % of the ribosomal subunits have undergone formation of 70S ribosomes. Molecular analysis of the corresponding three-dimensional reconstructions suggests that the inter-subunit bridges B2a, B2b, B3, and B7a form within 9.4 ms and bridges such as bridges B2c, B4, B5, and B6 take longer than 43 ms to form. This approach can be used to characterize the sequence of dynamic functional events on a complex macromolecular assembly, such as the ribosome.

L7

CHEMICAL CROSS-LINKING AND HYDROGEN-DEUTERIUM EXCHANGE –TOOLS FOR PRECISE DEFINITION OF HUMAN HAPTOGLOBIN STRUCTURE

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Haptoglobin (Hp) is acute phase plasma glycoprotein that binds hemoglobin (Hb) dimer to extraordinarily strong complex. The forming Hb-Hp complex is subjected to CD163-mediated endocytosis by macrophages and also prevents renal filtration of Hb in kidneys. Humans possess three phenotypes of Hp, reffered as Hp(1-1), Hp(2-1), and Hp(2-2). These variants differ in structure and function. The Hp(1-1) phenotype is composed of one 1 and subunits, the Hp(2-1) phenotype consists of 1 monomer and 2 monomer and Hp(2-2) phenotype is created by 2 and subunits. Although molecular model of human haptoglobin based on the X-ray structure of porcine hemoglobin-haptoglobin complex was described, unfortunately no structure of human haptoglobin has been revealed so far. The only structure was created based on the molecular modeling and CD spectroscopy.

In this study we present a workflow for detail structural characterization of human haptoglobin by using homobifunctional cross-linking reagents (BS2G, BS3, DSG, DSS and ADH) in combination with reverse-phase chromatography coupled to FT-ICR mass spectrometer. The knowledge of distance constrains between modified amino acids allowed us to re-model the structure of human haptoglobin monomer. On the other hand, cross-linkers between subunits enabled us to reconstitute the structure of haptoglobin multimer. The hydrogen-deuterium exchange approach was used to monitor the haptoglobin surface, which is partially covered by complex glycans. Combination of protein chemistry with analytical tools mentioned above can lead to better design of the structure of human haptoglobin and its multimers in solution.

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L8

COMBINATION OF XTAL, SAXS, DEER AND FRET DATA – THE STRUCTURE OF ESCRT-I/II SUPERCOMPLEX AND IMPLICATIONS FOR MEMBRANE BUDDING

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The ESCRT-I and ESCRT-II supercomplex induces membrane buds that invaginate into the lumen of endosomes, a process central to the lysosomal degradation of ubiquitinated membrane proteins. The solution conformation of the membranebudding ESCRT-I-II supercomplex from yeast was refined against small-angle X-ray scattering (SAXS), single-molecule Förster resonance energy transfer (smFRET), and double electron-electron resonance (DEER) spectra. These refinements yielded an ensemble of 18 ESCRT-I-II supercomplex structures that range from compact to highly extended. The crescent shapes of the ESCRT-I-II supercomplex structures provide the basis for a detailed mechanistic model, in which ESCRT-I-II stabilizes membrane buds and coordinates cargo sorting by lining the pore of the nascent bud necks. The hybrid refinement used here is general and should be applicable to other dynamic multiprotein assmeblies.