target for the first anti-HIV drugs, it still has potential for development of new drugs including the targeting of as yet unexploited regions such as the RNaseH active site and tRNA primer binding.

Keywords: HIV reverse transcriptases, inhibitor binding, drug resistance

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Structure-based Vaccine Design of Human Rhinovirus: HIV Chimeras as Candidate AIDS Vaccines

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Our laboratory team has developed a system for generating combinatorial libraries of cold-causing human rhinoviruses (HRVs) that effectively display immunogenic peptide segments from a variety of pathogens. We have used this system to generate chimeric HRV-HIV-1 viruses displaying regions of the HIV-1 membrane-spanning protein gp41 that are part of the conserved and critical viral fusion machinery. We have generated chimeric HRVs displaying the so-called ELDKWA epitope of this region of gp41 that elicit immune responses able to broadly and potently cross-neutralize HIV-1 primary isolates, the first neutralizing responses reported for any ELDKWA-based immunogens. Ultimately, such immunogens might serve as valuable constituents in an AIDS vaccine.

Structural considerations for this vaccine engineering system will be discussed. We have obtained diffraction data at CHESS and BNL for several HRV:HIV-1 chimeras; structure determination is in progress. We are also investigating structures of chimeric virus complexed with anti-HIV neutralizing antibodies or Fab fragments. An important long-term goal is to identify three-dimensional correlates of immunogenicity and apply the knowledge to facilitate vaccine design and development using a structure-based approach.

Keywords: virus structure, virus engineering, immunology

MS37 INTRACELLULAR TRAFFICKING OF BIOMOLECULES

Chairpersons: Yoshiro Yoneda, David Owen

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Transport out of the Nucleus and Beyond: Molecular Mechanisms

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The movement of proteins and RNAs between the nucleus and cytoplasm of eukaryotic cells is mediated by nucleo-cytoplasmic transport receptors. Most receptors belong to the karyopherin β family of protein, which are also known as importins or exportins according to whether they import or export cargo into/from the nucleus. The directionality of import and export processes depends on the small GTPase, Ran. In contrast to most proteins/RNAs, mRNAs are transported out of the nucleus by a transport factor unrelated to the karyopherin family. mRNA export is linked to quality control mechanisms that make sure that only correctly transcribed and processed mRNAs are exported and translated. A ubiquitous quality control mechanism is nonsense-mediated mRNA decay (NMD). NMD is a surveillance pathway that detects mRNAs containing premature translation termination codons (PTCs) and degrades them before they give rise to truncated protein products. In humans, detection and degradation of PTC-containing mRNAs is dependent on splicing. The splicing-dependence is correlated to the exon junction complex (EJC), a multiprotein assembly that is deposited on mRNAs at the end of splicing upstream of exon junctions. EJC components mark aberrant mRNAs for detection by the NMD machinery and deliver the targeted mRNA to degrading enzymes such as the exosome.

X-ray structures of components of the mRNA export/surveillance machinery give insights on the molecular mechanisms with which they function.

Keywords: protein-RNA interactions, macromolecular assemblies, intracellular trafficking

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Cse1: the Structure of an Exportin in its Closed, Cytosolic State

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Cse1 mediates nuclear export of importin-alpha, the nuclear localization signal (NLS) import adaptor. We report the 3.1Å resolution structure of cargo-free Cse1, representing this HEAT-repeat protein in its cytosolic state. Cse1 is compact, consisting of N- and C-terminal arches that interact to form a ring. Comparison with the structure of cargo-bound Cse1 shows a major conformational change leading to opening of the structure upon cargo binding.

The largest structural changes occur within a hinge region centered at HEAT repeat 8. This repeat contains a conserved insertion that connects the RanGTP and importin-alpha contact sites and that is essential for binding. In the cargo-free state, the RanGTP binding sites are occluded and the importin-alpha sites are distorted. Mutations that destabilize the N- to C-terminal interaction uncouple importin-alpha and Ran binding, suggesting that the closed conformation prevents association with importin-alpha.

Keywords: Cse1, exportin, nuclear transport

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Cracking of the Targeting Signal Embedded in Mitochondrial Presequences

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Most mitochondrial proteins are synthesized in the cytosol as precursor proteins with a cleavable N-terminal presequences and are imported into mitochondria. Protein import into mitochondria is mediated by protein assemblies in the mitochondrial membranes. A subunit, Tom20, functions as a general protein import receptor by recognizing presequences of preproteins. Although no consensus sequence is found, Tom20 recognizes a wide variety of presequences.

To understand the structural basis of the presequence recognition, we determined the NMR and crystal structures of Tom20 in a complex with a presequence peptide. Note that the presequence was fixed to Tom20 via a designed intermolecular disulfide bond to obtain crystals. The bound presequence forms an amphiphilic a-helix. NMR titration experiments indicated the presence of a unique presequence binding site in Tom20, and defined a common five-residue pattern in different presequences. To refine this pattern, we introduced a new peptide library approach using the formation of an intermolecular disulfide bond. We propose that a presequence is regarded as a collective entity of short amino acid sequences that are recognized by several proteins including Tom20. The organization (position, order, and overlapping) of these binding segments is unique for each presequence. This view explains why no consensus sequences are found by simple sequence comparisons.

Keywords: protein transport, molecular recognition, crystallographic and NMR solution state structures

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Vps29: a Phosphoesterase Fold that acts as an Interaction Scaffold in the Assembly of Retromer

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