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Tethering and integration of Murine leukemia virus integration: Structural studies of IN for viral and target recognition

Monica Roth[1], **Sriram Aiyer**[1], **Rongjin Guan**[2], **Swapna Gurla**[2] and **Gaetano T Montelione**[2,3]
[1]Department of Pharmacology, Robert Wood Johnson Medical School, Rutgers University, USA
[2]Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, Rutgers University, USA
[3]Department of Biochemistry and Molecular Biology, Robert Wood Johnson Medical School, Rutgers University, USA

Retroviral integration involves the recognition of the viral LTR sequences with the host DNA. Target-site selection has profound effects on viral pathogenesis and involves interaction with host proteins. Our research has focused on defining the structural elements within the MLV IN required for DNA interactions. All IN proteins have three domains, the N-terminal domain (NTD), the catalytic core domain (CCD), and the C-terminal domain (CTD). The MLV NTD contains an HHCC zinc-binding motif, which is conserved in all retroviral IN proteins and vary in their loop regions. Two crystal forms of the Moloney Murine leukemia virus (M-MLV) IN NTD have been analyzed and consist of an N-terminal extension domain (NED) before the HHCC domain, similar to prototype foamy virus (PFV) IN. The structure of the NED consists of three anti-parallel β-strands and α-helix and an extended β-sheet between HHCC β-strands and the NED. Differences between the PFV and MLV IN NEDs localize at regions identified to interact with the PFV LTR. A solution NMR structure of the M-MLV CTD and a structural homology model of the catalytic core domain (CCD) have been generated. The MLV IN CTD (PDB ID: 2M9U) adopts an SH3 domain fold followed by a long 28 residue unstructured tail. We have obtained a concordant structural model of the MLV IN CCD using SWISS-MODEL, MMM-tree and I-TASSER servers. Using the PFV IN target capture complex X-ray structure and structure-based sequence alignment, residues within the CCD α2 helical region and the CTD β1-β2 loop predicted to bind target DNA in the context of the active MLV intasome were identified. The role of these residues *in vivo* was analyzed by point mutations and motif interchanges. Next-generation sequencing and analysis of local integration target sites indicate the CCD α2 helical region interacts with the sequence outside the target site duplication (TSD), whereas the CTD β1-β2 loop binds to residues within TSD. On a global level, alterations to the MLV IN CTD successfully results in decreasing its integration frequency at transcription start sites (TSS) and CpG islands, thereby reducing the potential for insertional activation. The host BET proteins Brd2, 3 and 4 interact with the MLV IN protein primarily through the BET protein ET domain. We have characterized the IN CTD:Brd3 ET domain interaction using solution NMR in which a significant disorder-to-order transition of the unstructured C-terminal tail region of IN CTD occurs in the presence of the ET domain. Truncation of the C-terminal TP region of IN affects the global targeting profile of MLV vectors by decreasing the propensity to integrate near TSS and CpG islands. WT IN integration events show strong correlation within 100 bp of a known BET binding site. In the absence of the IN CTD TP region, targeting to these known BET binding sites is reduced. Recent structural studies have focused on the structure of the MLV IN CTD in complex with the Brd3 ET domain. The solution structure of the Brd3 ET domain will be presented in the presence and absence of IN sequences.

Biography

Monica Roth, PhD is the Merck Research Laboratory Professor in Clinical Pharmacology at Robert Wood Johnson Medical School, Rutgers University, Piscataway NJ USA.

roth@rutgers.edu

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