The human sliding clamp as a therapeutic target

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The human sliding clamp (also known as PCNA) controls access to DNA of many of the proteins involved in essential processes such as DNA replication, DNA repair and cell cycle control. Proteins compete for interaction with the PCNA surface by means of a short, conserved peptide sequence known as the PCNA-interacting protein motif (or PIP-box). Binding to PCNA via the PIP box allows access to DNA. For example, the major replicative polymerase, pol delta, requires PCNA for processive DNA synthesis, without interaction with PCNA the polymerase dissociates from DNA and is incapable of processive DNA synthesis. As such, many groups have proposed the usefulness of PIP box mimetics for use as cancer therapeutics given they would block upregulated PCNA form allowing interaction with pol delta and hence would inhibit DNA replication. However, no peptide mimetics of PCNA have been forthcoming to date. Here we describe the design and synthesis of the first PCNA peptidomimetic. Our mimetic, ACR2, was designed through synthetic lactam chemistry to constrain the secondary structure of the peptide for optimized binding to PCNA. NMR solution studies show that the wild type p21 peptide from which ACR2 was designed adopts no defined secondary structure in solution, while our mimetic adopts a 310 helix in solution, which has been shown in previous studies to be essential for PIP box binding to PCNA. Binding experiments determined a KD of 200 nM of ACR2 for PCNA, which is higher than the wild type peptide. A co-crystal structure of ACR2 bound to hPCNA revealed the mechanism of interaction of this mimetic with PCNA.

Recent Publications


Biography

John B Bruning received BSc from Texas A&M University in 1997. He began crystallography in the Laboratory of Yousif Shamoo at Rice University. During his graduate studies he worked on the structural mechanism of the human sliding clamp and its interactions with DNA replication proteins. He received his PhD in 2005 and completed two successful Post-docs; the first was at the Scripps Research Institute from 2005-2007 working on structural studies of nuclear receptors including PPAR, RXR, ER and TR and his second Post-doc was with Jim Sacchettini in the Houston Medical Centre and as a part of the TB structural genomics consortium. He received his first faculty position at the University of Adelaide as a Lecturer in 2012. He was tenured in 2015 and promoted as Senior Lecturer in 2016. Due to his continued collaboration with Scripps Research Institute, he was also appointed as Adjunct Professor of the Scripps Research Institute in 2016.

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