s1.m5.o4 **Conformational Variation of the DNA Holliday Junction**. Nicholas H. Hopcroft, James H. Thorpe, Anna L. Brogden, Benjamin C. Gale & Christine J. Cardin *School of Chemistry, University of Reading, Reading, UK. E-mail: n.h.hopcroft@reading.ac.uk*

Keywords: DNA Structure, Genetic Recombination, Metal Ion Binding

The Holliday junction [1] is a key intermediate in a number of cellular processes, including genetic exchange by homologous recombination, DNA repair and resetting of stalled replication forks at sites of DNA damage. The junction consists of four DNA double helical arms that are linked by the covalent continuity of individual strands shared between adjacent arms. It has long been known that the availability of metal ions has a strong effect on the overall conformation of the Holliday junction [2]. In the absence of significant concentrations of metal ions, the double helical arms of the junction radiate from the centre with approximately square planar geometry. However, in the presence of 100 μM Mg^{2+}, Ca^{2+}, Sr^{2+} or Ba^{2+}, the junction folds into the stacked-X conformation, in which pairs of arms stack with each other to form two pseudo-continuous double helices with strand exchange between them. The presence of the monovalent cations Na^{+} or K^{+} alone is insufficient to stabilise this stacked conformation. Several X-ray crystal structures of Holliday junctions in the stacked-X conformation have recently been determined, including one in which covalent modification by two intercalating psoralen drug molecules is required for junction formation [3]. We are designing and synthesising new bis-intercalating compounds and using competitive dialysis to study the binding of these and other molecules to DNA, to investigate the possibility that they stabilise the stacked-X conformation of the Holliday junction. We also report here the X-ray crystal structures of the Holliday junction forming DNA molecules $(CCGGTACCGG)_4$ and $(TCGGTACCGA)_4$ [4], in the presence of different divalent metal ions. In all cases the ions are located in the minor grooves, but are not coordinated directly by the DNA. Instead, they form interactions through their primary hydration shells, as well as other longer range contacts through more extensive networks of ordered water molecules. These results suggest that stabilisation of the stacked-X conformation by divalent metal cations involves specific hydrogen bond formation and ordering of the solvent cavities between the junction arms, rather than direct compensation of the phosphate negative charges alone. At the crossover point of each structure there are several largely conserved intramolecular and water mediated hydrogen bond contacts, but the conformational parameters of the double helical arms vary according to the DNA sequence and the type of metal ions present. The conformation adopted by the Holliday junction has important implications for the binding of the many proteins that specifically associate with this structure *in vivo*, including the p53 tumour suppressor and other proteins involved in the onset of cancer.

[1] Holliday (1964) *Genetic Research*, **5**, 282-304
[2] Duckett *et al.* (1990) *EMBO Journal*, **9**, 583-590
[3] Eichman *et al.* (2001) *Journal of Molecular Biology*, **308**, 15-26
[4] Thorpe *et al.* (2003) *Journal of Molecular Biology*, **327**, 97-109

s1.m5.o5 **Molecular basis of telethonin-mediated linkage of the N-terminus of titin within the sarcomeric Z-disc**. Nikos Pinotsis[1], Peijian Zou[1], Marco Marino[2], Stephan Lange[3], Alexander Popov[1], Irene Mavridis[4], Mathias Gautel[3], Olga M. Mayans[1,2] and Matthias Wilmanns[1], [1]*EMBL-Hamburg c/o DESY, Notkeststrasse 85, D-22603 Hamburg, Germany.* [2]*Biozentrum, University of Basel, Division of Structural Biology, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.* [3]*The Randall Centre and Cardiovascular Divisions, Muscle Cell Biology, New Hunt's House, King's College London, Guy's Campus, London, SE1 1UL, United Kingdom.* [4]*Institute of Physical Chemistry, National Center for Scientific Research "Demokritos", Aghia Paraskevi 15310, Athens, Greece. E-mail: pinotsis@embl-hamburg.de*

Keywords: Muscle sarcomere, Z-disc, Titin, Telethonin

Severe heart and muscle diseases, like dilated cardiomyopathy, result from mutations in components of multi-protein complexes, such as the N-terminus of titin, α-actinin and telethonin. These are localised at the Z-disk of skeletal and cardiac striated muscle sarcomeres and are essential for myofibril assembly and maintenance. To unravel the molecular basis of the titin/telethonin interactions in the Z-disk, we have determined the crystal structures of the titin N-terminus in the absence and presence of telethonin. The complex structure reveals a titin-telethonin-titin antiparallel arrangement, suggesting an unexpected telethonin-mediated linkage of the N-termini of two titin filaments.

The complex is formed by four independent binding sites, involving two α-hairpin wing motifs of telethonin, where each wing binds two IG-like domains from two different titin molecules in opposite orientations. The titin/telethonin complex is formed exclusively by intermolecular β-sheet interactions and is insensitive to single residue mutations, as shown by in-vitro binding data and colocalisation data of several titin/telethonin in neonatal rat cardiomyocytes. The titin/telethonin complex may serve as a hub to bind several other protein ligands inside and outside the Z-disk. For one of these components, MLP, a structural model is provided suggesting a telethonin-mediated functional linkage of MLP and titin. The availability of a second titin-filament cross-linking system by telethonin, in addition to that formed by α-actinin, allows to approach a structural model of the Z-disk providing rationales for anchoring and precise alignment of major sarcomeric filaments such as actin and titin.