s8a.m6.p3 **Structural studies of the transcription cofactor PC4**. S.H.W. Scheres[1], J.M.H. van den Elsen[1], M. Meisterernst[2], J. Kroon[1], P. Gros[1]; *[1]Dept. of Crystal and Strutural Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands; [2]Laboratory for Molecular Biology-Genzentrum, Ludwig-Maximilians-University, Munich, Germany.*
Keywords: PC4, transcription coactivation, ssDNA-binding protein.

PC4 (human positive cofactor 4) is a transcription cofactor that can both coactivate and repress activator dependent class II gene transcription. The structure of the C-terminal domain (residues 63-127) has been solved by X-ray diffraction in our lab[1]. This structure shows a structural motif reminiscent of the single stranded (ss)DNA-binding motif in human replication protein A. The orientation of the binding grooves on the dimer suggests that two ssDNA strands running in opposite directions can be bound to the protein. This agrees with the biochemical findings that heteroduplex DNA can be bound to the protein with high affinity. This binding mode is most likely involved in the repressing mode of PC4[2].

For its role as coactivator the N-terminal domain plays a more prominent role. Biochemical studies have shown that in the absence of transcription factor IIH (TFIIH) the activator function of PC4 is lost and transcription is strongly repressed by PC4[3]. In the same study it has been shown that PC4 can be phosphorylated by TFIIH and TFIID. Most likely, phosphorylation is to take place on the serine-rich N-terminal domain of PC4.

All crystallization attempts with full-length protein have failed so far. To understand more of the coactivating function of PC4, crystallization experiments were performed with a construct comprising residues 22-127. Here, we present the structures of two different crystal forms that appeared after degradation of the N-terminal part of this construct.

s8a.m6.p4 **The crystal structure of two UUCG tetraloops.** E. Ennifar[1], A. Nikulin[1,2], A. Serganov[1,2], M. Garber[2], C. Ehresmann[1], S. Nikonov[2] and P. Dumas[1]. *[1]UPR 9002 du CNRS, IBMC, 15 Rue René Descartes, 67084 Strasbourg cedex [2]Institute of Protein Research, Pushchino, Moscow Region, 142292 Russia.*
Keywords: RNA, tetraloop, hairpin.

Involved in several processes, hairpin loops can play various functional or structural roles. Tetraloops, loops containing four unpaired nucleotides in the loop, are the most common hairpins found in RNAs. Among them are found "unusually stable" tetraloops that belong to three classes, GNRA, UNCG and CUUG (N = U, A, C or G; R = G or A). They are all characterized by exceptional stability as their melting temperature is higher than for other similar DNA or RNA sequences.

The UUCG tetraloop is known to be the most stable one. As pointed out by thermodynamics and spectroscopic studies, 2'-hydroxyl groups are probably responsible of the gain in stability. NMR studies [F. Allain & G. Varani, *J.Mol.Biol.* **250**, 333-353] revealed the structure of a UUCG hairpin, showing the formation of a G-U mismatch and only two unpaired residues, the structural role played by 2'-OH groups remaining unknown. More recently, molecular dynamics simulations have tried to provide insight into the importance of such interactions [J.L. Miller & P.A. Kollman, *J.Mol.Biol.* **270**, 436-450 ; D.J. Williams & K.B. Hall, *Biophysical J.* **76**, 3192-3205]. Concerning X-ray studies of UUCG tetraloops, several attempts have been done, but all were unsuccessful as they all led, not to the expected hairpins, but rather to duplexes including C-U and G-U mismatches.

Here we present at 2.8 Å resolution the first crystal structure of two UUCG tetraloops. These loops were introduced as linkers into a complex RNA molecule of 57 nucleotides) representing a part of the 16S ribosomal RNA in interaction with the ribosomal protein S15 [A. Nikulin *et al*, Crystal structure of the S15-rRNA complex, *Nature Struct. Biol.* **7**, 273-277]. The observed tetraloops structure is very close to the one observed by NMR (rmsd = 1.1 Å). However, the crystal structure allows clear identification of several crucial 2'-hydroxyl interactions responsible for loop stability.

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