Jansen and co-workers have been involved in the prediction of the crystal structures of the binary alkali-metal nitrides for some time.[19] Interestingly, in the course of these studies the anti-ReO_3 type for the nitrides of Na, K, Rb, and Cs was not considered. From that fact it can be taken, that this structure type was not to be expected for Na_3N. Taking the synthetic conditions into account, one has to assume metastability for the obtained form of Na_3N. Since Na_3N was prepared only as a film on a sapphire substrate and since it is extremely moisture sensitive, no physical properties were determined so far. Surely, interesting results are to be expected.

With the synthetic technique presented by Fischer and Jansen it might be feasible to prepare the binary nitrides of K, Rb, and Cs, too. With the development of new synthetic routes further new (especially metastable) compounds of nitrogen might be expected in the near future. Such new compounds will inevitably have surprising structural chemistry and physical properties and, thus, provide new impulses in solid-state chemistry.

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Oligomerization of p53 upon Cooperative DNA Binding: Towards a Structural Understanding of p53 Function

Patrick Chene and Wolfgang Jahnke*

P53 is one of the most important gene products involved in cancer suppression, and has concomitantly been an interesting target for oncology research.[1] Despite its high pharmaceutical relevance, one of the basic biological principles of p53, its activation from a latent state to an active DNA-binding state, is still unclear. A recent paper now sheds light on some of the structural features associated with p53 activation and DNA binding.[2] The p53 protein is present in nonstressed cells in a latent form and at very low concentration. However, under different stress conditions—such as DNA damage, oncogene activation, hypoxia, or ribonucleotide depletion—it accumulates in the cell and is activated.[3] The activation of the p53 protein leads either to arrest of the cell cycle or to apoptosis.[4] The outcome of p53 activation, which is cell-type and/or stress dependent, prevents the damaged cells from dividing: p53 is a tumour-suppressor gene. Several lines of evidence show that the deletion or the mutation of the p53 gene favors development of cancer: mice homozygotes with inactivated p53 allele are highly sensitive to tumors,[5] p53 is mutated in about 50% of the human cancers,[6] and p53 germline mutations are associated with the Li – Fraumeni syndrome.[7]

Although part of the p53 activity is mediated by protein – protein interactions, much of it is linked to its ability to bind to DNA and to regulate the transcription of several genes. P53 is a transcription factor and, like other transcription factors, contains several domains: a transactivation and proline-rich domain (residues 1 – 43 and 61 – 94, respectively) at the N-terminus; a DNA-binding domain in the middle of the protein (residues 110 – 286); and a tetramerization domain (residues 326 – 355) and a regulatory region (residues 363 – 393) at the C-terminus of the protein. While no structure of full-length p53 is known, structures have been determined for the individual domains by X-ray structure analysis or NMR spectroscopy (Figure 1). In particular, the determination of

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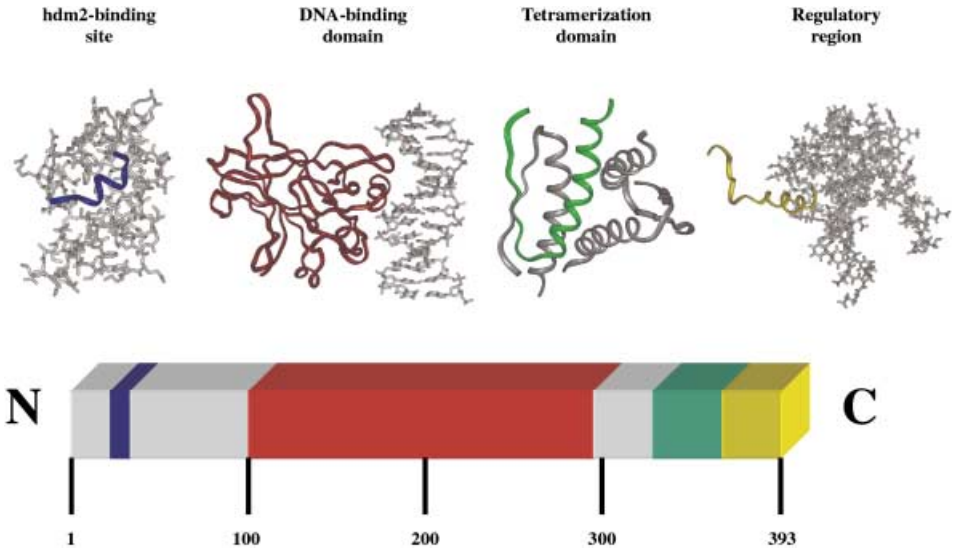

Figure 1. The domains of p53 and sketches of the experimental structures available for the individual domains. The structures of the binding partners are displayed in gray.

the three-dimensional structure of a p53–DNA complex has been a key step in the understanding of the interaction between p53 and DNA.[8] P53 binds to DNA through its central DNA binding domain to consensus DNA-binding sequences, which contain two copies of the 10-base pair motif 5′-Pu · Pu · Pu · C · (A/T) · (T/A) · G · Py · Py · Py-3′ (Pu = A/G, Py = T/C), which can be separated by up to 13 bases.[9] The internal symmetry of each ten-base pair motif made up of two oppositely oriented half-sites suggests that p53 binds to DNA as a tetramer, and indeed p53 exists as a tetramer in solution. The vast majority of p53 mutations occur in the DNA-binding domain.[6] The knowledge of its structure at least in the monomeric form together with an analysis of the thermodynamic stability of different p53 mutants[10] provide a framework for understanding the effect of these mutations on p53 activity. The mutations target either residues involved in the maintenance of the structure of the DNA-binding domain and/or residues that directly contact DNA. In all cases these mutations abolish DNA binding and therefore inactivate the p53 protein.

Two burning questions remain to understand the function of p53 on a molecular level: How is p53 activated from a latent to an active state, and what is the molecular arrangement of individual p53 subunits and p53 domains in the full-length tetrameric p53–DNA complex? Several lines of evidence have shown that the p53 protein exists in a latent conformation where it does not bind to DNA in a sequence-specific manner and in an active conformation where it does.[11] The finding that the shift between both conformations is mediated by activators acting either at its N- or its C-terminus led to the concept of an allosteric regulation of p53 activity.[12] This model assumes the hypothesis that in the latent conformation an interaction between the DNA-binding domain and another region of the protein (most likely its C-terminal 30 amino acids) would prevent DNA binding. The discovery that small peptides mimicking the p53 C-terminus activate the wild-type protein and some of its mutated forms for DNA binding supported this model and suggested that the allosteric activation could be utilized to design new anticancer agents.[13] However, in a recent NMR study researchers of Novaspin and Roche investigated the interaction between the p53 DNA-binding domain and various C- and N-terminal peptides by a variety of NMR methods, such as chemical shift perturbation, diffusion measurements, and saturation transfer difference spectroscopy, and showed that these peptides do not interact with the DNA-binding domain.[2] Therefore these results, which are in agreement with independent recent work[14] and other new findings,[15] show that the allosteric model is not valid for explaining p53 regulation. Instead, some form of competitive binding model is now favored to explain the experimental data.[16] Indeed, a weak (and probably unspecific) interaction between the C-terminal peptide and DNA was reported.[2]

It has long been demonstrated that p53 DNA-binding domains can cooperatively bind to DNA in solution.[17, 18] However, an exact understanding of the structural basis of this cooperative effect is limited because the structure of the (tetrameric) full-length p53 molecule in the complex with DNA is not known. Klein et al.[2] now present additional NMR structural data about the origin of the cooperative binding of the DNA-binding domains to DNA. By titrating unlabeled consensus DNA to ^{15}N-labeled p53 DNA-binding domain, it was possible to selectively observe changes in the chemical shift of the DNA-binding domain, with a spatial resolution at the level of individual amino acid residues. These changes in chemical shift reflect changes in the local environment of the respective amino acids, which is either caused by direct interactions with DNA or another p53 monomer, or by local conformational changes.[19] Chemical-shift peturbations are confined to distinct regions of the p53 DNA-binding domain. This observation indicates that its conformation is not drastically changed upon interaction with DNA, and confirms the site of interaction with DNA.[8] Most importantly, however, it defines the part of the p53 surface that is involved in the interaction between two p53 DNA-binding domains, and therefore provides experimental data for the generation of a model for the p53 tetramer bound to consensus DNA. As already suggested by Cho et al.,[8] the oligomerization interface includes the small helix H1, which spans residues proline-177 to cysteine-182. Very interestingly, some of the p53 mutations that were found in cancer cells are located in this H1 helix region. The involvement of the helix in the oligomerization of p53 explains the presence of these "hot spots" in and around a p53 region that is not in direct contact with DNA. It would be very interesting to determine whether these mutations indeed affect the assembly of the

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DNA-binding domains. In other words, can single mutations in the helix H1 prevent assembly of p53 and abolish DNA binding as some mutations in the tetramerization domain do?[20]

What is the structural organization of the complex between tetrameric full-length p53 and DNA? In the "clamp model"[21] (Figure 2A) the consensus DNA is surrounded by one p53 dimer on each side, so that the tetramer binds DNA as a pair

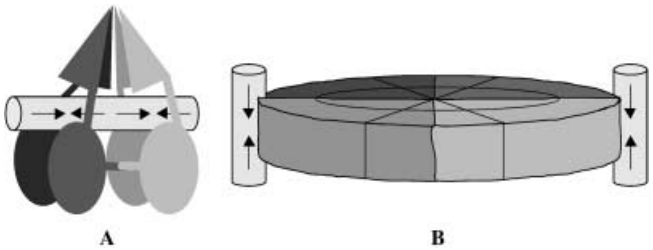

Figure 2. Models for the structures of the complex formed between full-length tetrameric p53 and DNA: A) "clamp model",[21] B) "sandwich model" as proposed by Klein et al.[2] Each p53 monomer is shown by a different shading, DNA is shown by tubes, and the orientation of the respective quarter-sites is indicated with arrows.

of clamps. On the basis of the reported data and on symmetry considerations, Klein et al. now propose a "sandwich model" for the p53–DNA interaction.[2] In this model, it is assumed that p53 tetramers link two separated, juxtaposed DNA consensus sites (Figure 2B). The tetramerization domains form the central part of the disc-shaped tetramer, while DNA-binding domains and transactivation domains are at the outside, with the DNA-binding domains contacting consensus DNA. This model is not only consistent with a D_2 symmetry of the complex and identical conformations of each p53 monomer, but is also supported by electron microscopy studies, which show that p53 can bridge two DNA consensus sites.[22]

Another aspect becomes clear from the work of Klein et al.: Dynamics play an integral role in understanding the p53–DNA interaction. The NMR signals of certain p53 residues broaden significantly after DNA binding. Since the authors provide evidence that the complex is tight enough to exclude chemical exchange as its source, the observed line broadening is likely the result of conformational exchange, that is, conformational flexibility of p53 residues when bound to DNA. This conformational exchange reduced the quality of the NMR spectra and precluded a more detailed NMR structural analysis—however, it is also interesting to note that it adds to the experimental evidence that molecular flexibility is a key driving force in protein–DNA interactions.[23, 24] Analysis of the thermodynamics of the formation of the p53–DNA complex may provide further insight into the function and activation of p53.

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