P53 conformational switching for selectivity may reveal a general solution for specific DNA binding

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DNA-binding proteins are integral members of the cell community, having indispensable roles in critical DNA processes such as gene expression regulation, replication, transcription, and genomic maintenance. Yet, how these critical proteins efficiently recognize their target binding sites in the vast excess of DNA within the genome has been perplexing. In this issue of *The EMBO Journal*, Petty *et al* (2011) provide insights into this enigma by combining structure, fluorescence anisotropy, and mutational analysis of the sequence-specific DNA-binding protein p53 to reveal a basis for discriminating between specific and non-specific DNA targets: a DNA-binding-mediated conformational switch regulates DNA off-rates independent of affinities. Besides explaining the prevalence of conformational switches in sequence-specific protein–DNA interactions, these findings could also be broadly relevant for specific DNA binding including such processes as DNA repair.

The p53 protein is a sequence-specific transcription factor that has a significant role in human cancer. It is a tumour suppressor that regulates cell-cycle progression, apoptosis, DNA repair, senescence, cellular metabolism, or autophagy in response to several cellular stress signals, such as DNA damage, abnormal oncogenic events, telomere erosion, and hypoxia (Dai and Gu, 2010). P53 also participates in transcription-independent stress responses in the cytosol (Dai and Gu, 2010). For ~half of all human cancer cases, the p53 gene contains mutations that disrupt p53 protein function; most of these are missense mutations within the DNA-binding domain that result in altered conformation and decreased DNA binding (Dai and Gu, 2010). In the remaining cases, other p53 pathway components are disrupted (Dai and Gu, 2010).

Full-length p53 has four domains: (1) N-terminal transactivation, (2) proline-rich, central DNA-binding core, (3) tetramerization, and (4) C-terminal regulatory (Dai and Gu, 2010). The DNA-binding and tetramerization domains are distinctly folded, whereas the remainder of p53 is unstructured (Joerger and Fersht, 2008). Although several p53 DNA-binding domain structures with and without DNA have been reported, all of these are of the DNA-binding domain only and none show a conformational change either within the DNA-binding domain or bound DNA, as seen for most other characterized sequence-specific DNA-binding proteins. This observation led Petty *et al* (2011), in this issue of *The EMBO Journal*, to ask

Figure 1 Model for how DNA-binding-mediated conformational switching may control selectivity for target DNA, pathway, etc. When bound to non-target DNA, no conformational change takes place within the DNA-binding protein (cyan; DNA-binding loop is shown in purple) or the DNA (grey), leading to a high protein off-rate from DNA. When bound to target DNA (target site is shown in green), a conformational switch within the protein and/or DNA results in a low off-rate, allowing for selectivity. Model based on the results from Petty *et al* (2011), in this issue of *The EMBO Journal*.

key questions: why had such a conformational change not been observed for p53, could such a conformational change be observed in p53 structures containing both the DNA-binding and oligomerization domains, and what is the significance of this potential conformational change, since it occurs for virtually all other characterized sequence-specific DNA-binding proteins?

To address these key questions, Petty *et al* (2011) used a combined experimental approach. X-ray crystallographic structure determination and comparison of the first DNA-free and bound p53 structures containing both DNA-binding and oligomerization domains showed, for the first time, a DNA-binding-mediated switch of DNA-binding domain loop L1 from a recessed to an extended conformation, respectively. The DNA-bound, extended conformation makes direct contacts with DNA and promotes a 3-Å DNA helical axis shift at the centre of the binding site to accommodate this loop in the major groove. Fluorescence anisotropy revealed an unexpected greater difference in off-rates rather than affinities for specific versus non-specific DNAs. Supporting mutational analysis showed even higher off-rate constants for mutants with substitutions facilitating loop L1 conformational switching than wild-type p53. Together, these results suggest conformational switching of loop L1 orchestrates an induced fit mechanism to impart DNA-binding specificity by regulating DNA binding off-rates independent of affinities.

Petty *et al* (2011) note that a conformational change to regulate DNA binding off-rates, rather than affinities could provide a general explanation for the universal prevalence of conformational switches in sequence-specific DNA-binding protein–DNA interactions (see Figure 1). They further propose that larger differences in DNA binding off-rates, rather than affinity, as previously thought, could account for how these proteins recognize their target binding sites among the vast excess of genomic DNA. These ideas bring a fresh twist into the long puzzling question of how these proteins locate target DNA *in vivo* and have implications for other DNA-binding protein systems. For example, similar loop conformational switches are seen for many prototypic DNA repair proteins,

which recognize DNA damage rather than specific sequences. In DNA base excision repair, DNA-binding characteristics for structurally characterized mammalian enzymes, such as uracil-DNA glycosylase (Slupphaug *et al*, 1996) and apurinic/apyrimidinic endonuclease (Mol *et al*, 2000), resemble those for sequence-specific DNA-binding proteins, including a conserved, electrostatically positive surface guides initial DNA binding; a grooved surface aids DNA bending and positioning; enzyme loops contact the DNA phosphodiester backbone; and the enzyme changes from an open, unbound conformation to a closed, bound conformation to favour catalysis and retard product release (Parikh *et al*, 1999). In DNA *O*⁶-alkylguanine damage repair, alkyltransferase-like protein uses lesion-binding cavity conformational plasticity and a DNA lesion-binding-mediated open-to-closed loop conformational switch to remain bound to damage and direct repair to one of two distinct DNA repair pathways, direct base repair or nucleotide excision repair (Mazon *et al*, 2009; Tubbs *et al*, 2009; Aramini *et al*, 2010; Tubbs and Tainer, 2010). Thus, conformational switches may have far-reaching roles in critical DNA processes, spanning from sequence and target recognition to DNA repair pathway selection (Figure 1). Indeed, structural analyses of proteins and nucleic acids in solution including p53 by evolving methods, such as small angle X-ray scattering results, suggest conformational variation is a general functional feature of macromolecules (Rambo and Tainer, 2010). So the insights from Petty *et al* (2011) on p53 may generally inform our views of specificity in macromolecular transactions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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