Where cancer meets calcium — p53 crosstalk with EF-hands

Mitsuhiko Ikura and Kyoko L. Yap

S100B, an EF-hand Ca^{2+}-binding protein, grasps the C-terminus of the tumor suppressor p53 and inhibits protein kinase C-dependent phosphorylation and acetylation of p53 in a Ca^{2+}-dependent manner. The mode of interaction between S100B and p53 is different from the interactions seen in S100A–annexin complex structures.

Calcium is a ubiquitous second messenger that regulates many cellular functions, including cell growth, differentiation and neural signal transduction. S100A and S100B proteins are members of a growing subfamily of the EF-hand calcium binding protein superfamily, possessing ~40% sequence identity. The S100B protein is one of the '*elite*' proteins in the field of calcium signaling, being implicated in cell cycle arrest and apoptosis[1] as well as in Down's syndrome and Alzheimer's disease[2]. On page 570 of this issue of *Nature Structural Biology*, Weber and coworkers[3] report the NMR-derived structure of S100B in complex with the C-terminal negative regulatory region of the tumor suppressor p53. The p53 protein is a transcriptional activator involved in ~50% of all human cancers[4]. This structure reveals how the S100B homodimer recognizes two molecules of p53 and inhibits post-translational modifications of p53 (ref. 5).

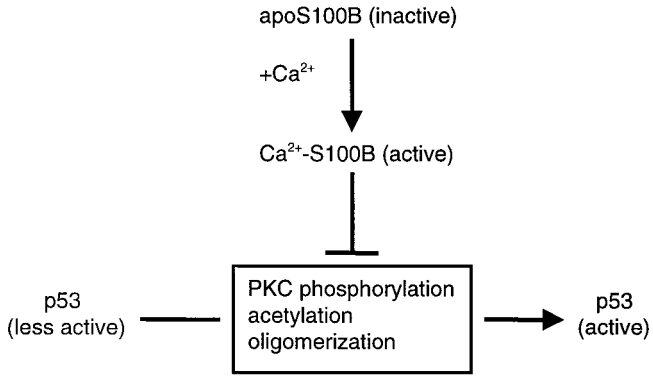

Fig. 1 A proposed action of S100B in p53 function. Post-translational modifications of p53 such as PKC phosphorylation and acetylation result in activation of p53 transcriptional activity. Tetramerization of p53 also enhances transcriptional activity. Ca^{2+} binding to S100B induces a conformational change in S100B, enabling S100B to inhibit PKC phosphorylation, acetylation and tetramerization.

How S100B regulates p53 function

There are a number of *in vitro* and *in vivo* studies that have addressed this question. First, Ca^{2+} binding to the EF-hand motifs of S100B induces a large conformational change that is absolutely required for its interaction with p53 (refs. 5,6). This Ca^{2+}-dependent interaction of S100B with p53 inhibits protein kinase C (PKC)-dependent phosphorylation at residues Ser 376 and Thr 377 of p53. By inhibiting these post-translational modifications of p53, S100B may regulate cellular functions of the tumor suppressor (Fig. 1). Second, S100B inhibits p53 tetramerization and promotes disassembly of the p53 tetramers[5]. In an allosteric regulatory model[4], PKC-dependent phosphorylation and acetylation of the C-terminal regulatory domain of p53 inhibit the interaction of this domain with the DNA-binding domain of p53, thus activating sequence-specific DNA binding required for transcription activation. More recently, S100B has been shown to protect p53 from thermal denaturation and aggregation *in vitro*[1]. In glial cells expressing a constitutive wild type p53, S100B synthesis in the G1 phase of the cell cycle is synchronized with the timing for p53 nuclear translocation and activation[1]. Based on these data, Scotto *et al.*[1] suggested that S100B could promote the p53-dependent growth arrest and apoptosis pathways by assisting p53 nuclear translocation in the G1 phase of the cell cycle.

S100B binds p53 regulatory domain

The p53 protein is composed of four structural/functional domains: an N-terminal transactivation domain, a central DNA-binding domain, a tetramerization domain and a C-terminal regulatory domain. The three-dimensional structures of the DNA-binding domain in DNA-bound[7] and 53BP2 (p53 binding protein 2)-bound[8] forms, the tetramerization domain[9-12] and the transactivation domain complexed with MDM2 (murine double minute 2) oncoprotein[13] have been determined previously by X-ray and/or NMR (Fig. 2). These structures contributed significantly to our understanding of how wild type p53 works and what structural consequences result from alterations that lead to cancer. The structure solved by Weber and colleagues shows that the C-terminal regulatory region containing PKC-dependent phosphorylation sites is α-helical when bound to S100B. The structure provides a possible conformation of this region (that is, α-helix) when p53 interacts with PKC, the acetylase domain of the transcriptional coactivator p300, and perhaps the DNA-binding of p53.

S100B is a homodimer of 91-residue polypeptides, each subunit consisting of a pair of the Ca^{2+}-binding EF-hand motif. For the structure determination[3], a short p53 peptide (residues 367–388) was used to form the p53–S100B quaternary complex (two p53 peptides per S100B homodimer). The α-helical peptide of p53, which is unstructured in absence of S100B, binds to a shallow wedge-shaped binding site on each S100B subunit. The NMR structures reveals a number of key electrostatic and hydrophobic interactions at the S100B–p53 interface. This interaction of S100B with p53 sterically masks two important PKC phosphorylation sites (Ser 376 and Thr 377) and two acetylation sites (Arg 379 and Lys 386) in p53. Perhaps binding of S100B removes the negative

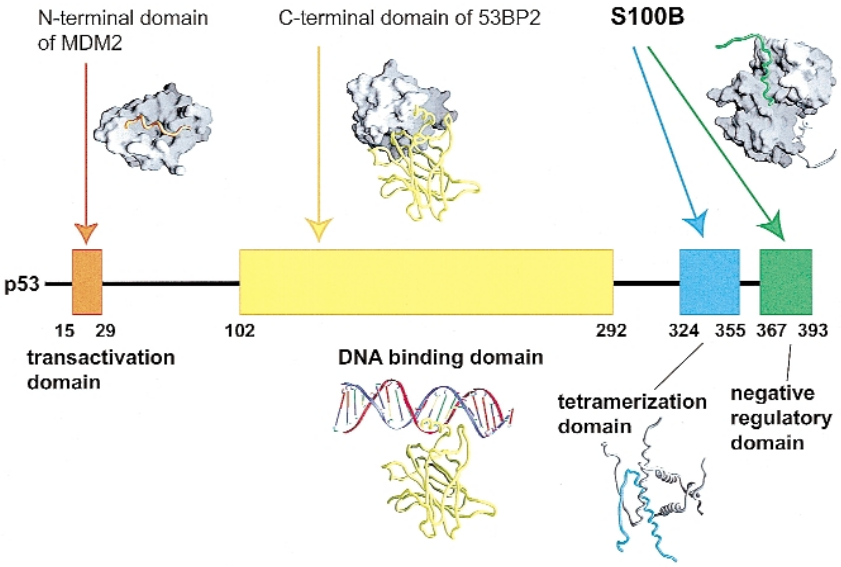

Fig. 2 Solved structures of p53 domains alone and in complex with interacting proteins. Arrows above the p53 functional domain layout indicate interaction between proteins (in surface representation) and p53 domains (in Cα trace). MDM2 (ref. 13) and 53BP2 (ref. 8) interact with one molecule of p53. S100B, a homodimer, interacts with two molecules of p53; only one p53 molecule is colored. S100B also interacts with the tetramerization domain, although the structure is not known. Below the domain layout are the structures of the DNA binding domain of p53 in complex with DNA[7] and the tetramerization domain, which is a stable tetramer even in isolation[9–11].

regulatory domain from the DNA-binding domain of p53 allowing it to bind DNA.

S100B interacts not only with the C-terminal regulatory domain but also with part of the tetramerization domain (residues 320–346)[6] (Fig. 2). This additional interaction with the tetramerization domain is most likely responsible for disruption of p53 oligomerization, which should result in reduction of p53 function. The net effect of S100B binding on p53 function is, however, under debate. It is also unclear how S100B protects p53 from thermal denaturation and aggregation.

Target recognition by S100 proteins

The solution structure of S100B in complex with the p53 peptide is the third S100 protein–target complex structure solved to date (Fig. 3). The general topology of these S100 proteins is very similar, which is not surprising given that their sequences are 37–40% identical. However, the position of the p53 peptide in complex with S100B is very different from those of the annexin peptides in the S100A10–annexin II (ref. 14) and S100A11–annexin I (ref. 15) complexes. (Annexins are phospholipid-associated, non-EF-hand calcium binding proteins that are known to interact with several members of the S100 protein family.) The p53 peptide does not lie deeply in a hydrophobic binding pocket, whereas the annexin peptides, with little sequence homology, both bind in a deeper binding pocket. The annexin peptides interact with the S100A proteins mainly *via* hydrophobic contacts and a few hydrogen bonds. On the other hand, the S100B–p53 interaction involves a high content of ionic and polar residues, including Arg 379 and Lys 386 of p53 forming a salt bridge with Glu 45 and Glu 86 of S100B, respectively[3]. This difference in the nature of the interaction between S100B–p53 and S100A–annexin complexes may explain why the former interaction is weaker (K_d ~10^{-6} M) than the latter ones (K_d ~10^{-8} M) (refs. 16,17).

The 'hinge' region (between the two EF-hands in the S100 monomer) and the C-terminal extension (which contributes to the S100 dimer interface) are crucial for S100–target interaction, and differences in sequence and length of these regions may be responsible for the specificity of target binding in S100 proteins[18]. As noted by Rustandi *et al.*[3], these regions are where the most significant structural changes occur when S100B binds the p53 peptide, and where the sequence is least conserved when S100B is compared to the other S100 proteins. The C-terminal extension also plays an important role in target binding by bovine S100A1 (60% sequence identity to rat S100B)[19], which is able to form heterodimers with S100B. S100A1 also binds to a peptide derived from the negative regulatory domain of p53, and this mode of interaction could be very similar to that observed in the S100B–p53 peptide complex.

Just as the S100A–annexin and S100B–p53 interactions appear to differ greatly, other S100–target complexes may also exhibit unique modes of interaction. Human S100A6 or calcyclin (42% sequence identity to rat S100B), which interacts with annexin XI, does so *via* its N-terminus[20], in contrast to the C-terminal interaction observed in the S100A10 and S100A11 complexes. Multiple target sites have been proposed for both S100A1 (ref. 21) and S100B (ref. 22), with the former utilizing different sites for Ca^{2+}-dependent and Ca^{2+}-independent target interactions. It has also been suggested that human S100A7 or psoriasin (26% sequence identity to rat S100B) binds its target proteins *via* a cleft formed by helices I and IV of both monomers[23]. However, since these helices in other S100 proteins do not undergo significant conformational change upon binding Ca^{2+}, the cleft is an unlikely region for Ca^{2+}-dependent target binding. On the other hand, the S100A-annexin interactions also occur far from helix III, which exhibits the largest Ca^{2+}-induced conformational change in S100 proteins. The

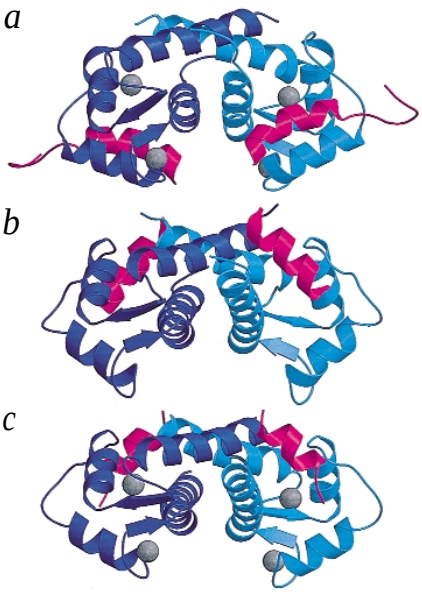

Fig. 3 Structures of S100–target peptide complexes. The two monomers are dark and light blue. Calcium ions are gray and the peptides derived from target proteins are pink. ***a***, S100B in complex with the negative regulatory domain of p53 (ref. 3). ***b***, S100A10 in complex with the N-terminus of annexin II (ref. 14). Note that S100A10 does not bind Ca^{2+}. ***c***, S100A11 in complex with the N-terminus of annexin I (ref. 15).

S100B–p53 peptide complex structure demonstrates that there is no one mode of S100–target interaction, making the structurally homologous family of S100 proteins much more diverse than previously believed.

Calcium everywhere

The structure of S100B complexed with the p53 peptide provides the first glimpse of how an EF-hand protein recognizes a transcription factor in order to regulate transcriptional activity. Other examples of EF-hand proteins involving transcription regulation include the down-stream regulatory element antagonist modulator DREAM, which functions as a Ca^{2+}-dependent DNA-binding transcriptional repressor[24]. Clearly Ca^{2+} signaling has diverse cellular functions ranging from transmembrane and cytoplasmic signal transduction to gene regulation. Ca^{2+}–S100B-mediated regulation of p53 transcription activity provides a possible link between Ca^{2+} signaling and oncogenic processes in which the tumor suppressor p53 plays key roles. Future questions on the p53–S100B interaction include how S100B interacts with the tetramerization domain of p53 and how S100B binding influences the structural stability of p53 both *in vitro* and *in vivo*.

Mitsuhiko Ikura and Kyoko L. Yap are in the Division of Molecular and Structural Biology, Ontario Cancer Institute and the Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9. email: Correspondence should be addressed to M.I. mikura@oci.utoronto.ca

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TolC, a macromolecular periplasmic 'chunnel'

Kathleen Postle and Hema Vakharia

The crystal structure of TolC, one of the most mysterious proteins in the outer membrane of Gram-negative bacteria, suggests a mechanism for its role in secretion of proteins and efflux of toxic chemicals.

Two concentric membranes, the cytoplasmic (or plasma) membrane and an outer membrane fenestrated with protein-based pores, surround Gram-negative bacteria, with an aqueous compartment termed the periplasmic space between the two membranes. Due to the nature of these membranes, the trafficking of proteins and nutrients in and out of Gram-negative bacteria is much more complex than if only a single membrane were present. The crystal structure of the outer membrane protein TolC (tolerance to colicins) has just been reported[1] in a recent issue of *Nature* and has greatly enhanced our ability to understand these processes.

To address these complex modes of transport, it has been important to make mutations in transport protein genes and attempt crystallization of the transport proteins — a daunting task since they are integral membrane proteins. Although relatively few bacterial cytoplasmic membrane proteins have been crystallized, it has been fairly straightforward to isolate mutations in their corresponding genes in order to understand their function, and even make reasonable guesses about their structures. For virtually all, characteristic and specific functions have been established.

The transmembrane domains of the cytoplasmic membrane proteins are almost certainly α-helical, and their locations can be predicted with some reliability by examining the primary amino acid sequence with algorithms that identify long (~20 amino acid) hydrophobic stretches. The situation is quite different for outer membrane proteins, which thus far, at least, use β-strands to span the outer membrane. Because as few as six amino acids are needed to form a β-strand, only some of which must be hydrophobic in order to traverse the outer membrane, the 'signature' of a β-strand is much less apparent. Thus, from the very beginnings of sequence-gazing efforts, it has been difficult to make accurate predictions regarding the occurrence and placement of secondary structures in outer membrane proteins. However, like cytoplasmic membrane proteins, outer membrane proteins have been fairly straightforward to characterize mutationally.

Pore proteins were the first outer membrane proteins to be crystallized, and it was discovered that they have a rather simple structure — a trimer of β-barrels[2]. The temptation since has been to predict that simple β-barrels are the only types of proteins to be found in the outer membrane. The first examples to contradict this notion were FepA (ferric uptake) and FhuA (ferric hydroxamate uptake), where a globular signaling domain fills the hole in the β-barrel[3–5]. The globular domain