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Interactions between BRCT repeats and phosphoproteins: tangled up in two

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The C-terminal region of the breast-cancer-associated protein BRCA1 contains a pair of tandem BRCA1 C-terminal (BRCT) repeats that are essential for the tumour suppressor function of the protein. Similar repeat sequences have been identified in many proteins that seem to mediate cellular mechanisms for dealing with DNA damage. The BRCT domain in BRCA1 has been recently shown to constitute a module for recognizing phosphorylated (phospho-) peptides, with a recognition groove that spans both BRCT repeats. The fact that many other BRCT-containing proteins have phosphopeptide binding activity suggests that BRCT repeats might mediate phosphorylation-dependent protein–protein interactions in processes that are central to cell-cycle checkpoint and DNA repair functions.

The repair of DNA damage in eukaryotic cells requires ordered assembly of the appropriate repair machinery at the site of damage and arrest of the cell cycle to enable the damage to be processed before DNA replication [1,2]. Assembly of the protein complexes that govern these processes is controlled by posttranslational modification of the proteins involved. Phosphorylation seems to be a crucial signal to trigger protein–protein interactions in many of these processes; until recently, however, the protein modules that recognize phosphorylation signals in the DNA repair pathways remained poorly understood.

The forkhead-associated and 14–3-3 domains were the first two phosphoprotein recognition modules shown to be involved in the DNA damage response [3]. Recent findings have shown that BRCT domains constitute a new class of phosphoprotein binding modules that have important roles in DNA repair pathways. The relative abundance of these domains in eukaryotic repair enzymes and cell-cycle checkpoint proteins further suggests that these domains have a central role in coordinating the DNA damage response.

Here we review the structure of BRCT repeats, their mechanisms of interacting with phosphorylated and non-phosphorylated protein targets, and the role of these interactions in key networks that regulate DNA repair.

Discovery of the BRCT repeat module

The BRCA1 protein, which is associated with hereditary breast and ovarian cancer, is intimately linked to mechanisms that enable mammalian cells to respond to and to repair DNA damage [4]. Detailed sequence analysis of the C terminus of BRCA1 detected the presence of a pair of repeats, which were subsequently termed BRCT repeats. Most mutations in BRCA1 that are known to cause cancer result in the partial truncation or complete loss of both BRCT repeats, indicating that this region is essential to the tumour suppressor function of the protein.

Sequence analysis has shown that BRCT repeats are present in a large superfamily of proteins that are implicated in the cellular response to DNA damage. Members of this family include several proteins that are directly linked to DNA repair, such as the base excision response scaffold protein XRCC1 and DNA ligase IV, and numerous proteins that, like BRCA1, localize to DNA damage foci and are thought to have a signalling role, including the p53-binding protein 53BP1 and MDC1. Currently there are 658 open reading frames recorded in the Pfam BRCT protein domain family database (http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF00533).

BRCT structure

Several groups began to pursue structural studies of BRCT proteins in the late 1990s. The isolated C-terminal BRCT repeat of XRCC1 was the first structure of a BRCT repeat to be solved [5]. The structure shows that the basic fold of a single repeat consists of a parallel four-stranded β-sheet, which is flanked on one side by a pair of α-helices (α1 and α3) and on the other side by a single α-helix (α2; Figure 1a). BRCT repeats are defined by conserved clusters of hydrophobic residues that occupy the core of the repeat structure and by glycine residues that facilitate a tight turn between α1 and β2 (Figure 2 and reviewed in Ref. [6]). So far, the structures of the BRCT repeats of BRCA1 [7,8] (Figure 1a), 53BP1 [8,9] (Figure 1b), DNA ligase III [10] and an nicotinamide adenine dinucleotide (NAD)-dependent DNA ligase [11] have been determined. The basic structure seems to be well conserved in all of these BRCT repeats, with the exception of the α2 helix, whose sequence is poorly conserved and is absent in the repeat of DNA ligase III.

Although the C-terminal BRCT repeat of XRCC1 can exist as a well-folded isolated domain, most of the BRCT repeats that have been detected occur in multiples. Structural studies of the BRCT repeats of BRCA1 have shown that the pair of repeats in this protein pack in a head-to-tail manner involving a large hydrophobic interface. This interface comprises the α2 helix of the N-terminal repeat, which is packed against α1 and α3 of the C-terminal

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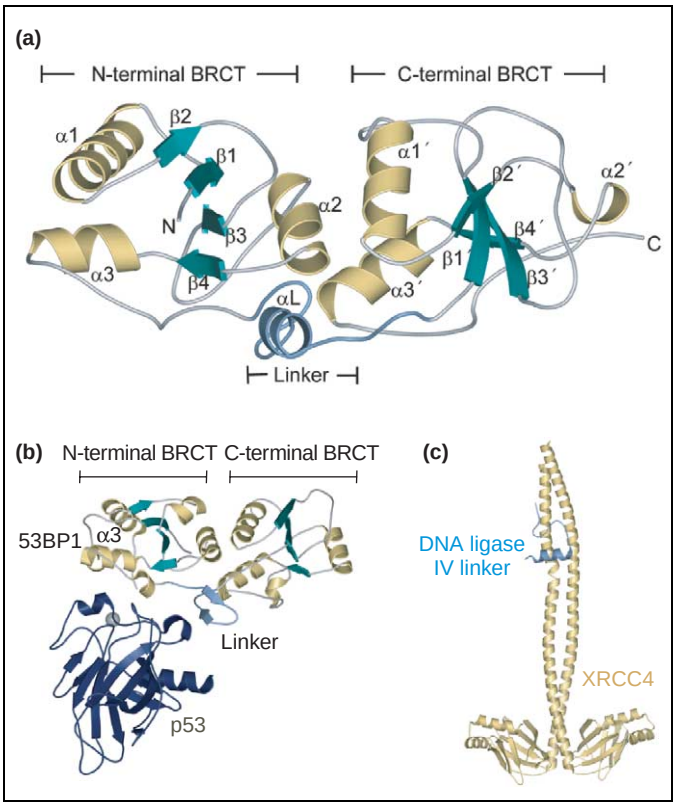

Figure 1. Structural studies of BRCT domains. **(a)** Structure of the tandem BRCT repeats of BRCA1, showing elements of secondary structure. Structural elements in the C-terminal repeat are indicated by primes. Reproduced, with permission, from Ref. [7]. **(b)** Structure of the tandem BRCT repeats of 53BP1 (shown in gold and green) bound to the DNA-binding domain of p53 (shown in blue). The complex is oriented so that the BRCT domain of 53BP1 is viewed in roughly the same orientation as the BRCT domain of BRCA1 in (a). **(c)** Structure of the BRCT repeat linker of DNA ligase IV (shown in blue) bound to XRCC4 (shown in gold).

repeat, and portions of the linker that connects the two repeats [7,8] (Figure 1a). Essentially the same packing arrangement is seen in the structure of the tandem BRCT repeats of 53BP1 [8,9] (Figure 1b), and the fact that the interface residues located in α1, α3 and to a lesser extent α2 are conserved in several BRCT repeats suggests that this mode of packing is used by other members of the family (Figure 2).

The importance of the interaction between the N- and C-terminal repeats in BRCA1 has been highlighted by the fact that several cancer-causing missense mutations involve residues in this interface [7]. In addition, several of these mutations markedly destabilize the protein fold [7,12–14], suggesting that, at least in BRCA1, the two BRCT repeats pack together to form a single protein domain.

BRCT domains: versatile protein–protein interaction modules

Genetic and biochemical studies have shown that several BRCT repeat domains mediate protein–protein interactions that are essential for cell-cycle checkpoint and DNA repair processes (Table 1). In some proteins, the BRCT repeats can specifically contact BRCT repeats in other proteins. For example, the two BRCT repeats of XRCC1 seem to contact distinct targets involved in base excision repair: the N-terminal BRCT repeat interacts with the BRCT repeat of poly(ADP-ribose) polymerase 1 (PARP1) [15], whereas the C-terminal BRCT repeat associates with the single BRCT repeat of DNA ligase III [16–18].

By far the most common form of interaction, however, is between BRCT repeats and protein domains of different structure. Three such complexes have been studied crystallographically: the complex formed between the tandem BRCT repeats of 53BP1 and the p53 DNA-binding domain [8,9]; the complex formed between the XRCC4 repair scaffold protein and the linker between the two BRCT repeats of DNA ligase IV [19]; and complexes formed between the BRCT repeats of BRCA1 and protein targets containing phospho-serine (see later).

Like BRCA1, 53BP1 is a large protein containing a pair of BRCT repeats at its extreme C terminus that becomes localized to DNA damage foci and is intimately involved in cell-cycle checkpoint activation in response to DNA damage. The structure of the p53–53BP1 heterodimer shows that the p53 DNA-binding domain contacts both the α3 helix of the N-terminal BRCT repeat and the inter-repeat linker. Binding to 53BP1 partially occludes the DNA recognition surface and inhibits the DNA-binding activity of p53 [20]. Paradoxically, however, binding to 53BP1 has been also shown to potentiate the transcriptional activation function of p53 [21,22]. This apparent contradiction might be explained by the fact that p53 exists as a tetramer in which only some of the subunits bind 53BP1, leaving the other subunits free to interact with DNA.

XRCC4 and DNA ligase IV are both essential components of the nonhomologous end joining pathway that is the primary route for the repair of double-strand breaks in mammalian cells [23]. XRCC4 forms a tight complex with the cognate DNA ligase IV and might function as a scaffold that mediates the correct spatial and temporal association of repair enzymes to foci of DNA damage during nonhomologous end joining repair. DNA ligase IV contains a pair of C-terminal BRCT repeats that are joined by a linker that is larger than those found in either 53BP1 or BRCA1. The isolated linker forms a tight complex with the coiled-coil domain of XRCC4 and does not seem to require the adjacent BRCT repeats [19].

Because of the clear importance of the BRCT repeats to the function of BRCA1, several approaches have been used to identify proteins that interact with this region and that might cooperate functionally with BRCA1. Some of these interactions are consistent with the idea that the BRCT domain of BRCA1 has a role in transcription. For example, BRCA1 has been shown to co-purify with the RNA polymerase holoenzyme, perhaps through interactions involving the BRCT repeats and the holoenzyme component RNA helicase A [24,25]. In addition, several studies have indicated that the BRCT repeats of BRCA1 interact with the CtIP transcriptional co-repressor in a manner that seems to be regulated by protein phosphorylation [26–31]. Furthermore, the BRCT domain has been shown to interact with histone deacetylases, suggesting that BRCA1 could be also associated with the regulation of chromatin structure [32]. Most recently, the BRCT domain of BRCA1 has been shown to interact with the DNA helicase BACH1, which has been implicated in regulating

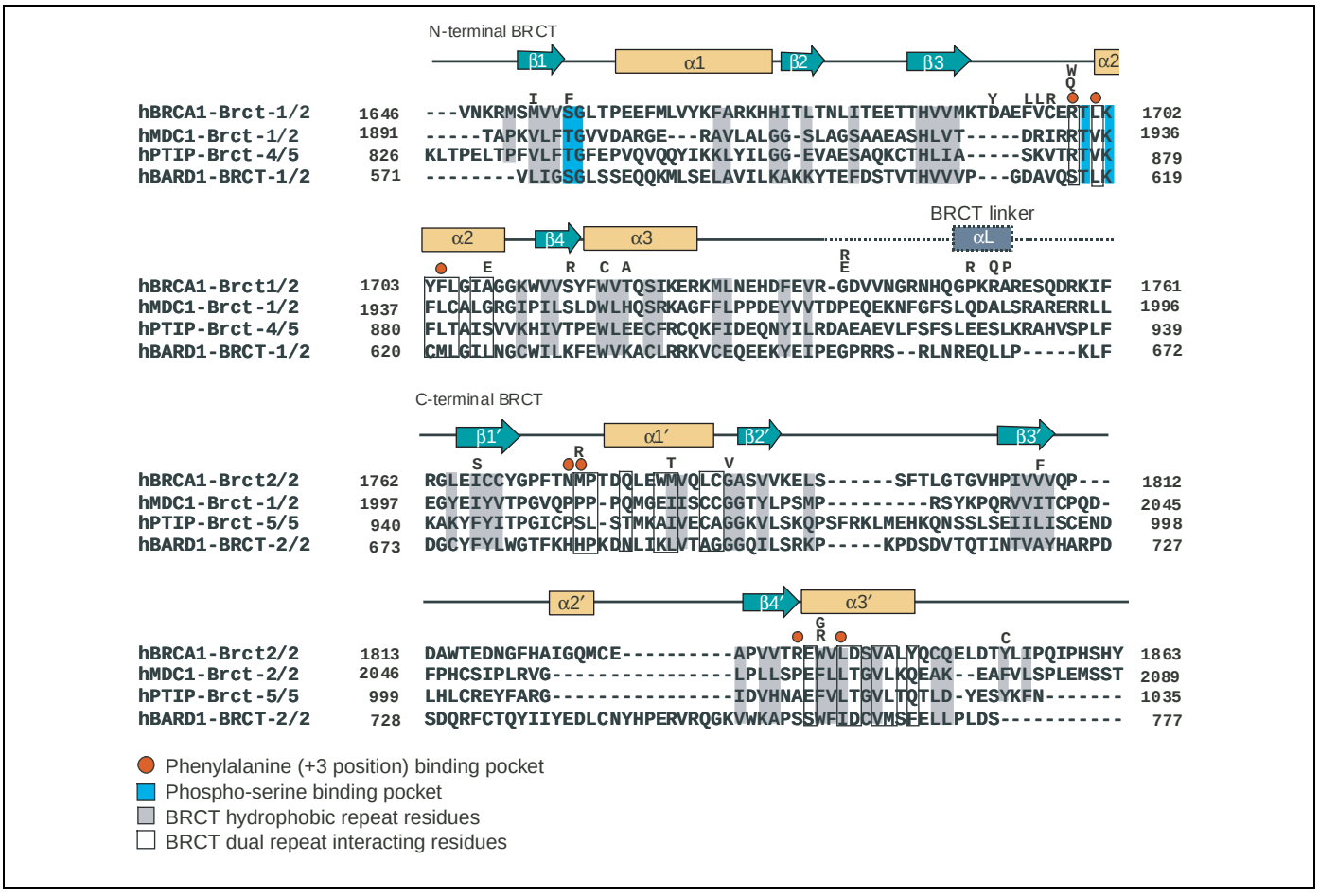

Figure 2. Alignment of the tandem BRCT repeats of BRCA1, MDC1, PTIP and BARD1. The structural elements shown are those of BRCA1, and the letters above the sequence indicate missense mutations in the BRCT repeats of BRCA1 that have been isolated from breast and ovarian cancer screening programs. Reproduced, with permission, from Ref. [37] (www.nature.com).

the G2/M cell-cycle checkpoint in response to DNA damage [33,34].

Phospho-peptide recognition by the BRCT repeats of BRCA1

Two key studies published in late 2003 have shown that the two BRCT repeats of BRCA1 function as a phospho-protein recognition domain [35,36]. Yu *et al.* [35] focused on the previously described interactions between BRCA1 and BACH1 and found that these interactions are dependent on the phosphorylation of BACH1. In addition, they identified the target of phosphorylation as Ser990, which is located in a C-terminal region that had been previously shown to interact with the BRCT domain of BRCA1 [33]. Both BACH1 and BRCA1 are essential for correct functioning of the G2/M cell-cycle checkpoint in response to ionizing radiation in human cells, and Yu *et al.* [35] found that the expression of wild-type BACH1, but not BACH1 containing a serine to alanine mutation at residue 990, could rescue the G2/M checkpoint defect in BACH1-knockdown cells, indicating that Ser990 and its interactions with BRCA1 are essential for checkpoint function.

In a parallel study, Manke *et al.* [36] used a proteomics approach to identify phospho-serine binding proteins from a library of about 10^5 *in vitro* translated polypeptides. The strongest phospho-serine binding peptide that was isolated corresponded to a BRCT-containing fragment of the transcriptional regulatory protein Pax transactivation-domain-interacting protein (PTIP), and further work indicated that the two BRCT repeats in BRCA1 also have phospho-peptide binding activity. Peptide selection studies, using the purified BRCT domain of BRCA1 and a randomized peptide library containing a dipeptide of phospho-serine and glutamine, showed that this domain binds specifically to peptides containing a pSer-X-X-Phe motif [36]. Notably, this motif is also present in the region of Ser990 in BACH1, and mutation of the phenylalanine at 993 to alanine blocks interactions between BRCA1 and BACH1 in human cells.

Structures of complexes between BRCT repeats and phospho-peptides

The way in which the BRCT repeats in BRCA1 bind to phospho-peptide targets has been recently revealed by three independent crystallographic studies [37–39]. Two of these studies used a phospho-peptide corresponding to the BACH1 target [38,39], whereas the third used a target that was optimized in a peptide selection study [37]. All three peptides contained the pSer-X-X-Phe recognition motif.

The structures of all three complexes are essentially identical and show that the phospho-peptide is bound in a

Table 1. BRCT-mediated protein–protein interactions[a]

BRCT–BRCT interactions			
BRCT-containing protein	**BRCT-containing binding partners**	**Cellular function**	**Refs**
XRCC1	PARP	Base excision repair	[15]
XRCC1	DNA ligase III	Base excision repair	[16–18]
*Sc*RAD9	*Sc*RAD9	DNA damage response	[52]
BRCT–non-BRCT interactions			
BRCT-containing protein	**Binding partners**	**Cellular function**	**Refs**
BRCA1	BACH1	G2/M checkpoint	[33–35]
BRCA1	CtIP and LMO4	Transcriptional regulation	[26–31,53]
BRCA1	HDACs	Chromatin structure	[32]
BRCA1	CBP	Chromatin structure	[54]
BRCA1	P53	Transcription	[55]
BRCA1	RNAP holo-enzyme (RHA subunit)	Transcription	[24,25]
BRCA1	Acetyl-CoA carboxylase	Fatty acid metabolism	[56]
*Sc*DNA ligase IV	LIF1	NHEJ	[57]
DNA ligase IV	XRCC4	NHEJ	[19,58,59]
TOPBP1 (similar to *Sp*Cut5/Rad4, *Sc*Dpb11, *Dm*Mus101)	E2F1	DNA repair/ checkpoint/ transcription repression	[60,61]
Rad4	Rad9 (PCNA-like)	Checkpoint	[62]
Dpb11	Ddc1 (PCNA-like)	Checkpoint	[63]
Dpb11	Drc1	S-phase checkpoint	[64]
53BP1	p53	DNA repair/ transcription/ checkpoint	[8,9,20]
*Sp*Crb2 (similar to 53BP1)	Rad3 kinase	Checkpoint	[65]
FCP1	RNAPII CTD	Transcription	[35]
Swift	Smad2	Transcription	[66]

[a]Abbreviations: 53BP1: p53 binding protein 1; BACH1: BRCA1-associated C-terminal helicase; BRCA1: breast-cancer-associated protein 1; BRCT: BRCA1 C-terminal; CBP: CREB-binding protein; CTD, C-terminal heptad repeat tail; *Dm*, *Drosophila melanogaster*; HDAC: histone deacetylase; NHEJ: non-homologous end joining; PARP: poly(ADP-ribose) polymerase; PCNA, proliferating-cell nuclear antigen; RNAP, RNA polymerase; *Sc*, *Saccharomyces cerevisiae*; *Sp*, *Schizosaccharomyces pombe*; TOPBP1; topoisomerase IIβ binding protein I.

groove that involves both the N- and C-terminal repeats (Figure 3). The structures show that there is a phospho-serine binding pocket in the N-terminal BRCT repeat; this pocket supplies ligands that recognize three of the phosphate oxygen atoms. Comparison of the residues that recognize the phosphate between the peptide-bound and peptide-free forms of the BRCT domain of BRCA1 indicates that these residues are relatively inflexible and present a pre-aligned recognition surface for the phospho-serine residue. The phenylalanine residue is recognized by a largely hydrophobic groove located at the interface between the N- and C-terminal repeats, which explains

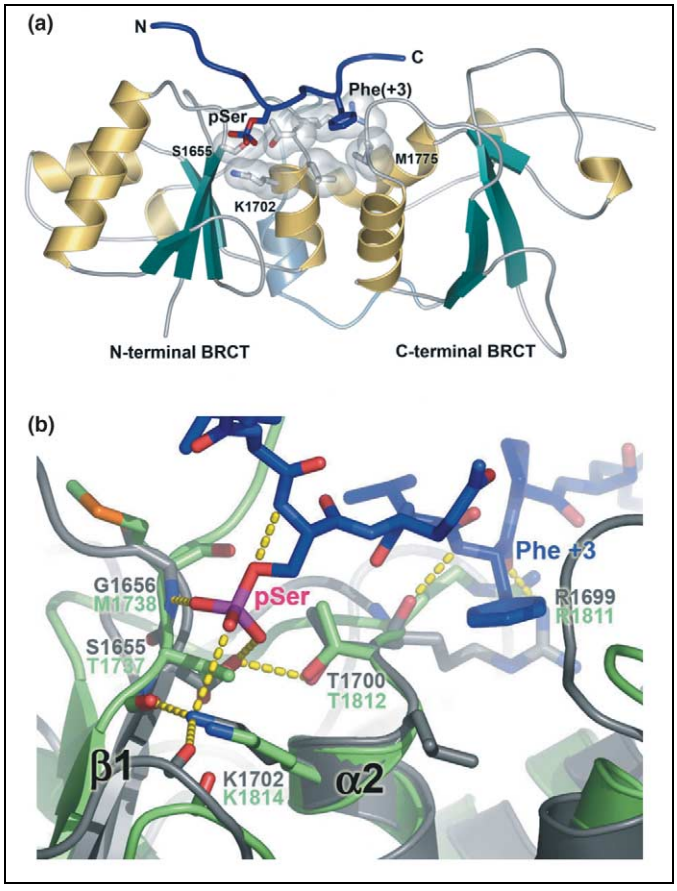

Figure 3. Phospho-peptide recognition by the BRCT domain of BRCA1. **(a)** Overview of the complex formed between the BRCT domain and phospho-peptide, highlighting the side chains that line the phospho-serine and phenylalanine (+3 position) binding pockets. **(b)** Detail of phospho-peptide recognition. The optimized phospho-peptide is shown in blue, the BRCT domain of BRCA1 is shown in grey, and hydrogen bonding and salt bridges are indicated by yellow dashes. Alignment of the structure of the BRCT domain of 53BP1 (shown in green) with that of BRCA1 reveals structural conservation of the peptide-binding interface. Modified, with permission, from Ref. [37] (www.nature.com).

why both repeats are required for BRCA1 function (Figure 3).

Numerous missense mutations in the BRCT domains of BRCA1 have been isolated in breast cancer screening programs, and a significant subset of these mutant proteins have been tested for their ability to bind peptides containing phospho-serine [37–39]. In general, these studies have provided support for the notion that the integrity of both the phospho-serine and phenylalanine binding pockets are essential for interactions with phospho-peptides *in vivo*. Particularly interesting is the behaviour of the methionine to arginine mutation at position 1775 (Met1775Arg). This mutation was among the first to be linked to breast cancer [40,41] and leads to defects in cell-cycle regulation [35], in double-strand break repair [42], and in the recognition of target proteins such as BACH1 [33,35], CtIP [27] and histone deacetylases [32].

The methionine at position 1775 forms the base of the recognition pocket for phenylalanine (Figure 3a), and its mutation to arginine essentially destroys the ability of the protein to interact with peptides containing the pSer-X-X-Phe motif *in vitro* [37–39]. The structure of the Met1775Arg mutant reveals the basis for this: the

substituted arginine side chain flips out, filling the pocket that would be normally occupied by the phenylalanine [13]. This pocket seems to be very sensitive to subtle mutations that are distant from the surface of the protein. For example, substitution of Val1809 with phenylalanine blocks peptide binding, and the structure of this variant reveals a cascade of structural rearrangements emanating from the core of the protein that block the phenylalanine binding pocket [37].

In addition, a complex of the BRCT repeats of BRCA1 and the BACH1 phospho-peptide has been recently characterized in solution by NMR spectroscopy [43]. The results are in agreement with the structures determined crystallographically and, furthermore, suggest that the BRCT domain of BRCA1 becomes more rigid on peptide binding.

Phosphoprotein binding: a common function of BRCT repeats?

Although structural information is available only for complexes of the BRCT repeats of BRCA1 and phospho-peptides, several other proteins involved in the DNA damage response possess both BRCT repeats and phospho-peptide binding activity. So far, the tandem BRCT repeats of MDC1, PTIP, BARD1, DNA ligase IV. *Saccharomyces cerevisiae* Rad9, 53BP1, Crb2, RAD4, Ect2 and TOPBP1 have been shown to have phospho-peptide binding activity *in vitro* [35,36,44]. Tandem BRCT repeats seem to be required for phospho-peptide binding in most proteins studied; however, single BRCT repeats present in terminal deoxynucleotidyl transferase (TDT), the Y-family DNA polymerase REV1, the phosphatase FCP1 and DNA ligase III have been also proposed to interact with phospho-peptides [35].

Consistent with the idea that phospho-peptide binding is a common function of BRCT modules, the residues that constitute the phospho-serine binding surface in BRCA1 (Ser/Thr-Gly in the β1–α1 loop, and Thr/Ser-X-Lys in α2 of the same repeat) are conserved in several BRCT domains [37] (Figure 2). For example, the N-terminal BRCT repeat of 53BP1 contains most of the conserved phosphate-binding residues, and analysis of the structure of this domain indicates that these residues are pre-aligned for phosphate binding (Figure 3b). Sequence analysis of the whole family of BRCT repeats suggests that other intriguing family members, such as the DNA repair scaffold protein XRCC1 and the large subunit of the clamp-loading complex, replication factor C, also contain BRCT repeats that could bind phospho-peptides [37].

Aside from the repeats in BRCA1, tandem BRCT repeats in two other proteins, MDC1 and PTIP, show significant sequence specificity for the +3 position of the bound phospho-peptide. MDC1 has an important role in DNA damage cell-cycle checkpoints and rapidly colocalizes to nuclear foci along with the phosphorylated form of the histone variant H2AX at sites of DNA double-strand breaks [45–47]. The BRCT repeats of MDC1 bind preferentially to peptides containing the motif pSer-Ile-Glu-Tyr (in which the phospho-serine, glutamic acid and tyrosine residues are the primary binding determinants) [44]. Intriguingly, the binding preference of MDC1 matches the phosphorylated tail of H2AX (pSer-Gln-Glu-Tyr), strongly suggesting that MDC1 could directly recognize the H2AX tail *in vivo*. Moreover, the fact that the tyrosine is the C-terminal residue of H2AX might be important in recognition, because the tyrosine carboxylate could form a salt bridge with the guanidinium group of an arginine residue (Arg1699 in BRCA1, Arg1933 in MDC1; Figure 3b) that is conserved in the tyrosine/phenylalanine-binding pocket. A pair of tandem BRCT repeats in PTIP also show strong binding selectivity for phospho-serine peptides that contain hydrophobic (Phe > Ile, Leu) side chains at the +3 position. This activity is probably responsible for the association of PTIP and 53BP1 in irradiated cells [36]. In addition, the tandem BRCT repeats of BARD1, DNA ligase IV and *S. cerevisiae* RAD9 have been shown to have more modest binding preferences in sequences immediately C-terminal to the phospho-serine, indicating that sequence selectivity might be a common feature of interactions between tandem BRCT repeats and phospho-peptides [44].

Notably, some single BRCT repeat domains have been suggested to show phospho-peptide binding activity, although whether any of these proteins have binding specificity other than the requirement for a phospho-serine has not been rigorously tested. A particularly interesting example is FCP1, a protein phosphatase that has been shown to dephosphorylate the C-terminal heptad repeat tail, or CTD, of the large subunit of RNA polymerase II, ultimately to regulate transcriptional elongation and the coupling of processes such as precursor mRNA processing to transcription [48]. In addition to its catalytic domain, FCP1 contains a BRCT repeat that has been shown to bind to phosphorylated forms of the heptad [35], which might help to recruit this protein to the CTD. The BRCT repeat might also have a more direct role in catalysis, because the presence of this domain is essential for the dephosphorylation of a CTD peptide by purified FCP1 [49]. For example, phospho-peptide binding to the BRCT domain might allosterically activate the catalytic domain or might supply part of the phosphatase active site.

Higher-order protein assemblies involving BRCT domains

The BRCT repeat structure provides a flexible framework for diverse interactions that are crucial in DNA damage signalling complexes, and the structural diversity of BRCT repeats make them particularly attractive as scaffolding elements at the heart of large, multiprotein complexes. Many BRCT proteins contain several BRCT repeats: the topoisomerase binding protein TOPBP1, for example, contains six. Multiple BRCT repeats could bring together several different phosphorylated target proteins or could be designed to recognize binding partners that contain several phosphorylated residues.

It is also clear that, whereas binding to phospho-proteins is a conserved function of these domains, in some situations BRCT domains can use other surfaces to contact proteins in ways that are not dependent on phosphorylation. The best-characterized examples are the interactions between 53BP1 and p53 [8,9], and those between DNA ligase IV and XRCC4 [19]

(Figure 1b,c). Both sets of interactions involve large flexible loops on the surface of the BRCT that are not well conserved in the family. These interactions might provide additional specificity in the recognition of a single protein or, alternatively, could facilitate the interaction of two or more different proteins on the surface of the BRCT scaffold.

The BRCT domain of BRCA1 bound to the optimized phospho-serine peptide crystallizes as a dimer containing two BRCT–peptide complexes [37]. The interface between the two complexes is relatively large ($\sim$2000 Å^2 of buried surface area), which suggests that this interaction might be physiologically relevant. The dimerization is largely mediated by the peptide and is stabilized by hydrogen-bonding interactions involving the glutamine side chain at the +1 position of the bound peptide. Notably, the BACH1 peptide contains a proline at this position and, probably for this reason, the BRCT domain of BRCA1 crystallizes as a monomer with this peptide [38,39]. This finding presents the intriguing possibility that the oligomeric state of the BRCT domain of BRCA1 might be regulated by its binding to specific phosphorylated targets. Moreover, BRCA1 is known to exist in a heterodimeric complex with BARD1, which also contains a tandem BRCT repeat at its C terminus [50,51]. Thus, it is possible that homo- or heterodimeric BRCT–BRCT interactions could be regulated in a manner that is dependent on phospho-peptide binding.

Concluding remarks

Clearly, we are beginning to understand the way in which BRCT repeats can be used to recognize isolated phosphorylated peptides. A challenge for future work will be to determine how BRCT repeat structures are integrated into large repair complexes, and how these complexes are dynamically regulated during the DNA damage response.

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