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REVIEW ARTICLE

Oligonucleotide/oligosaccharide-binding fold proteins: a growing family of genome guardians

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Abstract

The maintenance of genomic stability relies on the coordinated action of a number of cellular processes, including activation of the DNA-damage checkpoint, DNA replication, DNA repair, and telomere homeostasis. Many proteins involved in these cellular processes use different types of functional modules to regulate and execute their functions. Recent studies have revealed that many DNA-damage checkpoint and DNA repair proteins in human cells possess the oligonucleotide/oligosaccharide-binding (OB) fold domains, which are known to bind single-stranded DNA in both prokaryotes and eukaryotes. Furthermore, during the DNA damage response, the OB folds of the human checkpoint and DNA repair proteins play critical roles in DNA binding, protein complex assembly, and regulating protein–protein interactions. These findings suggest that the OB fold is an evolutionarily conserved functional module that is widely used by genome guardians. In this review, we will highlight the functions of several well-characterized or newly discovered eukaryotic OB-fold proteins in the DNA damage response.

Keywords: *OB fold; DNA damage; checkpoint; telomere; DNA repair*

Introduction

Eukaryotic cells have evolved elaborate mechanisms to faithfully duplicate the genetic information contained within the cell and accurately transmit it to the next generation. During each cell division, the processes of genome duplication and segregation are coordinated by a complex network of proteins, which function to ensure that incompletely replicated or damaged DNA is not passed on to subsequent generations. Through this network of genome guardians, cells can not only recognize intrinsic and extrinsic forms of DNA damage, but also initiate and propagate a DNA damage response when necessary (Zhou and Elledge, 2000; Harper and Elledge, 2007). Thus, the ability of cells to sense and respond to DNA damage is essential for coordinating cell cycle progression, preserving genomic integrity, and ultimately, sustaining cell survival.

A central component of the DNA damage response network is the protein kinase ataxia telangiectasia mutated and rad3-related (ATR; Abraham, 2001; Cimprich and Cortez, 2008; Shiotani and Zou, 2009). Following DNA damage, ATR and its associated partner ATRIP (ATR-interacting protein) are recruited to sites of damage where they launch a signaling cascade. This ATR-mediated signaling cascade, or the ATR checkpoint, regulates and coordinates cell cycle progression, DNA replication, DNA repair, and many other cellular processes critical for maintaining genomic stability. Although the exact mechanism by which ATR is activated by damaged DNA is not fully understood, generation of single-stranded DNA (ssDNA) has been implicated as a key step in the process. Data from others, and from our laboratory, have shown that the ssDNA coated by replication protein A (RPA), a common protein–DNA structure generated during DNA replication and DNA repair, is critical for the recruitment and activation of ATR-ATRIP at sites of DNA damage (Costanzo and Gautier, 2003; Zou and Elledge, 2003).

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The functions of ssDNA and ssDNA-binding proteins in DNA replication and repair have been well characterized in both prokaryotes and eukaryotes. Moreover, the critical role of ssDNA and RPA in the activation of ATR checkpoint further supports the concept that ssDNA and ssDNA-binding proteins are key coordinators of the DNA damage response. In addition to RPA, a number of eukaryotic ssDNA-binding proteins have been identified. Interestingly, like RPA, many of these proteins are involved in the maintenance of genomic stability and also possess a specific type of ssDNA- or protein-binding domain called the oligonucleotide/oligosaccharide-binding fold (OB fold; Murzin, 1993). The number of OB-fold proteins has grown rapidly during the past few years. To date, OB-fold proteins have been shown to play key roles in many cellular processes important for genomic stability, including DNA replication, recombination, repair, and telomere homeostasis. These findings suggest that the OB fold is an important functional module that is widely used by genome guardians. In this review, we will discuss the organization of the known eukaryotic OB-fold proteins, how OB folds regulate protein functions, and how these individual functions contribute to the maintenance of genomic stability.

The OB folds

The OB folds were originally identified from a group of bacterial and yeast proteins as domains that bind to oligonucleotides or oligosaccharides (Murzin, 1993). Subsequently studies have shown that various OB folds were capable of establishing protein–DNA, protein–RNA, or protein–protein interactions (Arcus, 2002; Agrawal and Kishan, 2003; Theobald *et al.*, 2003). Among these functions of OB folds, their roles in protein–ssDNA interactions are the most extensively characterized. The OB folds in different proteins vary in length (from 70–150 amino acids) and have a low degree of sequence similarity. However, all OB folds share several structural features. Structurally, the OB folds are β barrels consisting of five highly coiled, antiparallel β sheets (Murzin, 1993). These β barrels are capped by an α helix at one end, and present a binding cleft at the other end. The connecting loops between β sheets vary in sequence, length, and conformation, contributing to the binding specificities of the OB folds. Another common feature of the OB folds is that they are often present as tandem repeats in proteins or protein complexes. These tandem OB folds may interact with ssDNA in a cooperative fashion, providing increased affinity and/or sequence specificity for ssDNA binding. Through binding to DNA or proteins, the OB folds play important roles in orchestrating protein–DNA and protein–protein interactions during the DNA damage response, providing a crucial means to regulate the localization and function of DNA repair and signaling proteins.

RPA

RPA is a well-characterized ssDNA-binding protein complex that interacts with ssDNA using OB folds (Wold, 1997; Fanning *et al.*, 2006). Originally identified as an essential component of the DNA replication machinery, RPA is now known to play important roles in checkpoint signaling, DNA recombination, DNA repair, and telomere maintenance. RPA is a heterotrimeric protein complex composed of three distinct subunits of approximately 70, 32, and 14 KDa (Figure 1). *In vitro*, RPA exhibits much higher affinity to ssDNA than double-stranded DNA (dsDNA) or RNA. As a complex, RPA binds to ssDNA in a non-sequence specific manner with a dissociation constant of 10^{-9}–10^{-10} M (Kim *et al.*, 1994).

The binding of RPA to ssDNA is mediated by four OB folds commonly referred to as DNA binding domains DBD-A, -B, -C and -D (Figure 1). The DBD-A, DBD-B, and DBD-C domains all reside within the RPA70 subunit, whereas DBD-D is located in the RPA32 subunit. RPA is able to bind ssDNA in at least three distinct modes (Bochkareva *et al.*, 2001; 2002). The DBD-A and -B of RPA70 alone are able to establish a weak interaction with ssDNA, creating an occluded site of 8–10 nt. The binding of DBD-A and –B to ssDNA induces a conformational change in the RPA complex and facilitates DBD-C to bind ssDNA, extending the occluded site to 12–23 nt. Finally, through its interaction with RPA70, the DBD-D of RPA32 is positioned onto ssDNA, resulting in an occluded site of 28–30 nt. Complete disruption of DBD-A eliminates the ssDNA binding of RPA and abolishes all of its functions in cells (Haring *et al.*, 2008). Point mutations in DBD-A, DBD-B, or deletion of DBD-C, reduce the affinity of RPA to ssDNA to various extents. Interestingly, some RPA OB-fold mutants with substantially reduced affinity to ssDNA remain functional in DNA replication, repair, and checkpoint response, suggesting that these functions of RPA do not require the maximal affinity to ssDNA (Haring *et al.*, 2008).

Besides the four OB folds involved in ssDNA binding, RPA possesses two additional OB folds in RPA70 and RPA14. The OB fold located at the N terminus of RPA70 does not have a significant role in ssDNA binding, but is important for checkpoint signaling and DNA repair (Jacobs *et al.*, 1999; Binz and Wold, 2008). Rfa1-t11, a yeast Rpa70 mutant lacking a critical lysine residue in the N-terminal OB fold, is proficient for DNA replication, but defective of checkpoint activation (Umezu *et al.*, 1998). Unlike the wild-type RPA, the rfa1-t11 mutant is unable to interact with Ddc2 (yeast homolog of human ATRIP), and fails to recruit Ddc2 and Ddc1 (yeast homolog of human

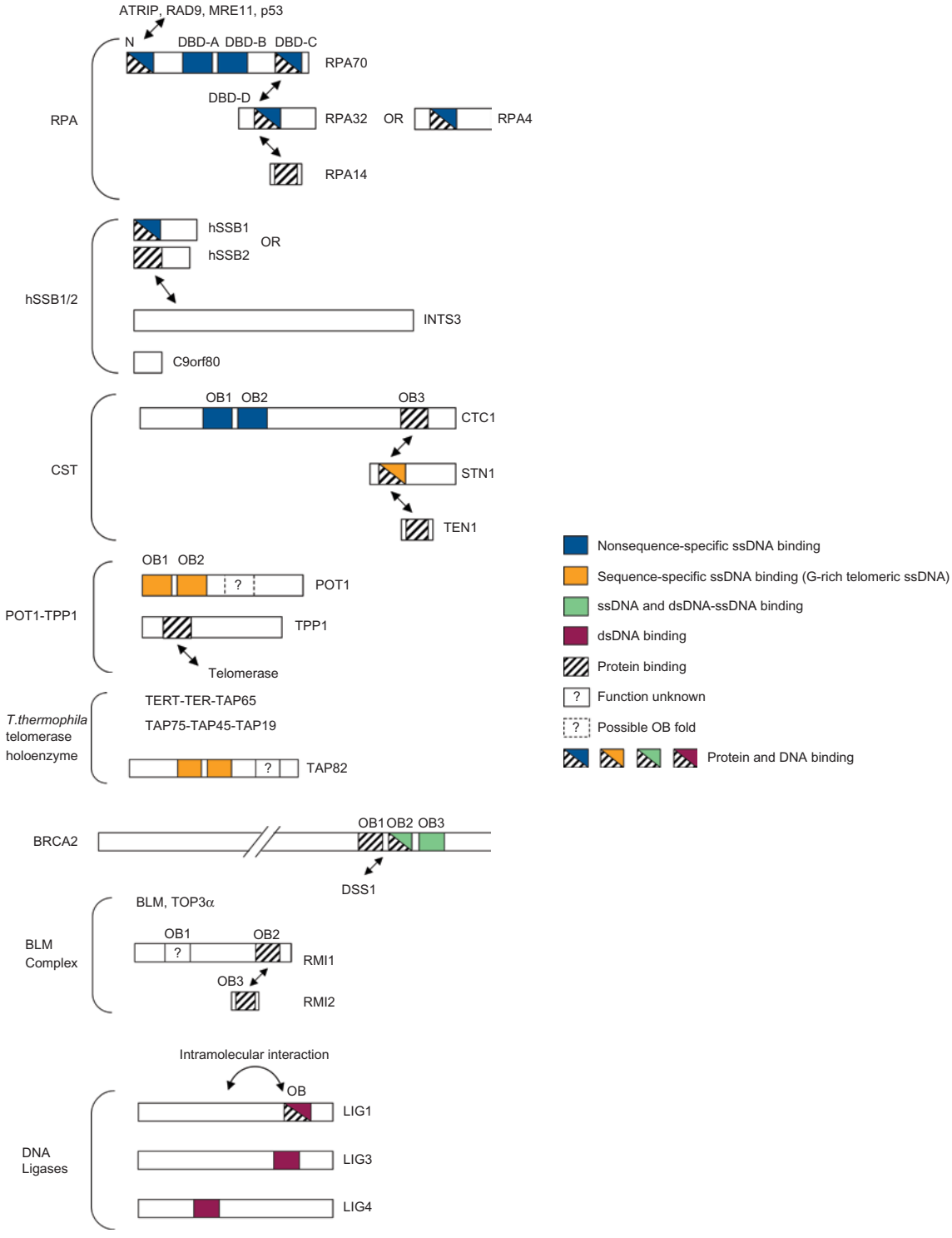

Figure 1. The OB-fold proteins and protein complexes involved in the DNA damage response and telomere maintenance. The OB folds are classified are colored according to their functions. For the OB folds that bind to DNA, their DNA sequence specificity (or lack of sequence specificity) and DNA structure specificity are indicated. For the OB folds involved in protein–protein interactions, their known interacting proteins are shown.

Rad9) to sites of DNA damage (Zou and Elledge, 2003; Zou *et al.*, 2003; Lucca *et al.*, 2004; Majka *et al.*, 2006). During homologous recombination (HR), the rfa1-t11 mutant is displaced by Rad51 more slowly than wild-type RPA, and it fails to support a Rad52-dependent annealing step after Rad51-mediated strand exchange (Kantake *et al.*, 2003; Sugiyama *et al.*, 2006). The N-terminal OB fold of human RPA70 is known to interact with a number of proteins

involved in DNA damage response, including p53, ATRIP, RAD9, and MRE11 (Jacobs *et al.*, 1999; Zou and Elledge, 2003; Ball *et al.*, 2005; 2007; Namiki and Zou, 2006; Olson *et al.*, 2007; Xu *et al.*, 2008b; Oakley *et al.*, 2009). These findings suggest that the N-terminal OB fold of RPA70 is a major protein–protein interaction module of RPA that recruits and/or coordinates checkpoint and DNA repair proteins. The OB fold of RPA14 is also involved in a protein–protein interaction, and specifically interacts with RPA32 subunit within the RPA complex (Bochkarev *et al.*, 1999; Deng *et al.*, 2007). The DBD-C of RPA70 and the DBD-D of RPA32 are also implicated in the interactions among the RPA subunits (Figure 1).

In addition to the three subunits of the canonical RPA complex, a 30-kDa homolog of RPA32 termed RPA4 has been detected in certain human tissues and cancer cells (Figure 1; Keshav *et al.*, 1995). RPA4 contains an OB fold at its N terminus like RPA32, and is able to form a stable complex with RPA70 and RPA14 and support efficient binding to ssDNA (Mason *et al.*, 2009). RPA4 is localized to sites of DNA damage in the absence of RPA32 (Haring *et al.,* 2010). The alternative RPA complex is able to perform some of the DNA repair functions of RPA (Kemp *et al.,* 2010), but is unable to support chromosomal DNA replication (Haring *et al.,* 2010). Domain swapping experiments suggest that the OB fold of RPA4 cannot substitute for the OB fold of RPA32 during DNA replication.

Thus, the distinct functions of the OB folds of RPA provide a good example of how OB folds can contribute to the assembly of a protein complex, the binding of a protein complex to ssDNA, and the interactions between protein complexes.

Human SSBs

Until recently, RPA was thought to be the functional homolog of the bacterial single-stranded binding protein, SSB, in mammalian cells. However, recent studies have identified two additional mammalian homologs of SSB, hSSB1 and hSSB2 (Richard *et al.*, 2008). Sequence alignments have revealed that hSSB1 and hSSB2 are more closely related to the bacterial SSB than RPA. Both hSSB1 and hSSB2 contain a single OB fold at the N terminus. These OB folds are highly conserved between hSSB1 and hSSB2, but exhibit a lower degree of sequence conservation from the bacterial and archaeal SSBs. *In vitro*, purified hSSB1 specifically binds to ssDNA, but not dsDNA (Richard *et al.*, 2008). The affinity of hSSB1 to ssDNA appears to be much lower than that of RPA, with a dissociation constant in the range of 10^{-5}–10^{-6} M. hSSB1 binds to ssDNA in a largely sequence-independent manner, but is unable to bind poly dA. Furthermore, hSSB1 binds to long ssDNA more efficiently than short ssDNA. Currently, there are no data detailing the ssDNA-binding capabilities of hSSB2.

Three independent studies reveal that hSSB1 and hSSB2 exist in two distinct complexes (Figure 1; Huang *et al.*, 2009; Li *et al.*, 2009; Zhang *et al.*, 2009). Like RPA, the hSSB1 and hSSB2 complexes are heterotrimers. Both of these complexes contain the integrator complex subunit 3, INTS3, and a previously uncharacterized protein, C9orf80. hSSB1 and hSSB2 are the only distinct subunits in the two complexes. Interestingly, hSSB1 and hSSB2 are similar to RPA32 in many ways: they are all similar in size, they all contain a single OB fold at the N terminus, and they are all the middle subunits in their corresponding complexes (Figure 1). However, the INTS3 and C9orf80 subunits of the hSSB1/2 complexes do not appear to contain additional OB folds like RPA70 and RPA14. When tested *in vitro*, the INTS3-hSSB1-C9orf80 complex and hSSB1 exhibit similar affinity to ssDNA (Huang *et al.*, 2009). This result infers that the affinity of the hSSB1 complex to ssDNA is much lower than that of RPA. While the hSSB1 complex is clearly capable of binding ssDNA *in vitro*, the functional significance of this activity *in vivo* remains unclear. It is worth noting that the OB fold in RPA32 (DBD-D) has only a minor role in ssDNA binding, but is important for the interactions among the RPA subunits. The analogy between hSSB1/2 and RPA32 raises the possibility that the main functions of the OB folds of hSSB1/2 are protein–protein interactions, rather than protein–ssDNA interactions. Consistent with this idea, Zhang *et al.* show that the N terminus of INTS3 directly binds to the OB folds of hSSB1 and hSSB2 (Zhang *et al.*, 2009).

The OB folds of hSSB1 and hSSB2 have been implicated in the recruitment of these proteins to sites of DNA damage in cells. Like RPA, hSSB1 and hSSB2 are localized to discrete nuclear foci in cells treated with ionizing radiation (IR), suggesting that they are recruited to double-stranded DNA breaks (DSBs; Richard *et al.*, 2008). Loss of the OB fold of hSSB1 results in complete loss of hSSB1 foci. Due to the fact that the OB fold of hSSB1 interacts with INTS3, it is plausible that hSSB1 can only be recruited to DSBs as part of the INTS3-hSSB1-C9orf80 complex. Indeed, the focus formation of hSSB1 and hSSB2 is dependent on INTS3 (Huang *et al.*, 2009). The accumulation of INTS3 at DSBs in turn requires a number of proteins involved in DNA damage response, including H2AX, MDC1, MRN (the MRE11-RAD50-NBS1 complex), RNF8, and 53BP1 (Huang *et al.*, 2009; Zhang *et al.*, 2009). Furthermore, INTS3 interacts with NBS1, providing a physical means to target the hSSB1/2 complexes to DSBs (Huang *et al.*, 2009).

Like RPA, the hSSB1/2 complexes are important for the repair of DSBs. Depletion of INTS3 reduces the recruitment of BRCA1 and RAD51 to DSBs, suggesting that the hSSB1/2 complexes are involved in HR (Huang *et al.*, 2009; Skaar *et al.*, 2009; Zhang *et al.*, 2009). The functions of RPA and the hSSB1/2 complexes in HR appear to be distinct.

Co-depletion of INTS3 and CtIP, a protein required for the localization of RPA to DSBs, impairs HR more than depletion of INTS3 or CtIP alone (Huang *et al.*, 2009). Although both RPA and hSSB1 are recruited to DSBs, they do not precisely coincide with each other. Unlike RPA, hSSB1 and hSSB2 are recruited to DSBs in the absence of CtIP (Huang *et al.*, 2009). Furthermore, depletion of INTS3 does not affect the localization of RPA. These results suggest that hSSB1/2 and RPA are independently recruited to DSBs. Interestingly, the localization of hSSB1 and hSSB2 to DSBs is only dependent on the MRN complex during S and G2 phases of the cell cycle, the period when cells are able to carry out HR (Huang *et al.*, 2009).

The hSSB1/2 complexes, like RPA, are also involved in the checkpoint response. However, while RPA specifically regulates the ATR checkpoint, hSSB1/2 appear to regulate both the ATM and the ATR checkpoints. Ablation of the components of the hSSB1/2 complexes compromises the phosphorylation of ATM, NBS1, CHK1, CHK2, and p53 after DNA damage (Richard *et al.*, 2008; Li *et al.*, 2009; Skaar *et al.*, 2009; Zhang *et al.*, 2009). The exact functions of the hSSB1/2 complexes in checkpoint response remain unclear. One intriguing clue is that depletion of INTS3 impairs the recruitment of TopBP1, a critical activator of ATR-ATRIP, to DSBs (Zhang *et al.*, 2009).

Taken together, the available data suggest that the OB folds of hSSB1/2 are important for the assembly and localization of the hSSB1 and hSSB2 complexes. However, although these OB folds are capable of binding ssDNA *in vitro*, whether they directly engage ssDNA during DSB repair or checkpoint activation remains to be investigated.

The CST complex

In addition to sites of DNA replication and repair, telomeres are also associated with ssDNA (Verdun and Karlseder, 2007; Palm and de Lange, 2008). In mammalian cells, telomeres are composed of repetitive sequences $(5'\ TTAGGG\ 3')_n$ approximately 5–10 Kb in length. During each cell division, approximately 50–100 nucleotides of telomeric DNA are lost due to incomplete lagging strand synthesis at telomere ends. With multiple cell divisions this end replication problem leads to telomere attrition, which eventually would lead to cellular senescence or death. Cells have evolved a specialized machinery to maintain telomere length. As a result of both the end replication problem and exonucleolytic resection, a 3′ssDNA overhang is generated at the end of each chromosome and serves as a scaffold for the reverse transcriptase telomerase to elongate the G-rich strand (TTAGGG). Subsequently, the complementary C-rich strand is synthesized by other replication proteins. In *Saccharomyces cerevisiae*, this process is regulated, in part, by an RPA-like complex composed of Cdc13, Stn1, and Ten1, all of which contain OB folds (Gao *et al.*, 2007). In mammalian cells, the protection of telomeres 1 protein, POT1, appears to perform similar functions as Cdc13 (see the next section). Recently, the human and plant homologs of the yeast Stn1 and Ten1 were identified (Martin *et al.*, 2007; Miyake *et al.*, 2009; Surovtseva *et al.*, 2009; Wan *et al.*, 2009). Interestingly, these proteins do not interact with POT1; however, they do form a complex with a novel OB-fold protein called CTC1 (conserved telomere maintenance component 1). This newly identified CTC1-STN1-TEN1 complex is named the CST complex (Figure 1).

Like RPA, the CST complex contains multiple OB folds. CTC1 possesses three OB folds, whereas STN1 and TEN1 each have one (Figure 1; Miyake *et al.*, 2009). CST preferentially binds to ssDNA in a length-dependent manner, with no obvious sequence specificity. The CST complex binds to ssDNA with a minimum length of 20–32 nt. The affinity of CST to ssDNA is approximately 3 to 8-fold lower than RPA, depending on the length of ssDNA. The N-terminal OB folds 1 and 2 of CTC1 form a tandem repeat, and are required for the binding of CST complex to ssDNA (Miyake *et al.*, 2009). These OB folds of CTC1 may function analogously to the DBD-A and B of RPA70. A C-terminal fragment of CTC1 that contains the OB fold 3 is able to form a complex with STN1 and TEN1, suggesting that this OB fold may play a structural role in the CST complex, analogous to the DBD-C of RPA70. A notable difference between RPA70 and CTC1 is that CTC1 is unable to bind ssDNA on its own (Miyake *et al.*, 2009). Whether STN1 can bind telomeric ssDNA by itself is controversial (Miyake *et al.*, 2009; Wan *et al.*, 2009). Like RPA32 in the RPA complex, STN1 is required for bridging the interaction between CTC1 and TEN1 (Miyake *et al.*, 2009). This role of STN1 in the assembly of CST complex requires not only its N-terminal OB fold, but also its C-terminal region. Recently structural analysis of the *S. cerevisiae* Stn1 revealed that its C terminus is structurally related to Rpa2 (Gelinas *et al.*, 2009). Furthermore, the *S. pombe* Stn1 and Ten1 form a heterodimer resembling the Rpa2–Rpa3 dimer. All these results suggest that the CST complex is an RPA-like complex (Sun *et al.*, 2009).

In cells, the STN1 subunit of the CST complex forms discrete nuclear foci that partially colocalize with telomeres (Miyake *et al.*, 2009; Wan *et al.*, 2009). The localization of STN1 to telomeres appears to be independent of POT1. Loss of STN1 does not affect overall telomere length or promote telomere end-to-end fusions. However, depletion of STN1 enhances the uncapping of telomeres induced by POT1 ablation, suggesting that STN1 and POT1 may function redundantly in protecting telomeres (Miyake *et al.*, 2009). Given this function of STN at telomeres, it is surprising that the CST complex does not exhibit any sequence specificity in ssDNA binding. Intriguingly, a complex of CTC1 and STN1 has been

purified as a stimulator of DNA polymerase α-primase using ssDNA templates of random sequences (Casteel *et al.*, 2009). However, STN1 does not appear to colocalize with sites of DNA synthesis in cells, suggesting that the CST complex is not part of the DNA replication machinery (Miyake *et al.*, 2009). How the CST complex functions at telomeres and whether it has any additional functions remains unclear.

POT1 and TPP1

In humans, telomeres are protected by shelterin, a six-component protein complex formed by TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 (Palm and de Lange, 2008). Three components of this complex are sequence-specific DNA-binding proteins. TRF1 and TRF2 specifically bind to double-stranded telomere DNA, whereas POT1 specifically binds to single-stranded telomere DNA. The components of shelterin have multiple roles at telomeres: they prevent telomeres from being recognized as DNA breaks and triggering a DNA damage response; they regulate DNA replication within the telomere regions; and they regulate the recruitment and action of telomerase. In this article, we will focus on POT1 and TPP1, two OB-fold proteins that form a ssDNA-binding complex (Figure 1).

POT1 contains two OB folds at its N terminus. A third OB fold at the C terminus of POT1 has been suggested by sequence analysis (Theobald and Wuttke, 2004) (Figure 1). *In vitro*, POT1 specifically binds to telomeric ssDNA with a minimum length of 10 nt and a preferred sequence of TTAGGGTTAG (Loayza *et al.*, 2004). The N-terminal OB folds of POT1 are essential for the binding to ssDNA. The crystal structure of the N terminus of human POT1 reveals that the OB folds of POT1 are structural related to the OB folds of archaeal SSB and human RPA70 (Lei *et al.*, 2003; 2004). However, it is important to note that the OB folds of POT1 and RPA70 bind to ssDNA in very different confirmations. In RPA70, DBD-A and DBD-B form a single channel creating a continuous groove for ssDNA binding. In contrast, the N-terminal OB folds of POT1 create a kink in the ssDNA, bending it at a 90° angle between the two OB folds. This structural difference may contribute to the sequence specificity of POT1 binding. Purified POT1 binds to telomeric ssDNA with a dissociation constant in the range of 10^{-7}–10^{-8} M (Wang *et al.*, 2007; Xin *et al.*, 2007). In cells, POT1 and TPP1 form a stable complex and function together. The binding of POT1 to ssDNA is enhanced by TPP1, which reduces the dissociation constant to 10^{-8}–10^{-9} M (Wang *et al.*, 2007; Xin *et al.*, 2007). TPP1 also contains an OB fold at the N terminus (Figure 1). However, TPP1 is unable to bind ssDNA in the absence of POT1. The OB fold of TPP1 is shown to interact with telomerase and play a role in recruiting telomerase to telomeres (Xin *et al.*, 2007).

In cells, the POT1–TPP1 complex localizes to telomeres; however, the OB folds of POT1 are neither necessary nor sufficient for this localization (Xin *et al.*, 2007). The interaction between POT1 and TPP1, and the interaction between TPP1 and TIN2, are important for the recruitment of POT1 to telomeres and its functions *in vivo* (Ye *et al.*, 2004; Hockemeyer *et al.*, 2007; Xin *et al.*, 2007). It is plausible that POT1–TPP1 is recruited by the shelterin components on double-stranded telomere DNA, and subsequently binds to telomere ssDNA. Depending on its position on telomeric ssDNA, POT1 can positively and negatively regulate telomerase (Lei *et al.*, 2005). When POT1 is bound to the 3′ terminus of the G-rich strand, it blocks the access of telomerase. When the binding of POT1 to the 3′ terminus is weakened by deletion of OB1, there is a dramatic increase in the length of the G-strand (Loayza and De Lange, 2003). Moreover, POT1 suppression results in an increase in G-strand overhang length, consistent with an inhibitory role of POT1 to telomerase. On the other hand, POT1 can positively regulate telomerase when it is bound internally to telomeric ssDNA. As described above, TPP1 in the POT1–TPP1 complex may directly recruit telomerase to telomeres (Xin *et al.*, 2007). In addition, purified POT1–TPP1 complex stimulates the processivity of telomerase by slowing primer dissociation and promoting translocation (Latrick and Cech, 2010; Wang *et al.*, 2007).

Another important function of POT1 at telomeres is to repress the activation of ATR checkpoint (Hockemeyer *et al.*, 2005; Wu *et al.*, 2006; Denchi and de Lange, 2007; Guo *et al.*, 2007). Intriguingly, ablation of POT1 specifically activates the ATR pathway, suggesting that POT1 antagonizes the formation of RPA-coated telomeric ssDNA, a key structure required for ATR activation. Given the low abundance of the POT1–TPP1 complex in cells (Takai *et al.*, 2010), and its relatively low affinity to ssDNA compared with RPA, how POT1 inhibits RPA binding to telomeric ssDNA is still unclear. POT1 is also implicated in the DNA replication of telomeres. In cells lacking the WRN helicase, which is involved in the lagging strand synthesis at telomeres, POT1 becomes indispensable for the synthesis of the C-rich strand of telomeres (Arnoult *et al.*, 2009).

Many of the functions of POT1 are linked to its ability to bind telomeric ssDNA, and are presumably dependent on its OB folds. However, the functions of POT1 cannot be fully explained by its sequence-specific binding to telomeric ssDNA. In mice, there are two homologs of human POT1, POT1a and POT1b (Hockemeyer *et al.*, 2006; Wu *et al.*, 2006). Although both of these mouse POT1 proteins exhibit virtually identical affinity and specificity to telomeric ssDNA and interact with the same mouse TPP1 protein, their functions are clearly distinct. POT1a appears predominantly to repress DNA damage response, whereas POT1b restricts 5′ end resection of

telomeres (Palm *et al.*, 2009). How POT1 carries out its different functions and how the OB folds contribute to these functions in mice remain to be investigated.

TAP82 and the telomerase holoenzyme

The recent investigation of the telomerase holoenzyme in *Tetrahymena thermophila* has identified three telomerase-associated proteins, TAP19, TAP50, and TAP82 (Min and Collins, 2009). These proteins join the previously identified telomerase holoenzyme components, the reverse transcriptase TERT, the telomerase RNA TER, and their associated proteins TAP75, TAP65, and TAP45 (Witkin and Collins, 2004). Among these proteins, TERT, TER, and TAP65 form the catalytic core. TAP75, TAP45, and TAP19 form a trimeric complex that associates with the catalytic core. TAP82 and TAP50 appear to be the peripheral subunits of the large complex. TAP82 exhibits significant sequence similarity to RPA70 (Min and Collins, 2009). Structural modeling of TAP82 predicts that it contains three OB folds, including a tandem OB-fold repeat (Figure 1). *In vitro*, TAP82 preferentially binds ssDNA. Moreover, TAP82 binds to G-rich telomeric ssDNA in a sequence-specific and length-dependent manner. Consistent with a role in regulating the telomerase holoenzyme, suppression of TAP82 causes a decrease in overall telomere length. *In vitro*, the high processivity of telomerase holoenzyme relies on the presence of TAP82 in the complex, suggesting that TAP82 functions to maintain telomerase on template DNA. Significant sequence homologs of TAP82 other than RPA70 have not been identified in non-ciliate cells. Whether the CST complex or the POT1–TPP1 complex in mammalian cells is the functional counterpart of TAP82 remains to be investigated. The discovery of an OB-fold protein as part of the telomerase holoenzyme again emphasizes the important roles of OB-fold proteins in telomere homeostasis and preservation of genomic stability.

BRCA2, RMI1, RMI2, and DNA ligases

The breast cancer associated gene 2 (BRCA2) and the RecQ-mediated genome instability 1 and 2 (RMI1 and RMI2) are three additional OB-fold proteins in mammalian cells that are implicated in the maintenance of genomic stability. BRCA2 is an important DNA repair/recombination protein that functions in HR (Pellegrini and Venkitaraman, 2004). Three OB folds (OB1, 2, and 3) have been revealed in the DNA-binding domain (DBD) of BRCA2 by structural studies (Figure 1) (Yang *et al.*, 2002). Among these OB folds, the structure of the OB2 of BRCA2 is most closely related to the DBD-A of RPA70. However, OB2 has an unusual 130-amino acid insert that adopts a tower-like structure (Tower domain) protruding away from the OB fold. The OB2 and OB3 of BRCA2 form a tandem repeat that binds ssDNA. The BRCA2–ssDNA interactions are very similar to those observed in RPA (Yang *et al.*, 2002). The OB1 and OB2 of BRCA2, together with a helix-turn-helix (HLH) domain at the C terminus of BRCA2, interact with the BRCA2-associated protein DSS1. A complex of the OB1 and HLH domains of BRCA2 and DSS1 has weak affinity to ssDNA. Interestingly, the DBD of BRCA2 not only binds ssDNA, but also dsDNA with short ssDNA overhangs or ssDNA gaps. This result suggests that dsDNA, in the context of ssDNA, may contribute to the binding to BRCA2 (Yang *et al.*, 2002).

During HR, the ssDNA overhangs of DNA breaks are first recognized by RPA. Subsequently, BRCA2 promotes the recruitment of RAD51 and the formation of RAD51 filament (Pellegrini *et al.*, 2002; Carreira *et al.*, 2009), providing a critical structure that mediates the strand invasion step of HR. In this process BRCA2 needs to interact with RAD51 through its BRC domains, and with DNA through its OB folds. Amazingly, a fusion protein of the BRC domains of BRCA2 and RPA70 is able to support HR, strongly suggesting that the function of the OB folds of BRCA2 is to target BRCA2 to ssDNA or junctions of ssDNA and dsDNA (Saeki *et al.*, 2006).

RMI1 and RMI2 were originally identified as components of a large protein complex containing the BLM helicase, topoisomerase 3α (Top3α), and RPA (Yin *et al.*, 2005). Loss of RMI1-2 leads to an increase in sister-chromatid exchange, suggesting that they are involved in the control of DNA recombination. RMI1 contains two OB folds, OB1 and OB2 (Figure 1) (Yin *et al.*, 2005). While OB1 sequence is similar to the bacterial RecG wedge domain, OB2 sequence is similar to RPA70 DBD-C. RMI2 also has an OB-fold, OB3, which has sequence similarity to RPA32 DBD-D (Figure 1) (Singh *et al.*, 2008; Xu *et al.*, 2008a). Analogous to RPA70 and RPA32, RMI1 and RMI2 interact with each other through OB2 and OB3 (Xu *et al.*, 2008a). RMI1 was shown to interact with both BLM and Top3α (Raynard *et al.*, 2006), to bind DNA (Mullen *et al.*, 2005; Raynard *et al.*, 2008), and to stimulate the binding of Top3α to ssDNA (Chen and Brill, 2007). However, the RMI1–RMI2 complex appears to lack the ability to bind ssDNA. *In vitro*, the RMI1–RMI2 complex stimulates the dissolution of Holliday junctions, an important HR intermediate, by the BLM helicase and Top3α (Xu *et al.*, 2008a). A point mutation in the OB3 of RMI2 disrupts the association of BLM with Top3α, RMI1, and RMI2, suggesting that the OB folds of RMI1/2 play critical structural roles in the large BLM complex (Xu *et al.*, 2008a).

LIG1, LIG3, and LIG4 belong to three distinct eukaryotic DNA ligase families critical for DNA replication and multiple DNA repair pathways. Members of the three DNA ligase families contain three distinct structural domains: the nucleotidyltransferase (NTase), OB-fold,

and DBD domains (Ellenberger and Tomkinson, 2008). Together these domains form a ring-shaped structure around dsDNA, enabling the ligases to encircle the DNA substrate. Crystal structure of human LIG1 on a nicked DNA substrate shows that the OB fold makes extensive interactions with the backbone of dsDNA, positioning the ligase to the DNA nick (Nair *et al.*, 2007; Pascal *et al.*, 2004). In addition, the crystal structure suggests that the interaction between the OB fold and the DBD domain is important for the overall ring structure of LIG1. During the process of ligation, the OB fold may undergo a large conformational change, orienting the DNA ends for specific steps of the ligation reaction (Pascal *et al.*, 2004). Interestingly, biallelic mutations within the NTase and OB domains of LIG1 have been identified in a patient with lymphoma (Prigent *et al.*, 1994). The mutation in the OB fold (R771W) caused an approximately 90% reduction in ligase activity.

Perspectives

The protein network that senses and responds to DNA damage is intricately regulated in cells. Recent studies have revealed that many proteins involved in this network use different types of functional modules to regulate and execute their functions. For example, many checkpoint and DNA repair proteins use the BRCT (BRCA1 C-terminal) and FHA (forehead-associated) domains to associate with phosphorylated binding partners (Durocher *et al.*, 2000; Glover *et al.*, 2004), use various ubiquitin-binding domains to associate with ubiquitylated binding partners (Hurley *et al.*, 2006), and use the PIP-box (PCNA-interacting peptide) domains to associate with the key DNA replication/repair protein PCNA (Moldovan *et al.*, 2007). The growing number of OB fold proteins in this network clearly suggests that the OB fold is another important functional module that is widely used in this context. Importantly, unlike the other functional modules mentioned above, many OB folds have the ability to directly associate with DNA, providing the crucial link between the DNA damage response network and damaged DNA. Many of the OB fold proteins may be directly involved in the sensing and processing of damaged DNA. Furthermore, OB fold proteins have prominent roles in the maintenance of telomere homeostasis, another DNA-directed process. In addition to their roles in DNA binding, the OB folds can also function as a protein–protein interaction module. Interestingly, several protein complexes contain multiple OB folds, which enable the complexes to execute multiple functions in a coordinated manner.

Although all OB folds are structurally related, they are sometimes difficult to identify by sequence analysis due to the poor sequence conservation. It is conceivable that the number of OB fold proteins involved in DNA damage response will continue to grow in the future. The OB folds have highly diverse functions, and they are put together in many different ways in different proteins and protein complexes. Understanding the functional specificities of different OB folds, and how these functional modules are used and regulated in proteins and protein complexes, will be a challenge for future studies. Another important problem that has yet to be explored is how various OB fold proteins function together in cells. Are they competitors for DNA binding? Do they function in a synergistic or coordinated fashion in specific settings? We anticipate that our knowledge of the OB fold genome guardians will continue to advance at a rapid pace in the next few years.

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