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Oligonucleotide/Oligosaccharide-Binding (OB) 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.

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 (Harper and Elledge, 2007; Zhou and Elledge, 2000). 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 our lab, and others' have

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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).

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 bound to oligonucleotides or oligosaccharides (Murzin, 1993). Subsequently studies have shown that various OB folds were capable of establishing protein-DNA, -RNA, or -protein interactions (Agrawal and Kishan, 2003; Arcus, 2002; 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 5 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 the 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 (Fanning et al., 2006; Wold, 1997). 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 (Fig. 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 (Fig. 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;Bochkareva et al., 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, 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 (Binz and Wold, 2008; Jacobs et al., 1999). 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 (Umezue et al., 1998). Unlike the wild-type RPA, the rfa1-t11 mutant is unable to interact with Ddc2 (yeast homologue of human ATRIP), and fails to recruit Ddc2 and Ddc1 (yeast homologue of human Rad9) to sites of DNA damage (Lucca et al., 2004; Majka et al., 2006; Zou and Elledge, 2003; Zou et al., 2003). During homologous recombination (HR), the rfa1-t11 mutant is displaced by Rad51 more slowly than wild-type RPA, and it fails to support an 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 (Ball et al., 2007; Ball et al., 2005; Jacobs et al., 1999; Namiki and Zou, 2006; Oakley et al., 2009; Olson et al., 2007; Xu et al., 2008b; Zou and Elledge, 2003). 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 (Fig. 1).

In addition to the three subunits of the canonical RPA complex, a 30-kDa homologue of RPA32 termed RPA4 has been detected in certain human tissues and cancer cells (Fig. 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.). The alternative RPA complex is able to perform some of the DNA repair functions of RPA (Kemp et al.), but is unable to support chromosomal DNA replication (Haring et al.). 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 homologue of the bacterial single-stranded binding protein, SSB, in mammalian cells. However, recent studies have identified two additional mammalian homologues 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 is no data detailing the ssDNA-binding capabilities of hSSB2.

Three independent studies reveal that hSSB1 and hSSB2 exist in two distinct complexes (Fig. 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 RPA34 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 the corresponding complexes (Fig. 1). However, the INTS3 and C9orf80 subunits of the hSSB1/2 complexes do not appear to contain additional OB folds like RPA70 and RPA34. 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 RPA34 (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 RPA34 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 are also involved in the checkpoint response like RPA. 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 (Li et al., 2009; Richard et al., 2008; 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 (Palm and de Lange, 2008; Verdun and Karlseder, 2007). In mammalian cells, telomeres are composed of repetitive sequences (5'TTAGGG3')_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 *S. 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 homologues 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 (Fig. 1).

Like RPA, the CST complex contains multiple OB folds. CTC1 possesses three OB folds, whereas STN1 and TEN1 each have one (Fig. 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 RPA34 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 STN1 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 (Fig. 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) (Fig. 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; Lei et al., 2004). However, it is important to note that the OB folds of POT1 and RPA70 bind to ssDNA in very different conformations. 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 (Fig. 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, as well as the interaction between TPP1 and TIN2, are important for the recruitment of POT1 to telomeres and its functions *in vivo* (Hockemeyer et al., 2007; Xin et al., 2007; Ye et al., 2004). 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 either 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, ; Wang et al., 2007).

Another important function of POT1 at telomeres is to repress the activation of ATR checkpoint (Denchi and de Lange, 2007; Guo et al., 2007; Hockemeyer et al., 2005; Wu et al., 2006). 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.), 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 homologues 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 to predominantly represses 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 (Fig. 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 homologues 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 (Fig. 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 (Carreira et al., 2009; Pellegrini et al., 2002), 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 3 α (Top3 α), 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 (Fig. 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 (Fig. 1) (Singh et al., 2008; Xu et al., 2008a). Analogous to RPA70 and RPA34, RMI1 and RMI2 interact with each other through OB2 and OB3 (Xu et al., 2008a). RMI1 was shown to interact with both BLM and Top3 α (Raynard et al., 2006), to bind DNA (Mullen et al., 2005; Raynard et al., 2008), and to stimulate the binding of Top3 α 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 Top3 α (Xu et al., 2008a).

A point mutation in the OB3 of RMI2 disrupts the association of BLM with Top3 α , 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 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 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 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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Reference

- Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 2001;15:2177–2196. [PubMed: 11544175]
- Agrawal V, Kishan KV. OB-fold: growing bigger with functional consistency. *Curr Protein Pept Sci* 2003;4:195–206. [PubMed: 12769718]
- Arcus V. OB-fold domains: a snapshot of the evolution of sequence, structure and function. *Curr Opin Struct Biol* 2002;12:794–801. [PubMed: 12504685]
- Arnoult N, Saintome C, Ourliac-Garnier I, Riou JF, Londono-Vallejo A. Human POT1 is required for efficient telomere C-rich strand replication in the absence of WRN. *Genes Dev* 2009;23:2915–2924. [PubMed: 20008939]
- Ball HL, Ehrhardt MR, Mordes DA, Glick GG, Chazin WJ, Cortez D. Function of a conserved checkpoint recruitment domain in ATRIP proteins. *Mol Cell Biol* 2007;27:3367–3377. [PubMed: 17339343]
- Ball HL, Myers JS, Cortez D. ATRIP binding to replication protein A-single-stranded DNA promotes ATR-ATRIP localization but is dispensable for Chk1 phosphorylation. *Mol Biol Cell* 2005;16:2372–2381. [PubMed: 15743907]
- Binz SK, Wold MS. Regulatory functions of the N-terminal domain of the 70-kDa subunit of replication protein A (RPA). *J Biol Chem* 2008;283:21559–21570. [PubMed: 18515800]
- Bochkarev A, Bochkareva E, Frappier L, Edwards AM. The crystal structure of the complex of replication protein A subunits RPA32 and RPA14 reveals a mechanism for single-stranded DNA binding. *Embo J* 1999;18:4498–4504. [PubMed: 10449415]
- Bochkareva E, Belegu V, Korolev S, Bochkarev A. Structure of the major single-stranded DNA-binding domain of replication protein A suggests a dynamic mechanism for DNA binding. *Embo J* 2001;20:612–618. [PubMed: 11157767]
- Bochkareva E, Korolev S, Lees-Miller SP, Bochkarev A. Structure of the RPA trimerization core and its role in the multistep DNA-binding mechanism of RPA. *Embo J* 2002;21:1855–1863. [PubMed: 11927569]
- Carreira A, Hilario J, Amitani I, Baskin RJ, Shivji MK, Venkitaraman AR, Kowalczykowski SC. The BRC repeats of BRCA2 modulate the DNA-binding selectivity of RAD51. *Cell* 2009;136:1032–1043. [PubMed: 19303847]
- Casteel DE, Zhuang S, Zeng Y, Perrino FW, Boss GR, Goulian M, Pilz RB. A DNA polymerase- α {middle dot} primase cofactor with homology to replication protein A-32 regulates DNA replication in mammalian cells. *J Biol Chem* 2009;284:5807–5818. [PubMed: 19119139]
- Chen CF, Brill SJ. Binding and activation of DNA topoisomerase III by the Rmi1 subunit. *J Biol Chem* 2007;282:28971–28979. [PubMed: 17693398]
- Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* 2008;9:616–627. [PubMed: 18594563]
- Costanzo V, Gautier J. Single-strand DNA gaps trigger an ATR- and Cdc7-dependent checkpoint. *Cell Cycle* 2003;2:17. [PubMed: 12695677]
- Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature* 2007;448:1068–1071. [PubMed: 17687332]
- Deng X, Habel JE, Kabaleeswaran V, Snell EH, Wold MS, Borgstahl GE. Structure of the full-length human RPA14/32 complex gives insights into the mechanism of DNA binding and complex formation. *J Mol Biol* 2007;374:865–876. [PubMed: 17976647]
- Durocher D, Smerdon SJ, Yaffe MB, Jackson SP. The FHA domain in DNA repair and checkpoint signaling. *Cold Spring Harb Symp Quant Biol* 2000;65:423–431. [PubMed: 12760058]
- Ellenberger T, Tomkinson AE. Eukaryotic DNA ligases: structural and functional insights. *Annu Rev Biochem* 2008;77:313–338. [PubMed: 18518823]

- Fanning E, Klimovich V, Nager AR. A dynamic model for replication protein A (RPA) function in DNA processing pathways. *Nucleic Acids Res* 2006;34:4126–4137. [PubMed: 16935876]
- Gao H, Cervantes RB, Mandell EK, Otero JH, Lundblad V. RPA-like proteins mediate yeast telomere function. *Nat Struct Mol Biol* 2007;14:208–214. [PubMed: 17293872]
- Gelinas AD, Paschini M, Reyes FE, Heroux A, Batey RT, Lundblad V, Wuttke DS. Telomere capping proteins are structurally related to RPA with an additional telomere-specific domain. *Proc Natl Acad Sci U S A* 2009;106:19298–19303. [PubMed: 19884503]
- Glover JN, Williams RS, Lee MS. Interactions between BRCT repeats and phosphoproteins: tangled up in two. *Trends Biochem Sci* 2004;29:579–585. [PubMed: 15501676]
- Guo X, Deng Y, Lin Y, Cosme-Blanco W, Chan S, He H, Yuan G, Brown EJ, Chang S. Dysfunctional telomeres activate an ATM-ATR-dependent DNA damage response to suppress tumorigenesis. *Embo J* 2007;26:4709–4719. [PubMed: 17948054]
- Haring SJ, Humphreys TD, Wold MS. A naturally occurring human RPA subunit homolog does not support DNA replication or cell-cycle progression. *Nucleic Acids Res* 38:846–858. [PubMed: 19942684]
- Haring SJ, Mason AC, Binz SK, Wold MS. Cellular functions of human RPA1. Multiple roles of domains in replication, repair, and checkpoints. *J Biol Chem* 2008;283:19095–19111. [PubMed: 18469000]
- Harper JW, Elledge SJ. The DNA damage response: ten years after. *Mol Cell* 2007;28:739–745. [PubMed: 18082599]
- Hockemeyer D, Daniels JP, Takai H, de Lange T. Recent expansion of the telomeric complex in rodents: Two distinct POT1 proteins protect mouse telomeres. *Cell* 2006;126:63–77. [PubMed: 16839877]
- Hockemeyer D, Palm W, Else T, Daniels JP, Takai KK, Ye JZ, Keegan CE, de Lange T, Hammer GD. Telomere protection by mammalian Pot1 requires interaction with Tpp1. *Nat Struct Mol Biol* 2007;14:754–761. [PubMed: 17632522]
- Hockemeyer D, Sfeir AJ, Shay JW, Wright WE, de Lange T. POT1 protects telomeres from a transient DNA damage response and determines how human chromosomes end. *Embo J* 2005;24:2667–2678. [PubMed: 15973431]
- Huang J, Gong Z, Ghosal G, Chen J. SOSS complexes participate in the maintenance of genomic stability. *Mol Cell* 2009;35:384–393. [PubMed: 19683501]
- Hurley JH, Lee S, Prag G. Ubiquitin-binding domains. *Biochem J* 2006;399:361–372. [PubMed: 17034365]
- Jacobs DM, Lipton AS, Isern NG, Daughdrill GW, Lowry DF, Gomes X, Wold MS. Human replication protein A: global fold of the N-terminal RPA-70 domain reveals a basic cleft and flexible C-terminal linker. *J Biomol NMR* 1999;14:321–331. [PubMed: 10526407]
- Kantake N, Sugiyama T, Kolodner RD, Kowalczykowski SC. The recombination-deficient mutant RPA (rfa1-t11) is displaced slowly from single-stranded DNA by Rad51 protein. *J Biol Chem* 2003;278:23410–23417. [PubMed: 12697761]
- Kemp MG, Mason AC, Carreira A, Reardon JT, Haring SJ, Borgstahl GE, Kowalczykowski SC, Sancar A, Wold MS. An alternative form of replication protein A expressed in normal human tissues supports DNA repair. *J Biol Chem* 285:4788–4797. [PubMed: 19996105]
- Keshav KF, Chen C, Dutta A. Rpa4, a homolog of the 34-kilodalton subunit of the replication protein A complex. *Mol Cell Biol* 1995;15:3119–3128. [PubMed: 7760808]
- Kim C, Paulus BF, Wold MS. Interactions of human replication protein A with oligonucleotides. *Biochemistry* 1994;33:14197–14206. [PubMed: 7947831]
- Lattrick CM, Cech TR. POT1-TPP1 enhances telomerase processivity by slowing primer dissociation and aiding translocation. *Embo J*.
- Lei M, Podell ER, Baumann P, Cech TR. DNA self-recognition in the structure of Pot1 bound to telomeric single-stranded DNA. *Nature* 2003;426:198–203. [PubMed: 14614509]
- Lei M, Podell ER, Cech TR. Structure of human POT1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection. *Nat Struct Mol Biol* 2004;11:1223–1229. [PubMed: 15558049]
- Lei M, Zaug AJ, Podell ER, Cech TR. Switching human telomerase on and off with hPOT1 protein in vitro. *J Biol Chem* 2005;280:20449–20456. [PubMed: 15792951]

- Li Y, Bolderson E, Kumar R, Muniandy PA, Xue Y, Richard DJ, Seidman M, Pandita TK, Khanna KK, Wang W. HSSB1 and hSSB2 form similar multiprotein complexes that participate in DNA damage response. *J Biol Chem* 2009;284:23525–23531. [PubMed: 19605351]
- Loayza D, De Lange T. POT1 as a terminal transducer of TRF1 telomere length control. *Nature* 2003;423:1013–1018. [PubMed: 12768206]
- Loayza D, Parsons H, Donigian J, Hoke K, de Lange T. DNA binding features of human POT1: a nonamer 5'-TAGGGTTAG-3' minimal binding site, sequence specificity, and internal binding to multimeric sites. *J Biol Chem* 2004;279:13241–13248. [PubMed: 14715659]
- Lucca C, Vanoli F, Cotta-Ramusino C, Pellicoli A, Liberi G, Haber J, Foiani M. Checkpoint-mediated control of replisome-fork association and signalling in response to replication pausing. *Oncogene* 2004;23:1206–1213. [PubMed: 14647447]
- Majka J, Binz SK, Wold MS, Burgers PM. Replication protein A directs loading of the DNA damage checkpoint clamp to 5'-DNA junctions. *J Biol Chem* 2006;281:27855–27861. [PubMed: 16864589]
- Martin V, Du LL, Rozenzhak S, Russell P. Protection of telomeres by a conserved Stn1-Ten1 complex. *Proc Natl Acad Sci U S A* 2007;104:14038–14043. [PubMed: 17715303]
- Mason AC, Haring SJ, Pryor JM, Staloch CA, Gan TF, Wold MS. An alternative form of replication protein A prevents viral replication in vitro. *J Biol Chem* 2009;284:5324–5331. [PubMed: 19116208]
- Min B, Collins K. An RPA-related sequence-specific DNA-binding subunit of telomerase holoenzyme is required for elongation processivity and telomere maintenance. *Mol Cell* 2009;36:609–619. [PubMed: 19941821]
- Miyake Y, Nakamura M, Nabetani A, Shimamura S, Tamura M, Yonehara S, Saito M, Ishikawa F. RPA-like mammalian Ctc1-Stn1-Ten1 complex binds to single-stranded DNA and protects telomeres independently of the Pot1 pathway. *Mol Cell* 2009;36:193–206. [PubMed: 19854130]
- Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. *Cell* 2007;129:665–679. [PubMed: 17512402]
- Mullen JR, Nallaseth FS, Lan YQ, Slagle CE, Brill SJ. Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. *Mol Cell Biol* 2005;25:4476–4487. [PubMed: 15899853]
- Murzin AG. OB(oligonucleotide/oligosaccharide binding)-fold: common structural and functional solution for non-homologous sequences. *Embo J* 1993;12:861–867. [PubMed: 8458342]
- Nair PA, Nandakumar J, Smith P, Odell M, Lima CD, Shuman S. Structural basis for nick recognition by a minimal pluripotent DNA ligase. *Nat Struct Mol Biol* 2007;14:770–778. [PubMed: 17618295]
- Namiki Y, Zou L. ATRIP associates with replication protein A-coated ssDNA through multiple interactions. *Proc Natl Acad Sci U S A* 2006;103:580–585. [PubMed: 16407120]
- Oakley GG, Tillison K, Opiyo SA, Glanzer JG, Horn JM, Patrick SM. Physical interaction between replication protein A (RPA) and MRN: involvement of RPA2 phosphorylation and the N-terminus of RPA1. *Biochemistry* 2009;48:7473–7481. [PubMed: 19586055]
- Olson E, Nievera CJ, Liu E, Lee AY, Chen L, Wu X. The Mre11 complex mediates the S-phase checkpoint through an interaction with replication protein A. *Mol Cell Biol* 2007;27:6053–6067. [PubMed: 17591703]
- Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet* 2008;42:301–334. [PubMed: 18680434]
- Palm W, Hockemeyer D, Kibe T, de Lange T. Functional dissection of human and mouse POT1 proteins. *Mol Cell Biol* 2009;29:471–482. [PubMed: 18955498]
- Pascal JM, O'Brien PJ, Tomkinson AE, Ellenberger T. Human DNA ligase I completely encircles and partially unwinds nicked DNA. *Nature* 2004;432:473–478. [PubMed: 15565146]
- Pellegrini L, Venkitaraman A. Emerging functions of BRCA2 in DNA recombination. *Trends Biochem Sci* 2004;29:310–316. [PubMed: 15276185]
- Pellegrini L, Yu DS, Lo T, Anand S, Lee M, Blundell TL, Venkitaraman AR. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature* 2002;420:287–293. [PubMed: 12442171]
- Prigent C, Satoh MS, Daly G, Barnes DE, Lindahl T. Aberrant DNA repair and DNA replication due to an inherited enzymatic defect in human DNA ligase I. *Mol Cell Biol* 1994;14:310–317. [PubMed: 8264597]

- Raynard S, Bussen W, Sung P. A double Holliday junction dissolvasome comprising BLM, topoisomerase III α , and BLAP75. *J Biol Chem* 2006;281:13861–13864. [PubMed: 16595695]
- Raynard S, Zhao W, Bussen W, Lu L, Ding YY, Busygina V, Meetei AR, Sung P. Functional role of BLAP75 in BLM-topoisomerase III α -dependent holliday junction processing. *J Biol Chem* 2008;283:15701–15708. [PubMed: 18390547]
- Richard DJ, Bolderson E, Cubeddu L, Wadsworth RI, Savage K, Sharma GG, Nicolette ML, Tsvetanov S, McIlwraith MJ, Pandita RK, et al. Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. *Nature* 2008;453:677–681. [PubMed: 18449195]
- Saeki H, Siaud N, Christ N, Wiegant WW, van Buul PP, Han M, Zdzenicka MZ, Stark JM, Jasin M. Suppression of the DNA repair defects of BRCA2-deficient cells with heterologous protein fusions. *Proc Natl Acad Sci U S A* 2006;103:8768–8773. [PubMed: 16731627]
- Shiotani B, Zou L. ATR signaling at a glance. *J Cell Sci* 2009;122:301–304. [PubMed: 19158338]
- Singh TR, Ali AM, Busygina V, Raynard S, Fan Q, Du CH, Andreassen PR, Sung P, Meetei AR. BLAP18/RMI2, a novel OB-fold-containing protein, is an essential component of the Bloom helicase-double Holliday junction dissolvasome. *Genes Dev* 2008;22:2856–2868. [PubMed: 18923083]
- Skaar JR, Richard DJ, Saraf A, Toschi A, Bolderson E, Florens L, Washburn MP, Khanna KK, Pagano M. INTS3 controls the hSSB1-mediated DNA damage response. *J Cell Biol* 2009;187:25–32. [PubMed: 19786574]
- Sugiyama T, Kantake N, Wu Y, Kowalczykowski SC. Rad52-mediated DNA annealing after Rad51-mediated DNA strand exchange promotes second ssDNA capture. *Embo J* 2006;25:5539–5548. [PubMed: 17093500]
- Sun J, Yu EY, Yang Y, Confer LA, Sun SH, Wan K, Lue NF, Lei M. Stn1-Ten1 is an Rpa2-Rpa3-like complex at telomeres. *Genes Dev* 2009;23:2900–2914. [PubMed: 20008938]
- Surovtseva YV, Churikov D, Boltz KA, Song X, Lamb JC, Warrington R, Leehy K, Heacock M, Price CM, Shippen DE. Conserved telomere maintenance component 1 interacts with STN1 and maintains chromosome ends in higher eukaryotes. *Mol Cell* 2009;36:207–218. [PubMed: 19854131]
- Takai KK, Hooper S, Blackwood S, Gandhi R, de Lange T. In vivo stoichiometry of shelterin components. *J Biol Chem* 285:1457–1467. [PubMed: 19864690]
- Theobald DL, Mitton-Fry RM, Wuttke DS. Nucleic acid recognition by OB-fold proteins. *Annu Rev Biophys Biomol Struct* 2003;32:115–133. [PubMed: 12598368]
- Theobald DL, Wuttke DS. Prediction of multiple tandem OB-fold domains in telomere end-binding proteins Pot1 and Cdc13. *Structure* 2004;12:1877–1879. [PubMed: 15458635]
- Umez K, Sugawara N, Chen C, Haber JE, Kolodner RD. Genetic analysis of yeast RPA1 reveals its multiple functions in DNA metabolism. *Genetics* 1998;148:989–1005. [PubMed: 9539419]
- Verdun RE, Karlseder J. Replication and protection of telomeres. *Nature* 2007;447:924–931. [PubMed: 17581575]
- Wan M, Qin J, Songyang Z, Liu D. OB fold-containing protein 1 (OBFC1), a human homolog of yeast Stn1, associates with TPP1 and is implicated in telomere length regulation. *J Biol Chem* 2009;284:26725–26731. [PubMed: 19648609]
- Wang F, Podell ER, Zaug AJ, Yang Y, Baci P, Cech TR, Lei M. The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* 2007;445:506–510. [PubMed: 17237768]
- Witkin KL, Collins K. Holoenzyme proteins required for the physiological assembly and activity of telomerase. *Genes Dev* 2004;18:1107–1118. [PubMed: 15131081]
- Wold MS. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu Rev Biochem* 1997;66:61–92. [PubMed: 9242902]
- Wu L, Multani AS, He H, Cosme-Blanco W, Deng Y, Deng JM, Bachilo O, Pathak S, Tahara H, Bailey SM, et al. Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. *Cell* 2006;126:49–62. [PubMed: 16839876]
- Xin H, Liu D, Wan M, Safari A, Kim H, Sun W, O'Connor MS, Songyang Z. TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. *Nature* 2007;445:559–562. [PubMed: 17237767]
- Xu D, Guo R, Soback A, Bachrati CZ, Yang J, Enomoto T, Brown GW, Hoatlin ME, Hickson ID, Wang W. RMI, a new OB-fold complex essential for Bloom syndrome protein to maintain genome stability. *Genes Dev* 2008a;22:2843–2855. [PubMed: 18923082]

- Xu X, Vaithiyalingam S, Glick GG, Mordes DA, Chazin WJ, Cortez D. The basic cleft of RPA70N binds multiple checkpoint proteins, including RAD9, to regulate ATR signaling. *Mol Cell Biol* 2008b; 28:7345–7353. [PubMed: 18936170]
- Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* 2002;297:1837–1848. [PubMed: 12228710]
- Ye JZ, Hockemeyer D, Krutchinsky AN, Loayza D, Hooper SM, Chait BT, de Lange T. POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. *Genes Dev* 2004;18:1649–1654. [PubMed: 15231715]
- Yin J, Sobeck A, Xu C, Meetei AR, Hoatlin M, Li L, Wang W. BLAP75, an essential component of Bloom's syndrome protein complexes that maintain genome integrity. *Embo J* 2005;24:1465–1476. [PubMed: 15775963]
- Zhang F, Wu J, Yu X. Integrator3, a partner of single-stranded DNA-binding protein 1, participates in the DNA damage response. *J Biol Chem* 2009;284:30408–30415. [PubMed: 19759019]
- Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000;408:433–439. [PubMed: 11100718]
- Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003;300:1542–1548. [PubMed: 12791985]
- Zou L, Liu D, Elledge SJ. Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc Natl Acad Sci U S A* 2003;100:13827–13832. [PubMed: 14605214]

Figure 1.

The OB-fold proteins and protein complexes involved in the DNA damage response and telomere maintenance. The OB folds are classified and 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.