DNA processing involves multiple biochemical steps requiring far more functions than can be easily incorporated into a single protein. Consequently, replication, repair, and recombination are performed by multiprotein assemblies. Remarkably, there is growing evidence that these processes share many common features. The enzymes active in replicating DNA during S phase are needed to synthesize new stretches of DNA during various types of recombination and repair. Repair of all types must occur in tandem with replication and transcription, because the exposure of DNA during these processes renders it open to discovery of common lesions. Recombination is used to catalyze genetic crossover during meiosis and is also a major DNA repair mechanism during replicative and later phases of the cell cycle. Thus, the processes of DNA replication, repair, and recombination are being increasingly viewed as integrated events in cellular life.

Considerable progress has been made in developing techniques to investigate at the atomic level how the DNA processing machinery functions. We are now in a position to ask very basic questions: what are the structural mechanisms that regulate progression from initiation through termination of a DNA processing pathway, and how does the DNA processing machinery facilitate cross-talk between replication, recombination, and repair? We highlight here some of the trends and concepts that are emerging as the structural mechanisms that regulate DNA processing begin to be elucidated.

Multiple dynamic protein interactions coordinate the ordered progression of DNA processing events. Working from a basic description of the hand-off of DNA from one protein to another, we introduce the concept of modularity of DNA processing proteins and the versatility of the common folds found in these proteins. This is followed by recently reported examples of direct competition and allosteric mechanisms promoting specific steps of DNA processing. These concepts are integrated into a simple working model that provides a general framework for thinking about the forward progression of DNA replication, repair, and recombination assemblies.

The Concept of Handing Off

Evidence is mounting in favor of dynamic assembly and disassembly of the DNA processing machinery at the time it is needed and against the existence of preformed holoenzyme complexes that meander around the cell until they are needed (1–3). Multiple dynamic protein interactions are fundamental to the nature of DNA processing and to progression through each processing pathway. The weak and/or transient nature of many of the protein interactions is thought to be the basis for the ability of the DNA processing machinery to assemble dynamically and to hand off the DNA from one subcomplex to the next. From the perspective of the DNA, the proteins are sometimes described as “trading places” (4).

A well known example from replication is the competitive polymerase switching observed during lagging strand synthesis (Fig. 1A) (5). In eukaryotes, DNA polymerase α-primase complex (pol-prim) synthesizes an RNA-DNA hybrid primer. For processive DNA synthesis to follow, the sliding clamp, proliferating cell nuclear antigen (PCNA), must be loaded by the clamp loader protein, replication factor C (RFC). Loading of PCNA triggers the displacement of pol-prim from the 3' end of the primer. Processive synthesis by DNA polymerase δ (pol δ) requires its direct association with PCNA. RFC is subsequently displaced from PCNA but remains stably associated with the PCNA-pol-prim complex during synthesis. Each polymerase switch is facilitated by protein interactions with replication protein A (RPA), a highly abundant ssDNA-binding protein in eukaryotes (6).

Hand-off is also well documented in eukaryotic nucleotide excision repair (NER), during which a wide variety of DNA lesions are removed and replaced (Fig. 1B) (7). In the global genome repair pathway, damage is detected by the XPC/hHR23B/centrin 2 heterotrimer. Local unwinding is effected by recruited TFIIH, a heterodimer composed of XPD and XPB subunits. Next, XPA and RPA associate with the complex and orient the incoming excision protein, ERCC1/XPF and XPG. Binding of XPG signals the release of XPC/hHR23B, whereas ERCC1/XPF triggers the excision of damaged DNA and the release of XPA and TFIIH. Of these proteins, only RPA remains stably bound after excision to facilitate association of the synthetic factors RFC, PCNA, pol δ, and DNA ligase.

Even in recombination, for which detailed knowledge lags somewhat behind that of replication and repair, there are reports of proteins trading places on DNA (Fig. 1C) (8, 9). Homologous recombination (HR) is one pathway used to repair double strand DNA breaks. In eukaryotes, the 5' ends around the break are resected by the Mre11/Rad50/NBS1 nuclease, leaving 3'-ssDNA overhangs. These are coated by RPA, which binds with high affinity to ssDNA. The displacement of RPA from ssDNA is effected by Rad51, the recombinase, with the help of Rad52, a recombination mediator protein. Nucleation of Rad51 on ssDNA promotes formation of the nucleoprotein filament that catalyzes the homology search and subsequent strand exchange.

In each pathway, the sequential nature of the recruitment and dissociation of DNA processing proteins is well established. The advantages of dynamic assembly and disassembly are many, not the least of which is that fewer copies of ubiquitous proteins such as RPA and PCNA are needed, because they can be recycled for use in other reactions. In addition, the ordered hand-off of DNA from protein to protein (or complex to complex) allows for several levels of temporal and spatial regulation of the processing pathways, whereas parallel steps between two (or more) pathways allow for cross-talk.

Modular Proteins with Multiple Binding Sites

A common feature of proteins involved in DNA processing is modularity, the physical connection of structural domains that perform different biochemical functions. This characteristic facilitates the coordination of biochemical activities without constraining functional modules to fit tightly together. Flexible tethering of domains in modular proteins allows multiple steps to be carried out independently yet remain coupled. An essential feature of modularity is the use of multiple contact points between one or more pairs of interacting proteins. This facilitates the hand-off of processing proteins in situations where rearrangement is required rather than full dissociation. It also provides a means to achieve a high overall affinity from a set of weak individual interactions (see below). The use of multiple binding sites also allows for more than one type of regulation at a single step of DNA processing.
During replication, the clamp loader protein RFC binds to RPA using three of its five subunits (6). Evidence from replication studies suggests that there are two distinct binding sites for RFC on RPA: one that overlaps with the pol δ binding site and one that does not. Multiple points of interaction between RFC and RPA facilitate hand-off of RFC without causing it to completely dissociate. pol δ competes with RFC for binding to RPA during the second polymerase switch of lagging strand synthesis, but after pol δ displaces RFC, RFC remains bound to the polymerase holoenzyme complex. The maintenance of RFC in the holoenzyme is mediated by a second, species-specific interaction with RPA (Escherichia coli SSBRPA cannot substitute) and is essential for processive DNA synthesis.

During NER, XPA binds to both the 32- and 70-kDa subunit of RPA (RPA32 and RPA70, respectively) (10, 11). The $K_d$ for the overall interaction measured by surface plasmon resonance was $1.9 \times 10^{-8}$ M (12). Enzyme-linked immunosorbent assay results suggested that the binding affinity is partitioned, with 80% of the affinity assigned to RPA32 and 20% to RPA70 (11). The expected dissociation constant for the XPA-RPA70 interaction was therefore on the order of $10^{-10}$ M. However, NMR experiments used to characterize the interaction of the isolated domains revealed that their binding affinity is several orders of magnitude weaker than that of the intact proteins (13). What factors make the total affinity significantly stronger than the sum of the isolated domain interactions? The key here is the linkage effect (14), which provides a means to obtain a high affinity interaction between binding partners from the tethering together of two or more components that by themselves interact very weakly. For example, the isolated A and B domains of RPA70 each bind ssDNA with relatively modest affinity ($K_a$ is micromolar). However, when tethered together by a flexible ~10-residue linker, their overall affinity for ssDNA is nearly 3 orders of magnitude higher ($K_a$ is nanomolar) (15).

In recombination events, Rad52 has been shown to bind to both the A domain of RPA70 (RPA70A) and the C-terminal domain of RPA32 (RPA32C) (16). In this case, the different points of contact between Rad52 and RPA are associated with different regulatory mechanisms. NMR chemical shift perturbation assays demonstrated that residues 257–274 of Rad52 bind to RPA32C at the same location as two other repair proteins: XPA and uracil DNA glycosylase (17). Overlapping binding sites between proteins from three different repair pathways suggested a competitive mechanism for switching between pathways. The interaction between Rad52 and RPA32C is also responsible for an allosteric enhancement (5-fold) of the ssDNA binding affinity of RPA, an effect mediated through the DNA binding domain in RPA32 (16). The same segment of Rad52 (residues 218–303) binds to RPA70A, and sequence comparison between RPA32C and RPA70A has predicted that the Rad52 binding site overlaps with the ssDNA binding site on RPA70A. This interaction is expected to be competitive with both DNA and other proteins known to bind at this site.

**Versatile Protein Domains**

Another striking observation about DNA processing proteins is the repeated utilization of specific interaction modules (folded domains). Remarkably, these domains may interact exclusively with proteins in one context but will interact with both proteins and DNA in another, and their sites of interaction may be similar, overlapping, or unique. Modules that interact with both proteins and DNA are critical to the recruitment and positioning of peripheral processing proteins as they assemble on and dissociate from the DNA.

The oligonucleotide/oligosaccharide binding (OB)-fold, the canonical ssDNA binding domain (18, 19), is one example of such a commonly used interaction module. OB-fold domains are found in DNA processing proteins ranging from nucleases and pyrophosphatases to ssDNA- and RNA-binding proteins (20). RPA has six OB-folds (and one winged helix domain, see below), four of which are involved in binding to ssDNA. In the crystal structure of the tandem RPA70AB domains (residues 181–422 of RPA70), an oligonucleotide is bound in the continuous cleft created by alignment of the tandem OB-fold domains (Fig. 2A) (21). Although OB-fold domains are characterized and, in fact, named for binding to nucleotides, they are also involved in protein interactions. The N-terminal domain of RPA70, for instance, is dedicated to interactions with pol-prim and p53 (23), whereas the tandem RPA70AB domains are known to interact with both ssDNA and proteins, including the SV40 large T antigen, XPA, and Rad51 (22).

A recent crystal structure of BRCA2, one of two well known human breast cancer-associated proteins, reveals a fundamentally different mode of protein binding to an OB-fold domain (23). BRCA2 possesses three OB-fold domains in its C-terminal region, two of which are bound to a 9-mer nucleotide in the same manner as observed for RPA70AB (Fig. 2B). The third domain is bound to a fragment of a binding partner, DSS1, which is essential for recombinational DNA repair (24). The DSS1 peptide binds away...
from the usual nucleotide binding site into a positively charged groove that traverses the domain from front to back. Hence, this OB-fold domain appears to have been adapted for a purpose other than nucleotide binding.

A second example of a versatile protein fold is the winged helix (WH) domain, a member of the common helix-turn-helix superfamily (25). WH domains were first characterized in 1993 and are best known for their role in dsDNA binding in transcription factors (26). Numerous examples of DNA-bound structures are available for a wide functional variety of proteins. A recently determined structure of the RecQ helicase from *E. coli* revealed that it contains a WH domain that possesses the characteristic structural features of a DNA binding WH domain (27). Interestingly, a homologous portion of human Werner's syndrome protein (WRN) is known to bind proteins, such as Bloom's syndrome protein and FEN-1, a eukaryotic endo/exonuclease (28, 29). The interaction of WRN with FEN-1 is functionally relevant, as it increases the DNA cleavage activity of the syndrome protein and FEN-1, a eukaryotic protein responsible for the interaction is the conserved C-terminal domain (30). In eukaryotic replication, competition for binding sites on RPA, although the precise locations of binding are unknown (6). The ability of proteins acting at later steps in the pathway to displace those present at earlier steps suggests overlapping binding sites, which is a well established feature of RPA. Mer et al. (17) showed that the C-terminal domain of the 32-kDa subunit of RPA binds to peptides from three different repair proteins, all at the same site. This early finding suggested that competition might facilitate hand-off of the DNA from one repair protein to another.

Two recent studies identify overlap between protein and ssDNA binding sites on RPA. NMR chemical shift perturbation experiments have indicated that both XPA and Rad51 bind to the canonical oligonucleotide binding site on RPA70A (13, 34). NMR competition experiments demonstrated that ssDNA could disrupt the preformed complex between RPA70A and the N-terminal domain of Rad51 (Rad51N), indicating that the binding of Rad51N and ssDNA is mutually exclusive (34). The competition between Rad51 and ssDNA for RPA is the basis for the displacement of RPA during initiation of homologous recombination, whereas XPA affects the preference of RPA for undamaged ssDNA during NER.

Homologous recombination is also regulated by competition in the case of the interaction between BRCA2 and Rad51 (35). BRCA2 possesses eight BRC repeats in its central region, six of which are capable of binding to Rad51 with varying affinities. The interaction is essential for the translocation of Rad51 into the nucleus and is inhibitory to Rad51 filament formation. The basis for the inhibition is simply that the BRC sequence binds to the site on Rad51 at which it (Rad51) self-associates. Thus, BRCA2 and Rad51 compete for an overlapping binding site on Rad51. Still, Rad51 forms oligomeric filaments in vivo. How? One possible explanation is that the low affinity BRC repeats release Rad51 first, followed by release of the remaining tightly bound molecules by allosteric or cooperative means.

**Allostery**

Allostery (action at a remote site) is a second common mechanism that promotes progression through a DNA processing pathway. Allostery takes on many forms. Some protein interactions serve to orient the binding partners for optimal activity at a distant site. In other cases, ATP binding and/or hydrolysis are coupled to conformational changes that affect the oligomerization state of the ATP-binding protein or its interactions with other proteins. Allostery can be used in conjunction with other types of regulation to catalyze a single step of DNA processing.

Allostery is evident in the interactions between the eukaryotic sliding clamp protein, PCNA, and its binding partners. PCNA is a recruitment factor for repair proteins from several different pathways, including XPG (NER), uracil DNA glycosylase (base excision repair), several MutS homolog proteins (mismatch repair), and the Werner helicase (recombination) (36). The homotrimeric structure of PCNA allows for several regulatory mechanisms, including the use of multiple binding sites, competition for overlapping sites, and allosteric control of binding partners. The type of DNA damage to which PCNA is bound plays a role in attracting binding partners, several of which specifically recognize kinked or bent DNA (37, 38). PCNA stimulates the nuclelease activity of flap endonuclease-1

---

**Fig. 3. AllostERIC effect of ATP binding on the subdomain orientation of Rad50.** Lobe 1 of Rad50 (purple oval) contacts both DNA (black helix) and lobe II (blue oval). When ATP binds (red cross), lobe I rotates with respect to lobe II, bringing the DNA into position for nucleolytic cleavage by Mre11 (yellow diamond) bound to lobe II.

**Fig. 4. Key concepts in DNA processing.** The upper panel symbolizes the interaction between two multidomain proteins (A, two open rectangles; B, two filled ovals) that have two contact points. The overall affinity between the two proteins results from two modest affinity interactions, each of which has an appreciable off rate. This corresponds to one representation of the linkage effect. The lower panel represents the facilitation of hand-off in this system. A third protein (checked circle) needs to bind to only one of the two domains of protein B to drastically reduce the overall binding affinity and promote release of B from A.
The interaction between these two proteins and its effect on the DNA binding ability of FEN-1 was recently characterized (40). The PCNA-FEN-1 interaction induces the formation of a β-zipper, with strands contributed by the C-terminal segments of each protein. On FEN-1, the β-zipper links the PCNA binding site to a DNA binding site. Formation of the β-zipper likely enhances the nucleic acid activity of FEN-1 through allosterically properly positioning it for cleavage of flapped DNA substrates.

Allosteric regulation is also observed for the Mre11-Rad50 complex, which generates the 3'-ssDNA used as templates for homologous recombination (41). Mre11 contains nucleic acid specificity for ssDNA, dsDNA ends, or hairpin DNA structures, whereas Rad50 plays a structural role in bringing sister chromatids together. Recent crystal structures have elucidated the allosteric mechanism whereby the ATPase activity of Rad50 is coupled to strand exchange via residues in loop L1. ATP binding has recently been shown to affect the pitch and orientation of subunits in the bacterial RecA filament (42). In eukaryotic Rad51, ATP has recently been shown to affect the pitch and orientation of subunits in the bacterial RecA filament (42). In eukaryotic Rad51, ATP binding is coupled to strand exchange by the DNA-binding loop L1.

Concluding Remarks

The accumulated evidence to date confirms that the DNA processing machinery is dynamically assembled and disassembled in conjunction with the cell cycle and in response to DNA damage. Assembly and rearrangement of protein assemblies is facilitated by the hand-off of proteins and DNA from one step to the next. We have discussed five key concepts relevant to understanding the progression of DNA processing assemblies: 1) Modular organization: DNA processing proteins contain multiple structural domains with distinct biochemical functions. 2) Multiple contact points: DNA processing proteins interact with each other through multiple contacts. 3) Modest affinity: the majority of protein interactions characterized to date have dissociation constants in the micromolar range. 4) Versatile structural modules: DNA processing proteins utilize a common set of structural frameworks that can interact with DNA and/or protein. 5) Multiple structural mechanisms promote hand-off: direct competition for binding sites and allosteric structural rearrangements have been characterized.

The emerging view is that DNA processing assemblies are composed of modular proteins with multiple, structurally independent functional domains. Individual contact points are characterized by relatively low (micromolar) binding affinities, which promotes hand-off of DNA and proteins. The structural modules commonly used by DNA processing proteins are versatile in their binding preferences and modes. These characteristics are integrated into the structural mechanisms that mediate the forward progression of DNA processing.

In this model, linked weak interactions provide high overall affinity while maintaining a significant off-rate for each contact point (Fig. 4). This in turn provides a ready means for rapid disassembly: by simply blocking one of the weak interactions, the overall affinity between two interacting molecules can be reduced by several orders of magnitude almost instantly. The rapid transition between high and low affinity states promotes hand-off and forward progression of DNA processing. This simple model provides a framework for developing a more sophisticated and detailed understanding of the structural mechanisms used by the multiprotein assemblies involved in DNA replication, repair, and recombination.

REFERENCES

1. Kowalczykowski, S. C. (2000) Nat. Struct. Biol. 7, 1087–1089
2. Redekopers, S., Volker, M., Fleckstraten, D., Nigg, A. L., Mone, M. J., Van Leeuwen, A. A., Hoeijmakers, J. H., Houtsmuller, A. B., and Vermeulen, W. (2003) Mol. Cell. Biol. 23, 5755–5767
3. Essers, J., Houtsmuller, A. B., Van Veelen, L., Paulusma, C., Nigg, A. L., Pastink, A., Vermeulen, W., Hoeijmakers, J. H., and Kanaar, R. (2002) EMBO J. 21, 2030–2037
4. Yuzhakov, A., Kelman, Z., and O’Donnell, M. (1999) Cell 96, 153–163
5. Daughdrill, G. W., Buchko, G. W., Botuyan, M. V., Arrowsmith, C., Wold, M. F., Kennedy, M. A., and Lowry, D. F. (2003) Nucleic Acids Res. 31, 4719–4724
6. Daughdrill, G. W., Buchko, G. W., Botuyan, M. V., Arrowsmith, C., Wold, M. F., Kennedy, M. A., and Lowry, D. F. (2003) Nucleic Acids Res. 31, 4719–4724
7. VanLoock, M. S., Yu, X., Yang, S., Lai, A. L., Low, C., Campbell, M. J., and Tainer, J. A. (2004) J. Biol. Chem. 279, 10505–10514
8. Lee, J. Y., Chang, C., Song, H. K., Moon, J., Yang, J. K., Kim, H. K., Kwon, S. T., and Yoon, S. W. (2000) EMBO J. 19, 1119–1125
9. Tom, S., Henrekson, L. A., and Bambara, R. A. (2000) J. Biol. Chem. 275, 10498–10507
10. Chapados, B. R., Hosfield, D. J., Han, S., Qiu, J., Yelent, B., Shen, B., and Tainer, J. A. (2004) Cell 116, 39–50
11. Hopfer, K. P., and Tainer, J. A. (2003)Curr. Opin. Struct. Biol. 13, 249–255
12. Hopfer, K. P., Karthier, A., Craig, L., Woo, T. T., Carney, J. P., and Tainer, J. A. (2001)Cell 105, 473–485
13. VanLoock, M. S., Yu, X., Yang, S., Lai, A. L., Low, C., Campbell, M. J., and Egelman, E. H. (2003) Structure (Camb.) 11, 187–196
14. Shin, D. S., Pellegrini, L., Chen, P. L., Lee, W. H., and Pavletich, N. P. (2002)Science 297, 11215–11225
15. Arunkumar, A. I., Stauffer, M. E., Bochkareva, E., Bochkarev, A., and Chazin, W. J. (2004) J. Biol. Chem. 279, 41077–41082
16. Donnell, M. (1999)Cytogenet. Cell Genet. 86, 201–211
17. Mer, G., Bochkarev, A., Gupta, R., Bochkareva, E., Frappier, L., Ingles, C. J., Edwards, A. M., and Chazin, W. J. (2004) J. Biol. Chem. 279, 42729–42739
18. Giraldo, R. (2003)Curr. Opin. Struct. Biol. 13, 420–427
19. Liu, J., Smith, C. L., De Ryckere, D., DeAngelis, K., Martin, G. S., and Berger, J. M. (2000)Mol. Cell 6, 637–648
20. Piotrowski, J., Dianova, I., Dianov, G. L., and Bohr, V. A. (2001)EMBO J. 20, 4566–4576
21. Blundell, T. L., Venkitaraman, A. R., and Tainer, J. A. (2003)Nature 420, 287–293
22. Yang, H., Jeffrey, P. D., Miller, J., Kinnucan, E., Sun, Y., Thoma, N. H., Zheng, C., Chen, P. L., Lee, W. H., and Pavletich, N. P. (2002)Science 297, 1837–1848
23. Stocker, E., Ukeleie, S. C., Heese, F. H., Vollenweider, P., Weibel, C., and Moser, G. (2002)Mol. Cell 10, 11205–11215
24. Yang, J. H., Grabowski, B., Kauisvarwanathan, R., Bell, S. D., and Kelman, Z. (2003)J. Biol. Chem. 278, 40805–40807
25. Blundell, T. L., Venkitaraman, A. R., and Tainer, J. A. (2003)J. Biol. Chem. 278, 83065–83067
26. Yang, J. H., Grabowski, B., Kauisvarwanathan, R., Bell, S. D., and Kelman, Z. (2003)J. Biol. Chem. 278, 83065–83067
27. Zhang, C., Wang, Y., Gutierrez, V., DeAngelis, K., Martin, G. S., and Berger, J. M. (2000)Mol. Cell 6, 637–648
28. Pelttari, V., and Tainer, J. A. (2003)Curr. Opin. Struct. Biol. 12, 1160–1166
29. Yang, J. H., Grabowski, B., Kauisvarwanathan, R., Bell, S. D., and Kelman, Z. (2003)J. Biol. Chem. 278, 40805–40807
30. Lopez de Sato, F. J., Georgescu, R. E., Goodman, M. F., and O’Donnell, M. (2003)EMBO J. 22, 6408–6418
31. Stauffer, M. E., and Chazin, W. J. (2004)J. Biol. Chem. 279, 25638–25645
32. Pellegrini, L., Yu, D. S., Lo, T., Anand, S., Lee, M., Blundell, T. L., and Venkitaraman, A. R. (2002)Nature 420, 287–293
33. Sugiura, M., and Hasegawa, M. (2003)J. Mol. Biol. 331, 153–158
34. Stauffer, M. E., and Chazin, W. J. (2004)Curr. Opin. Struct. Biol. 14, 36–42
