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Movement and Specificity in a Modular DNA Binding Protein

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The single-stranded DNA (ssDNA) binding protein RPA binds to and protects ssDNA while simultaneously recruiting numerous replication and repair proteins essential for genome integrity. In this issue of *Structure*, Brosey et al. (2015) show that the flexibility and interactions of the modular domains of RPA are altered by ssDNA binding and suggest that these changes in configurational freedom are important for the many functions of RPA.

The maintenance and propagation of the eukaryotic genome requires melting of the double helix and the exposure of single-stranded DNA (ssDNA). DNA must be in single-stranded form to be accessed by the proteins involved in its replication, recombination, and repair. However, ssDNA is quite susceptible to damage, formation of aberrant secondary structure, and attack from nucleases. To prevent these deleterious side reactions, eukaryotic cells employ an ssDNA binding protein called Replication Protein A (RPA). RPA tightly binds ssDNA in a non-sequence-specific manner, and in doing so it limits attack by nucleases and the formation of undesirable DNA secondary structures. However, although ssDNA must be protected, it must also simultaneously be accessible to the myriad of enzymes involved in the aforementioned DNA processing reactions. This necessitates complex ssDNA binding behavior, as RPA must not only relocate along ssDNA to allow interactions with target enzymes but also dissociate from the DNA when needed. Indeed, some fluorescence-based work has demonstrated that RPA diffuses across ssDNA, repositioning itself to allow for interactions between ssDNA and various globular proteins, and aids in DNA melting. (Nguyen et al., 2014)

Given RPA's role in recruiting a diverse nature of proteins that act upon DNA, it is critically important not only to DNA processing pathways but also to DNA repair pathways. Typically, RPA is the first component in a repair pathway to bind to DNA, acting as a sensor for damage. After binding, RPA recruits damage- and repair-pathway-specific enzymes such as glycosylases and other damage repair enzymes. The wealth of different repair processes requires RPA to specifically bind any of a large number of enzymes on a contextual basis. Moreover, given RPA's additional roles in DNA replication and processing, it must be equipped with a means to recruit enzymes relevant to these processes as well. The resultant diversity in RPA function requires it to be able to recruit an immense variety of enzymes in a situational manner, as demonstrated by Hass et al. (2012). Thus, all told, RPA must possess a means to bind ssDNA, an anionic linear polymer, as well as any of the large variety of globular proteins, and coordinate their interactions with ssDNA.

RPA's ability to differentially interact with proteins and DNA arises in part from its modular architecture. RPA is comprised of three proteins and seven globular domains (Figure 1). These three proteins, RPA14, RPA32, and RPA70, associate to form a trimer core between domains RPA14, RPA32D, and RPA70C (Figure 1). RPA32 is comprised of a flexible N-terminal tail (32N) and a globular (32C) protein, with 32C known to bind a variety of DNA repair enzymes and 32D possessing DNA binding activity. RPA70 is composed of four domains (70A, 70B, 70C, and 70N) connected by flexible linkers of varying length, each domain exhibiting a number of different functions. 70C has DNA binding activity and 70N binds p53, with additional very weak DNA binding affinity. In addition, 70A and 70B have high-affinity binding sites for ssDNA but also bind a number of DNA processing enzymes. The RPA domains also participate in numerous intra-domain interactions. The function of RPA is delicately balanced between DNA interactions, intra-domain interactions, and interactions with the DNA processing and repair proteins, and the poise of these multiple equilibria is instrumental in RPA function, especially given the contextual manner in which RPA operates. In part, RPA navigates the complexity of these interactions by possessing conformational flexibility that facilitates the modulation of its protein and DNA binding affinities.

To investigate the nature in which RPA domains interact with each other and with ssDNA, Chazin and coworkers utilize ^{15}N nuclear magnetic resonance (NMR) spin-relaxation measurements to examine the flexibility in RPA70A/B, which are connected by a short, 13-residue linker, and RPA70A/B/N, in which 70N is linked to the A and B subunits by a longer, 61-residue linker. These NMR experiments are a sensitive probe of the stochastic (internal and overall rotational) motions of the amide backbone N-H bond vector in the RPA protein on the picosecond to nanosecond timescale. These motions are described by a spectral density function ($J(\omega)$), which is a gauge of the frequency spectrum of these motions. The measured longitudinal ($R_1 = 1/T_1$) and transverse ($R_2 = 1/T_2$) NMR spin-relaxation rate constants are primarily determined by linear combinations of $J(\omega)$ values. The dynamics of N-H bond vector motions are thus encoded in these measured relaxation rates, which then depend on the orientations of the ^{1}H-^{15}N

vector and on their fluctuations relative to the protein's diffusion tensor. (Brüschweiler et al., 1995) In addition, heteronuclear nuclear Overhauser effects (het-nOe) are a measure of fast internal motions and are insensitive to rotational diffusion of the protein. Thus, Chazin and coworkers directly address the biophysics of overall rotational motion as well as internal bond fluctuations using these NMR relaxation experiments.

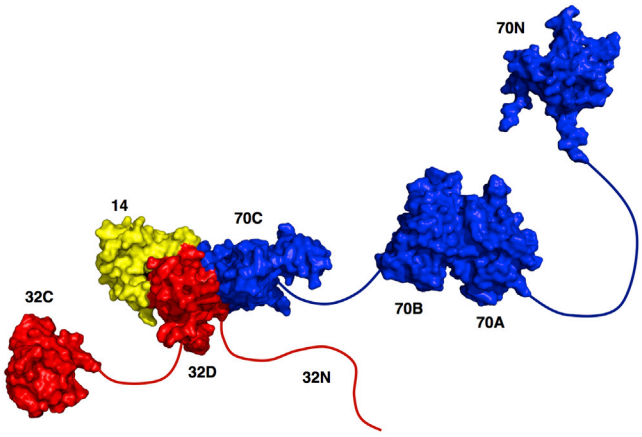

Figure 1. Modular Architecture of RPA
Individual proteins share the same color. Crystal structures for the trimer core (1L1O), 70A/B (1JMC), 70N (1EWI), and 32C (1DPU) were used for this depiction. Linkers were manually added and are for visual purposes.

Individually, 70A and 70B bind ssDNA with low micromolar affinity. This affinity increases by approximately 10- to 100-fold when 70A and 70B are linked. (Fanning et al., 2006) Yet the affinity of the linked proteins is less than additive of the two isolated domains, suggesting some compensating effects when A/B are connected by a 13-residue linker. Interactions between 70A and 70B modulate its affinity for DNA and would therefore be of functional relevance. Brosey et al. use NMR experiments to investigate the interactions between 70A and 70B in the absence of and when bound to ssDNA. The ratio of measured R_1 and R_2 values are sensitive to overall rate of rotational diffusion of the macromolecule. When not bound to DNA, the ratio of these relaxation rates is different for 70A and 70B (Figure 2A) indicating different rates of rotational diffusion. Extending this analysis, the calculated rotational diffusion tensors for 70A and 70B are axially symmetric in nature (Figure 3), and the NMR data suggest partially coupled domain motions due to the relatively short linkage between them. Moreover, reduced het-nOe values are evidence of enhanced flexibility of DNA binding loops (Figure 2A). When bound to 10-nt ssDNA, the rotational diffusion of 70A and 70B are the same (Figure 2B), as indicated by the uniform R_1/R_2 ratios. Diffusion tensor calculations show similar rotation for both domains and primary axes of diffusion that are distinct from the free protein values. These data suggest that binding of ssDNA increases the degree of coupling of macromolecular tumbling between 70A and 70B. Likewise, the het-nOe values indicate that there is restriction in the mobility of the 70A/B DNA binding loops upon interaction with ssDNA.

Building on this work, Brosey et al. next examine the dynamics of RPA70A/B/N, in which the N domain is connected to A and B via its 61-residue linker. The distinct R_1/R_2 ratios (Figure 6A) for the N domain relative to the A and B domains are consistent with independent motion of the N domain. Furthermore, the extra hydrodynamic burden of the N domain on A and B increases the complexity of the diffusion of A and B. As in the A/B construct, ssDNA binding recouples the rotational diffusion of the A and B domains but has little to no effect on 70N (Figure 6B). Neither the chemical shifts nor the relaxation rates of 70N are altered in the presence of ssDNA, indicating that 70N can operate independently of the rest of RPA.

RPA must deftly interact with ssDNA and the enzymes that process it. Furthermore, RPA not only recruits the proper processing enzymes but must also hand the DNA off, dissociating to facilitate this process. RPA must utilize some of the binding energy of protein interactions to alter its conformation and modulate its affinity for DNA. In this elegant work, Chazin and coworkers show in part how the flexibility of RPA enables such a complex set of interactions to occur. Domain modularity allows diversity in the number and types of specific interactions that can occur, and it appears that the linker length may allow varying degrees of coupling between domains that can further impact ligand interactions. Therefore, unlike a tightly packed globular protein, RPA domains exist in a set of semi-coupled interactions that are readily modified upon binding DNA and accessory proteins. While this work highlights some features of domain-domain dynamics, it also presents numerous questions for future work. The detailed mechanisms of how protein or DNA binding alters RPA conformation and dynamics and ligand affinity remain to be determined. This work also suggests a possible avenue toward elucidating how phosphorylation of RPA, known to occur in vivo, alters its function.

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