The initiation of DNA replication requires the melting of chromosomal origins to provide a template for replisomal polymerases. In bacteria, the DnaA initiator plays a key role in this process, forming a large nucleoprotein complex that opens DNA through a complex and poorly understood mechanism. Using structure-guided mutagenesis, biochemical, and genetic approaches, we establish an unexpected link between the duplex DNA-binding domain of DnaA and the ability of the protein to both self-assemble and engage single-stranded DNA in an ATP-dependent manner. Intersubunit cross-talk between this domain and the DnaA ATPase region regulates this link and is required for both origin unwinding in vitro and initiator function in vivo. These findings indicate that DnaA utilizes at least two different oligomeric conformations for engaging single- and double-stranded DNA, and that these states play distinct roles in controlling the progression of initiation.

Large multisubunit molecular machines, known as replisomes, couple the unwinding of parental DNA with the synthesis of new daughter strands to replicate host chromosomes (1–3). Replisome assembly is coordinated, in part, by a class of proteins known as initiators, which bind and remodel replication origins and recruit replicative components to the site of initiation (4–6). Although initiator proteins in the three cellular domains of life are diverse, they all utilize one or more ATPases associated with various cellular activities (AAA+) subunits to help with origin recognition, loading of replicative helicases, and the control of replication onset (7, 8). Although evolutionary and structural commonalities among cellular initiators hint at similar assembly and origin-recognition mechanisms, how ATP controls these functions, and how initiators directly or indirectly participate in origin opening, remain central unanswered questions.

In bacteria, replication begins with the sequence-specific recognition of the chromosomal origin, oriC, by the initiator protein DnaA. In Escherichia coli, some DnaA protomers remain stably bound to several high affinity, duplex DNA-binding sites (the R1, R2, and R4 loci) within oriC throughout most of the cell cycle (9). During initiation, additional ATP-bound DnaA protomers populate intermediate affinity sites (R5 and R3) as well as lower affinity sites known as ATP-DnaA boxes and I sites (I1, I2, and I3) (10–12). Occupation of these sites occurs with the ATP-dependent formation of a large, nucleoprotein complex (13–15), which, in the presence of architectural factors and negatively supercoiled DNA, stimulates the melting of an adjacent AT-rich DNA-unwinding element (DUE) (16–20). Following melting, DnaA collaborates with DnaC, the bacterial helicase loader, to deposit two hexamers of the DnaB helicase onto the origin (14, 21–24). DnaB in turn nucleates replisome assembly and processively unwinds the chromosome (3, 25).

The means by which DnaA melts oriC is not understood. The initiator itself is a complex, modular protein composed of four domains, including an N-terminal helicase-interaction domain (23), a variable linker element, a central AAA+ fold that binds single-stranded (ss) DNA (26), and a C-terminal, helix-turn-helix DNA-binding domain (DBD) that recognizes duplex origin sites (Fig. 1A) (27, 28). Clues to the DnaA assembly mechanism have come from structural studies conducted with a truncation lacking the helicase-interaction region (domain I). Thus far, DnaA has been imaged in two distinct states: a monomeric ADP-bound form (26, 29) and a higher order oligomer complexed with the ATP analog, AMPPCP (30). Unexpectedly, the DnaA oligomer structure revealed that the initiator self-assembles into an open-ended helical particle (Fig. 1B), as opposed to a closed-ring oligomer typically seen for AAA+ proteins (31). Although this configuration correctly positions neighboring AAA+ domains in contact with one another to form a bipartite ATPase site, how the spiral architecture relates to the distinct functions and assembly states accessed by DnaA during oriC recognition and open complex formation is unknown (10, 15, 16). The means by which the AAA+ element and the DBD coordinate their respective ssDNA- and double-stranded (ds)DNA-binding activities within the higher order DnaA complex similarly has not been resolved.
To address these issues, we conducted a series of mutagenesis, biochemical, and genetic studies of DnaA using available structures as a guide. Surprisingly, we found that crystallographically observed interactions occurring in \textit{trans} between the AAA\textsubscript{+/H11001} domain of one DnaA protomer and the DBD of a partner subunit are necessary for both higher order initiator assembly and the binding of ssDNA. Moreover, this cross-talk is required for origin unwinding \textit{in vitro} and global DnaA function \textit{in vivo}, and can be modulated by the binding of dsDNA substrates. Together, our data indicate that DnaA assemblies adopt at least two distinct conformations to associate productively with melted and duplex DNA segments, and that these states likely play a role in the transition from origin recognition to open complex formation.

EXPERIMENTAL PROCEDURES

Expression and Purification of \textit{Aquifex aeolicus} DnaA—Various \textit{A. aeolicus} DnaA (AaDnaA) constructs (residues 2–399, 76–399, 290–399, and 1–80) were expressed as tobacco etch virus (TEV) protease-cleavable His\textsubscript{6}-maltose-binding protein (MBP) fusions. All AaDnaA proteins were purified as previously described (30), except for a few modifications described in supplemental Experimental Procedures. As a final step, untagged AaDnaA proteins were run over an S-200 size-exclusion column (GE Healthcare) in gel-filtration buffer (50 mM HEPES, pH 7.5, 500 mM KCl, 10% (v/v) glycerol, 5 mM MgCl\textsubscript{2}). Monomeric species were pooled, concentrated, and flash-frozen as per AaDnaA, except that 50 mM PIPES-KOH, pH 6.8, 10 mM magnesium acetate, 200 mM ammonium sulfate, 20% (v/v) sucrose, 0.1 mM EDTA, and 2 mM DTT was used as a gel-filtration buffer. Mutations were introduced into His\textsubscript{6}-EcDnaA using QuikChange mutagenesis.

\textit{AaDnaA} Cross-linking Assays—Cross-linking was performed by incubating 50 \mu{g}/ml of various AaDnaA proteins in 80 \mu{l} of a reaction buffer (50 mM HEPES, pH 7.5, 10% (v/v) glycerol, 125 mM KCl, 5 mM MgCl\textsubscript{2}, 2 mM DTT) containing 2 \mu{M} nucleotide at 25 °C for 5 min. Glutaraldehyde (Poly-sciences, Inc.) was then added to 8.8 \mu{l} of a 0.1 M stock. Reactions were incubated at 25 °C for an additional 1 min before quenching with 8 \mu{l} of 200 mM glycine, followed by the addition of 30 \mu{l} of gel loading buffer (100 mM Tris, pH 6.8, 24% (v/v) glycerol, 8% (w/v) SDS, 200 mM DTT, 0.02% (w/v) bromophenol blue). Cross-linked proteins were loaded in a volume of 15 \mu{l} and separated on denaturing 4.5% polyacrylamide gels (80:1 acrylamide:bisacrylamide) in 0.1 M sodium phosphate, 0.1% SDS buffer (pH 7.2) (62, 63) and visualized by silver staining. Gel data were quantified using...
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Kodak Molecular Imaging software (version 4.0.5). Ratios comparing the amount of various cross-linked species were calculated with data obtained from the same experiment using the same glutaraldehyde stock solution.

**AaDnaA ssDNA Binding Assays**—Binding of 25-bp fluorescein-tagged oligonucleotides to AaDnaA was monitored by fluorescence polarization using a Victor3 V (PerkinElmer Life Sciences) multilabel plate reader. Measurements were carried out in 20 μL of a buffer containing 50 mM HEPES, pH 7.5, 125 mM KCl, 2% (v/v) glycerol, 0.1 mg/ml BSA, 2 mM DTT, 5 mM MgCl₂, 2 mM nucleotide. The substrate concentration was held constant at 10 nM, while the protein concentration was varied. A detailed description of the substrates can be found in the supplemental Experimental Procedures.

**Complementation Assays**—EcDnaA was cloned into a modified pET24 plasmid (pLIC-TrPP-NTK), generating a vector containing untagged wild-type EcDnaA (NT-EcDnaA) under T7 promoter control. Mutations were introduced into NT-EcDnaA using QuikChange mutagenesis. Wild-type NT-EcDnaA and mutant NT-EcDnaA vectors were transformed into the host strain MM294 dnaA5 (40) and grown to mid-log in LB/Kan medium at 30 °C. Following growth, cells were serially diluted and plated at 30 °C and 42 °C to assess survival resulting from leaky expression.

**OriC Unwinding Assay**—Following the basic protocol developed by Bramhill and Kornberg (16), EcDnaA was added to reactions containing 40 nM HEPES, pH 7.6, 8 mM magnesium acetate, 40 mM potassium glutamate, 15% (v/v) glycerol, 0.1 mg/ml BSA, 5 mM ATP, 20 nM Integration Host Factor, and 3.5 nM (−) supercoiled pBlueScript (pBS) plasmid containing the E. coli origin of replication (oriC) and 200 nM protein in a final reaction volume of 20 μL on ice. After incubation at 38 °C for 6 min, 0.6 unit of P1 endonuclease (Sigma) in 30 mM sodium acetate, pH 5.3, 1 mM ZnCl₂, was added to each reaction and incubation continued for 2 min. Reactions were quenched by the addition of SDS to 0.5% and EDTA to 28 mM. Reactions were analyzed on 1% agarose gels in Tris acetate/EDTA (TAE) buffer and stained with ethidium bromide. The percentage of linear DNA in each lane was quantified using a Molecular Dynamics Typhoon PhosphorImager.

**EcDnaA Double- and Single-stranded DNA Competition Experiments**—EcDnaA-ssDUE complexes were formed by incubating 800 nM EcDnaA with 40 nM fluorescein-labeled E. coli ssDUE fragment (supplemental Table S3) for 10 min at room temperature in 20 μL of competition buffer (50 mM HEPES, pH 7.5, 50 mM potassium acetate, 10% (w/v) sucrose, 0.1 mg/ml BSA, 2 mM DTT, 5 mM magnesium acetate, 2 mM ATP). After incubation, the concentration of the EcDnaA-ssDUE complex was diluted by half with increasing amounts of two 13-mer dsDNA fragments (0.002–20 μM) in the same buffer, one containing the R1 box sequence and the other containing a random sequence.

Cross-linking experiments were performed by incubating 800 nM EcDnaA, alone, in the presence of 266 nM E. coli ssDUE fragment, or in the presence of 266 nM E. coli ssDUE fragment with 10 μM unlabeled R1 box added (80-μL reactions). Samples were incubated at 25 °C for 5 min, after which glutaraldehyde was added to a final concentration of 10 mM using 8.8 μl of a 100 mM stock. Reactions were incubated at 25 °C for an additional 1 min before quenching with 8 μl of 200 mM glycine followed by the addition of 30 μl of gel loading buffer (described above). Cross-linked proteins were separated and analyzed as described above.

**RESULTS**

**Nucleotide Status Influences DnaA Assembly in the Absence of DNA**—The importance of ATP for stimulation of DnaA oligomerization on oriC is well known (10–15). What has been less clear is the extent to which nucleotide predisposes DnaA toward oligomerization in the absence of DNA, or how assembled initiator complexes might associate with specific nucleic acid substrates. To begin to examine this dependence, we first subjected full-length DnaA from the organism A. aeolicus (AaDnaA) to glutaraldehyde cross-linking in the presence of various nucleotide analogs. Analysis of the species formed by silver-stained denaturing polyacrylamide gels (Fig. 2A) shows that ATP, as well as the nonhydrolyzable analogs AMPPNP, ATPγS, and ADP-BeF₃, all strongly induce the appearance of higher order DnaA species (up to hexamer and beyond), consistent with the formation of a range of AaDnaA oligomers in solution. By contrast, the addition of ADP does not robustly stimulate assembly, yielding >70% less tetramer and 40% more monomer compared with reactions with ATP (Fig. 2B). Interestingly, AMPPPC generates an intermediate level of cross-linking compared with the ATP and ADP reactions, suggesting that this nucleotide only moderately stimulates DnaA assembly. This finding is consistent with our prior observation that crystallization of AaDnaA using AMPPPC often generates two different crystal forms in the same solution conditions, one of which harbors the DnaA oligomer, and the other DnaA monomers structurally similar to the ADP-bound initiator. Taken together, these results show that ATPs are by themselves sufficient to promote DnaA assembly and that the type of ATP mimic can significantly influence oligomerization propensity.

**Nucleotide and Sequence Dependence of ssDNA Binding**—Given the need for ATP-like molecules in promoting AaDnaA assembly, we next assessed the influence of these analogs on ssDNA binding using fluorescence polarization. Binding of the ssDUE by EcDnaA is known to be ATP-dependent and likely involves several DnaA molecules (11, 32). To find an optimal substrate for our studies with AaDnaA, we first screened a panel of fluorescein-labeled ssDNA substrates, including both homopolymers and two different regions of both complementary strands of the predicted Aquifex DUE for their ability to bind AaDnaA. Unlike EcDnaA, which preferentially binds one strand of the oriC DUE over other DNA sequences (11, 26), the Aquifex initiator showed no preference for any one substrate (Fig. 2C). We therefore turned to a labeled 25-mer dT substrate (F-dT₂₅) as a simplified mimic for the T-rich sequences typically found in DUEs (20) and varied the type of nucleotide added to the reaction. The ability of AaDnaA to associate with this oligonucleotide is strongly influenced by nucleotide, with ATP and most ATP mimetics giving rise to an

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apparent dissociation constant ($K_{d,app}$) of 0.15 ± 0.02 μM (Fig. 2D and supplemental Table S1). By contrast, ADP-DnaA mixtures bind ssDNA ~10-fold more weakly, and AMPPCP, which does not induce robust DnaA assembly, binds substrate only marginally better than ADP-AaDnaA. These findings show that avidity for ssDNA is linked to the ATPase status of DnaA and, in conjunction with our cross-linking data, suggest that this property correlates with initiator assembly.

AAA+ Domain and DBD Comprise the Minimal Assembly and ssDNA-binding Determinants of DnaA—Although domain I of DnaA can form a weak dimer that is believed to play a role in initiator function (33–36), the structure of the DnaA domains III/IV oligomer indicates that the ATPase region likely comprises the dominant assembly nexus (30). To test this assumption biochemically and determine the minimal regions responsible for supporting DnaA self-association in the presence of ATPs, we prepared a suite of AaDnaA fragments and tested them by cross-linking (Fig. 3, A and B). Prior to glutaraldehyde exposure, we established that all of these constructs both eluted as monomers of the expected size by gel-filtration chromatography and remained soluble under our experimental conditions (not shown). Inspection of these reactions by SDS-PAGE shows that although most AaDnaA constructs exhibit a relatively low degree of interaction that can be trapped by cross-
linker treatment, a fragment consisting of both domains III and IV robustly assembles into an observable array of higher order species. Although we note that relative accessibility of reactive groups on different elements of a protein can influence the outcome of cross-linking studies, our data suggest that the AaDnaA DBD synergizes with the ATPase region to promote initiator assembly.

We next set out to determine which of our AaDnaA constructs could bind ssDNA using fluorescence polarization (Fig. 3C). Domain I proved unable to bind a labeled dT25 oligonucleotide in the presence of ADP-BeF3. Domains III and IV show some ability to interact with the substrate on their own; however, their binding is significantly weaker than full-length AaDnaA and for domain IV, it also is nonsaturable. In the end, only a fragment containing both domains III and IV could bind ssDNA comparably to wild-type protein. As with our observations for intact AaDnaA, these findings indicate that there is a correlation between the nucleotide-dependent assembly state of domains III/IV and their ability to engage single-stranded substrates.
Intersubunit Cross-talk Controls Initiator Assembly and ssDNA Binding—The AAA+ domain of DnaA contains the nucleotide-binding center of the initiator (27, 29, 37), forms the largest interaction surface between protomers in the DnaA assembly (30), and possesses amino acids important for binding ssDNA (26). We were thus surprised to discover that this domain on its own could neither significantly self-assemble nor interact strongly with single-stranded substrates in the presence of the ATP mimetic ADP-BeF$_3$. We therefore reexamined the available AaDnaA structures for possible clues as to why the DBD might affect DnaA assembly and ssDNA association. Interestingly, this analysis revealed that in the oligomeric DnaA model, the DBD orients toward the filament interior, docking against the AAA+ domain of an adjacent protomer to sequester ~26% of the total buried surface area in the assembly (Fig. 4A and supplemental Fig. 1A) (30). This conformation is preserved in all four protomers in the asymmetric unit and is unimpeded by crystal packing contacts, suggesting that it is a natural structural state that might stabilize the DnaA assembly directly.

To test this idea, we made a large panel of amino acid substitutions throughout the protomer/protomer interface and assessed the ability of the altered proteins to assemble using cross-linking (Fig. 4B and supplemental Fig. S2). We divided the interface into three interaction regions for this analysis: AAA+/AAA+, DBD/AAA+, and DBD/DBD. As expected, certain mutations in the AAA+/AAA+ interface disrupt assembly, causing up to an 80% reduction in trimer and tetramer formation. Among these is an arginine finger mutant (R230A), which is known to abolish oriC melting by EcDnaA (38), as well as a G281Q substitution that has not been studied previously. Significantly, several amino acid changes to the DBD/AAA+ interface (e.g., S350D and K219D) also disrupt assembly. By contrast, mutations in the small DBD/DBD interface have relatively little effect on self-association.

We next tested whether the integrity of the DBD/AAA+ interface is important for binding ssDNA (Fig. 4C and supplemental Table S2). Although assembly and ssDNA-binding propensities do not always track together perfectly, those...
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To confirm that residues identified in the DBD/AAA+ interface interfere with origin melting directly, we next tested Ser\textsuperscript{421} and Asp\textsuperscript{280} substitution variants in an oriC unwinding assay, using the P1 nuclease as a probe for DUE opening (16, 41) (Fig. 5B). Consistent with the observed loss of activity in vivo, mutations at Ser\textsuperscript{421} severely impair or abolish DNA unwinding. Likewise, whereas EcDnaA-D280A retains more than 50% of the wild-type level of origin melting capacity, D280K and D280Y show significantly less activity. To exclude the possibility that these mutations disrupt origin melting simply because they interfere with the recognition of duplex DNA-binding sites in oriC, we tested the ability of our EcDnaA mutants to interact with a fluorescein-labeled dsDNA 13-mer containing an R1 box using fluorescence polarization (supplemental Fig. S4). All mutants bind dsDNA within error of wild-type, exhibiting $K_{\text{d,app}}$ values in good agreement with previous studies (42) (supplemental Table S4).

Single- and Double-stranded DNA Binding Modes Rely on Distinct DnaA States—Thus far, our studies show that protomer DBD/AAA+ interactions formed during the ATP-dependent self-association of DnaA are critical for productive initiation events. However, inspection of the DnaA oligomer structure also shows that these self-same contacts bury the helix-turn-helix element of the DBD required for association with duplex DNA-binding sites in oriC (30, 42, 43) (Fig. 6A). A straightforward way to resolve this paradox is to posit that the DDBD undergoes a positional rearrangement from the state seen in the crystal structure to one that can bind dsDNA (30). One movement capable of exposing the helix-turn-helix motif is a simple, outward rigid-body rotation of the DDBD about the linker helix that connects the DDBD to the AAA+ module (Fig. 6B). A structural transition of this nature has been seen previously in the ADP state of AaDnaA (supplemental Fig. 51B) (29); however, this extended conformation also disrupts the DDBD/AAA+ interface that our data establish as critical for several DnaA activities.

This line of reasoning suggested to us that the necessity for DBD/AAA+ contacts in supporting particular DnaA activities could be probed by using short duplex substrates as exogenous competitors for the protein/protein interaction surface. To test this idea, we used cross-linking to assess the ability of EcDnaA to self-assemble in the absence of DNA and in the presence of a ssDUE fragment either with, or without, a duplex R1 box substrate that is recognized and bound with high affinity by the DDBD (42–45). Although the cross-linking signal for the EcDnaA protein is not as robust as for AaDnaA, higher order species consistent with oligomerization are nonetheless clearly observable (Fig. 7A). Moreover, assembly is notably enhanced when EcDnaA forms a complex with the ssDUE fragment and is in
turn dampened by the presence of the R1 box substrate (Fig. 7B and supplemental Table S3).

As an orthogonal test, we used a similar strategy to see whether short duplex DNAs could interfere with ssDNA binding by directly sequestering the DBD. We first prepared complexes of EcDnaA and a fluorescein-labeled ssDUE fragment in the presence of ATP (supplemental Table S3). Preliminary studies revealed that ATP-bound EcDnaA binds this fragment with a $K_{\text{d,app}}$ of $\approx 85$ nM (supplemental Fig. S5), so we used high protein concentrations (400 nM) in our competition assay to ensure complete complex formation. We then added increasing amounts of two unlabeled duplex DNA 13-mers, one containing a strong DnaA-binding site (the R1 box substrate), and the other containing a random sequence (Fig. 7C and supplemental Table S3). Both DNAs interfere with ssDNA binding, but the R1 dsDNA fragment is $\approx 10$-fold more effective in doing so than random DNA, indicating that the effect is specific for the DBD ($K_{\text{d,app}}$ of 230 nM versus 2100 nM for a 50% reduction in complex formation). Together, these findings are consistent not only with the idea that DBD/AAA interactions are important for assembly and interacting with single-stranded substrates, but that the DBD can transition between distinct conformational states with respect to the oligomerized ATPase core depending on the DNA binding status of the initiator.

**DISCUSSION**

**Origin Melting by DnaA**—Despite extensive study, the mechanisms by which cellular initiators process origins and facilitate replisome assembly have remained obscure. To understand the dynamics of some of these events during initiation in bacteria, we developed a series of assays that connect distinct initiator conformations to key oligomeric and functional states. In particular, we uncovered a new mode of intersubunit communication between the DBD and AAA domain of DnaA that is a critical determinant of initiator assembly, ssDNA binding, origin melting, and *in vivo* activity. Because the conformation of the DBD as observed in the DnaA oligomer structure is required for this cross-talk, but is not compatible with engaging duplex origin sites, we conclude that at least two ATP-assembled DnaA conformations are employed during initiation.

How might these physical states work together to promote replication onset? During initiation, DnaA progressively renders *oriC* competent for replisome formation in at least three stages: (i) continual occupancy of high affinity dsDNA sites (9); (ii) cell cycle-dependent binding of additional low affinity duplex sites, along with the formation of a higher order nucleoprotein complex (10, 12–15, 46); and (iii) a melted state in which the DUE has been opened, likely through direct ssDNA contacts with the initiator (11, 16, 18). During the first two stages, we propose that the DBD extends away from the body of the initiator to expose its helix-turn-helix motif for engaging high and low affinity duplex sites, along with the formation of a higher order nucleoprotein complex (10, 12–15, 46); and (ii) cell cycle-dependent binding of additional low affinity duplex sites, along with the formation of a higher order nucleoprotein complex (10, 12–15, 46); and (iii) a melted state in which the DUE has been opened, likely through direct ssDNA contacts with the initiator (11, 16, 18). During the first two stages, we propose that the DBD extends away from the body of the initiator to expose its helix-turn-helix motif for engaging high and low affinity duplex DNA sites in *oriC* (Fig. 8). At this point, the stability of the initiator assembly likely would be compromised to some degree by the loss of DBD/AAA interactions but rescued by the close proximity and phasing of duplex DnaA-binding sites, which would serve to increase the local concentration of the initiator on DNA and assist protomer association. Such behavior would help explain why ATP pro-
motes the binding of DnaA to weak dsDNA sites (10, 12, 46, 47), in that subunits situated at strong oriC loci could serve as anchor points for recruiting additional subunits to less favorable sites through nucleotide-dependent, ATPase domain contacts. Engagement of the melted DUE would then be accomplished by a different oligomer state in which the free DBD docks against a partner AAA+ domain in trans to stabilize the DnaA assembly, rendering it competent to bind ssDNA. Although our data do not reveal whether the DUE is actively melted by such dynamics or captured as it spontaneously breathes from superhelical tension, they do suggest that a conformational switch within DnaA, from an extended to a compact conformation, may help regulate its function. We speculate that these interconversions may underlie two distinct “initial” and “open” DnaA-dependent nucleoprotein complexes that appear to form during origin recognition and melting (10, 15, 16).

Implications for Other Initiator Systems—It has long been established that all cellular initiators, as well as the initiators of certain viruses, are controlled by nucleotide turnover. In each of these systems, AAA+ proteins form the nexus of the initiation machinery (7). For DnaA and systems like the eukaryotic Origin Recognition Complex (ORC), both of which belong to the same AAA+ subgroup, the conformation, assembly, and origin recognition properties of the initiator all can be altered by ATP (17, 31, 48–51). For the more distantly related SFIII helicases, a group of AAA+ helicases that serve as initiators for certain dsDNA viruses, ATP is required for DNA melting as it is for DnaA (52–56). How nucleotide elicits these various responses is a highly active area of debate.

Combined with prior studies, our work highlights a previously unsuspected structural transition in DnaA that links DNA-binding status with the ATP-dependent control of initiator oligomerization. This behavior is reminiscent of ORC, whereby the specific binding of origins is known to regulate initiator ATPase activity (48, 57). Given the phylogenetic kinship between cellular initiators, it seems likely
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that allosteric control mechanisms analogous to those observed here for DnaA broadly underpin the assembly and function of each of these AAA+ systems. However, functional congruencies between initiator families need not be restricted solely to the ATPase domain. For instance, the DBD of DnaA is required for bacterial replication because of its origin localization properties (28, 42, 43), yet we find that this domain also participates in subunit oligomerization and DNA melting. This auxiliary role has parallels with the SFIII proteins of papillomavirus and SV40 (7, 58), which localize to replication origins through sequence-specific DBDs (59, 60) and then assemble into hexameric rings that processively unwind the viral genome (52–54, 61). During this process, the DBDs of the viral initiators transition from an initial duplex-bound state to a final configuration in which they dissociate from their dsDNA-binding sites to assist with both initiator assembly and the engagement of ssDNA. Future studies will be needed to define these themes further and to determine the extent to which the molecular mechanisms of these evolutionarily connected initiation systems are both similar and distinct.

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