Protein-induced DNA linking number change by sequence-specific DNA binding proteins and its biological effects

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Abstract Sequence-specific DNA-binding proteins play essential roles in many fundamental biological events such as DNA replication, recombination, and transcription. One common feature of sequence-specific DNA-binding proteins is to introduce structural changes to their DNA recognition sites including DNA-bending and DNA linking number change (ΔLk). In this article, I review recent progress in studying protein-induced ΔLk by several sequence-specific DNA-binding proteins, such as *E. coli* cAMP receptor protein (CRP) and lactose repressor (LacI). It was demonstrated recently that protein-induced ΔLk is an intrinsic property for sequence-specific DNA-binding proteins and does not correlate to protein-induced other structural changes, such as DNA bending. For instance, although CRP bends its DNA recognition site by 90°, it was not able to introduce a ΔLk to it. However, LacI was able to simultaneously bend and introduce a ΔLk to its DNA binding sites. Intriguingly, LacI also constrained superhelicity within LacI–*lac OI* complexes if (–) supercoiled DNA templates were provided. I also discuss how protein-induced ΔLk help sequence-specific DNA-binding proteins regulate their biological functions. For example, it was shown recently that LacI utilizes the constrained superhelicity (ΔLk) in LacI–*lac OI* complexes and serves as a topological barrier to constrain free, unconstrained (–) supercoils within the 401-bp DNA loop. These constrained (–) supercoils enhance LacI's binding affinity and therefore the repression of the *lac* promoter. Other biological functions

include how DNA replication initiators λO and DnaA use the induced ΔLk to open/melt bacterial DNA replication origins.

Keywords DNA linking number change (ΔLk) · *lac* repressor (LacI) · cAMP receptor protein (CRP) · λO · DNA-bending · DNA topological barrier

Sequence-specific DNA-binding proteins play critical roles in many essential biological events, such as transcription, DNA replication and recombination. Very often, these sequence-specific DNA-binding proteins cause DNA structural changes to their recognition sites, including bending, looping, winding or unwinding, wrapping and other distortions (Bates and Maxwell 2005; White et al. 1992). Among these DNA structural changes, protein-induced DNA bending has been extensively studied using different biochemical and biophysical techniques (Chen et al. 2010b; Crothers et al. 1991; Kim et al. 1989; Leng 2013; Maher 1998; Wu and Crothers 1984; Zinkel and Crothers 1990). For instance, the *E. coli* cAMP receptor protein (CRP) or catabolite activator protein (CAP) sharply bends its binding sites (Chen et al. 2010b; Gartenberg and Crothers 1988; Gaston et al. 1992; Kim et al. 1989; Passner and Steitz 1997; Schultz et al. 1991; Zinkel and Crothers 1990). The 90° DNA bend stems from two ~45° DNA kinks by the dimeric DNA-binding protein (Schultz et al. 1991). Another well-studied DNA-bending protein is the *E. coli* lactose repressor or inhibitor (LacI) (Bell and Lewis 2000; Kercher et al. 1997; Kim et al. 1989; Lewis 2011; Lewis et al. 1996; Matthews 1996; Matthews and Nichols 1998; Zwieb et al. 1989). This tetrameric protein causes a 40–60° DNA bend to its binding site (Kim et al. 1989; Lewis et al. 1996). It also forms a DNA loop upon binding to two of its natural operators, i.e., *lac OI*, *O2* and *O3* (Becker

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et al. 2014; Eismann and Muller-Hill 1990; Kramer et al. 1988; Matthews 1992; Mossing and Record 1986; Muller-Hill 1998; Oehler et al. 1990; Saiz et al. 2005; Swigon et al. 2006; Wu and Liu 1991). DNA bending and looping by LacI have been considered to play an essential role in the regulation of expression of the *lac* operon (Adhya 1989; Becker et al. 2005, 2007, 2013, 2014; Bond et al. 2010; Fulcrand et al. 2016b; Muller et al. 1996; Muller-Hill 1998; Oehler et al. 1990, 1994).

DNA-binding proteins also result in DNA unwinding or linking number changes (ΔLk) to their recognition sites. For example, there have been several attempts to determine LacI-mediated ΔLk (Douc-Rasy et al. 1989; Kim and Kim 1983; Wang et al. 1974), but the results were inconsistent due to using different approaches (Douc-Rasy et al. 1989; Kim and Kim 1983; Wang et al. 1974). Recently, we developed an experimental strategy to determine protein-induced ΔLk by sequence-specific DNA-binding proteins (Chen et al. 2010a). Our approach has been to construct plasmid DNA templates that carry many tandem copies of a DNA-binding protein recognition site for one sequence-specific DNA-binding protein. In this way, protein-induced ΔLk is greatly amplified and can be determined by agarose gel electrophoresis. Utilizing this method, we found that protein-induced ΔLk is an intrinsic property for the tested DNA-binding proteins. Further, we demonstrated that protein-induced ΔLk did not correlate to protein-induced DNA bending (Chen et al. 2010a). This result is not surprising because ΔLk is a topological property and DNA bending is a geometric property (Cozzarelli et al. 1990). These two properties should not correlate to each other. As pointed out by White et al. (Cozzarelli et al. 1990; White and Bauer 1986), linking number (Lk) can be described by two geometric terms, writhe (Wr) and twist (Tw) in the following equation: $Lk = Wr + Tw$ (Bates and Maxwell 2005; Cozzarelli et al. 1990; White et al. 1988). If DNA bending does not affect Tw , it should yield a change to Wr and also to Lk . In this case, protein-induced DNA bending should correlate to protein-induced ΔLk . However, DNA bending may cause a change to both Tw and Wr , which result in an overall small or zero change to Lk (Tw – Wr compensation). In this scenario, protein-induced DNA bending should not correlate to protein-induced ΔLk . For protein-induced ΔLk , White and coworkers further pointed out that ΔLk is the sum of two experimentally accessible, geometrical terms: the surface linking number (SLk) and the winding number (ϕ): $\Delta Lk = \Delta SLk + \Delta \phi$ (White et al. 1988, 1992). They showed that only ΔSLk is related to DNA bending (White et al. 1992). They also proposed an experimental procedure to determine protein-induced ΔLk and explained how ΔLk should be interpreted in turn of ΔSLk and $\Delta \phi$ (White et al. 1992). Our previously published work is an experimental extension of this theoretical work (Chen et al. 2010a; White et al. 1992) although similar procedures have been used to study protein-

induced DNA bending by other groups (Kahn and Crothers 1998; Lutter et al. 1996). In this article, we will review our current understanding about protein-induced ΔLk for several sequence-specific DNA binding proteins and discuss how protein-induced ΔLk affects different biological processes.

E. coli LacI

E. coli LacI is a paradigmatic transcriptional factor that controls the expression of *lacZYA* in the *lac* operon (Muller-Hill 1996; Lewis 2013). This tetrameric protein specifically binds to the *O1*, *O2* and *O3* operators of the *lac* operon and forms a DNA loop to repress transcription from the *lac* promoter (Oehler et al. 1990, 1994). The first attempt to measure LacI-mediated DNA unwinding was performed by Wang et al. (1974). Using membrane filter binding assay, they showed that LacI causes unwinding of the *lac O1* operator by 40°–90°, which corresponds to a ΔLk of -0.11 to -0.25 . Later, Kim and Kim utilized a plasmid DNA template carrying 15 *lac O1* operators and showed that each LacI unwinds 54.5° of the *lac O1* operator corresponding to a ΔLk of -0.15 (Kim and Kim 1983). However, the LacI concentration used in their DNA relaxation assays was too high and may cause nonspecific binding of LacI to the supercoiled DNA template (Kim and Kim 1983; fig. 2). Douc-Rasy et al. obtained a much smaller unwinding angle of LacI upon binding to the *lac O1* operator using a 722 bp DNA minicircle carrying only one *lac O1* operator in their gel mobility shift assays ($\sim 13^\circ$ to 16° corresponding to a ΔLk of -0.036 to -0.044) (Douc-Rasy et al. 1989).

Recently, we constructed several plasmid DNA templates that contain many tandem copies of *lac O1* operator (5'-AATTGTGAGCGGATAACAATT-3'). Plasmid pYZX46 is a plasmid DNA template that carries 19 *O1* operators and was used in our previously published results (Chen et al. 2010a). We utilized two methods, i.e. the ligation method and the topoisomerase I relaxation method, to determine the LacI-induced ΔLk . We also used subnanomolar concentrations of LacI to avoid nonspecific binding of LacI to the plasmid DNA template (Chen et al. 2010a). Our results showed that ΔLk determined by these two methods are different. The LacI-induced ΔLk was determined to be -0.081 ± 0.016 by the ligation method. In contrast, the LacI-induced ΔLk was determined to be -0.299 ± 0.015 by the topoisomerase I relaxation method. Since supercoiled DNA templates were used in the topoisomerase I relaxation method, these results demonstrated that LacI is able to keep certain superhelical energy within the LacI–DNA complexes (Chen et al. 2010a). Interestingly, our results showed that IPTG did not inhibit the LacI-induced ΔLk . In contrast, IPTG stimulated the LacI-induced ΔLk for the ligation method. Since LacI in the presence of IPTG is still able to bind to multiple tandem

copies of *lac* operators and form DNA loops as shown previously (Kramer et al. 1987, 1988), our results suggest that the DNA topology in the LacI–DNA complexes is different in the presence and absence of IPTG (Chen et al. 2010a).

In order to further study how LacI traps supercoils in the LacI–*lac OI* complexes, we constructed four plasmid DNA templates, pCB107, pCB108, pCB147, and pCB149 each containing 16 *OI* operators (Fulcrand et al. 2016b). Plasmids pCB107 and pCB147 carry 16 *OI* operators in one location. In contrast, plasmid pCB108 and pCB149 carry 16 *OI* operators equally distributed between two different locations. An additional difference between these plasmids is the space separating the neighboring *OI* operators and the phase match of the neighboring *OI* operators: 20 bp in pCB147 and pCB149, and 25 bp in pCB107 and pCB108. Because the *lac OI* operator is a 21-bp DNA sequence and the head-to-tail distance of the *lac OI* operators for pCB107 and pCB108 is 46 bp, the neighboring *OI* operators were cloned in opposite directions such that LacI cannot simultaneously bind to the neighboring *OI* sites. For pCB147 and pCB149 which have a 41-bp head-to-tail distance of the *lac OI* operators, each LacI tetramer simultaneously binds to the neighboring *OI* operators of these two DNA templates due to the location of these DNA-binding sites on the same side of the helix. We used two different DNA templates, (–) supercoiled and nicked DNA templates, to examine how LacI traps superhelical tension in the LacI–*lac OI* complexes (Fig. 1). Our results are summarized in Fig. 2 and Table 1. As expected, in the absence of IPTG, LacI was able to trap 4 (–) supercoils in the LacI–*lac OI* complexes for (–) supercoiled plasmids pCB107 and pCB108 as the starting DNA templates. In contrast, for the nicked DNA templates of pCB107 and pCB108, LacI was only able to trap 1 or 2 (–) supercoils to the LacI–*lac OI* complexes. These results suggest that LacI was able to constrain superhelical energy within the LacI–*lac OI* complexes, which is consistent with our previous results (Chen et al. 2010a). In the presence of IPTG, LacI was also able to trap 4–5 (–) supercoils within the tandem LacI–*lac OI* complexes. These results are also consistent with our previous results (Chen et al. 2010a).

For plasmids pCB147 and pCB149, due to the fact that the LacI tetramer is able to simultaneously bind to the neighboring *OI* operators, LacI did not trap significant amounts of (–) supercoils in the LacI–*lac OI* complexes (Fig. 2b, d; Table 1). For instance, in the absence of IPTG, LacI constrained only one (–) supercoil to LacI–*lac OI* complexes for the (–) supercoiled DNA templates (compare lane 2 to lane 1 of Fig. 2b, d; Table 1). LacI did not introduce any supercoils in the LacI–*lac OI* complexes on the nicked DNA templates (Table 1). Nevertheless, these results clearly demonstrated that LacI was able to constrain superhelicity within LacI–*lac OI* complexes if the (–) supercoiled DNA templates were provided (Table 1).

Fig. 1 An experimental procedure to determine DNA supercoils constrained within LacI–*lacOI* complexes. A DNA template, either (–) supercoiled or nicked, which contains recognition sites for the nicking endonucleases Nt.BbvCI and Nb.BtSI and 16 *lac OI* operators in one or two different locations, was used in these experiments. After *E. coli* LacI binds to the operators to form LacI–*lacOI* DNA-looping complexes, the DNA template was digested by Nt.BbvCI and Nb.BtSI. Two DNA nicks are formed to remove supercoils in both loops of the plasmid DNA templates. In this case, supercoils trapped in the LacI–*lacOI* complexes could be determined. A large excess of oligonucleotides containing Nt.BbvCI and Nb.BtSI recognition sites was then added to the reaction mixture to inhibit both enzymes' activities. After ligation by T4 DNA ligase and phenol extraction, the linking number change (ΔLk) of the DNA molecule was determined by gel electrophoresis

Based on our published results, we proposed a model to explain how DNA-looping proteins, such as LacI, bring two groups of the DNA-binding sites together to fold into a topologically-constrained nucleoprotein complex (Fig. 3a). Details of the model were provided in our previous publication (Chen et al. 2010a). This model was confirmed by our AFM imaging studies in which multiple LacI tetramers brought two groups of *lac OI* operators together and formed a topologically constrained filament along these two groups of tandem copies of *lac OI* operators (Fig. 3b; Leng et al. 2011). We believe that this is a general model for many different nucleoprotein complexes that constrain and utilize superhelicity for certain biological functions (Ding et al. 2014). For instance, constraining superhelicity within the LacI–*lacOI* complexes allows LacI to block supercoil diffusion and confines supercoils in a defined region (Leng et al. 2011). These LacI–*lacOI* nucleoprotein complexes behave as DNA topological barriers, block transcription-coupled DNA supercoiling (TCDS), and stimulate TCDS in the in vitro-defined protein systems (Fig. 4a; Leng and McMacken 2002).

Recently, we showed that LacI forms a topological barrier upon binding to the *OI* and *O2* operators of the *lac* promoter

Fig. 2 The LacI-mediated Δ Lk in the presence or absence of IPTG. The experiments were performed as described in Fig. 1. Details of the experimental procedures have been published previously in Chen et al. (2010a). In addition to the DNA samples, the reaction mixtures also contained LacI, IPTG, Nt.BbvCI, and Nb.BtsI. The DNA molecules were isolated and subjected to agarose gel electrophoresis in the presence of 0.5 μ g/ml of chloroquine. The plasmid templates pCB107 (a), pCB147 (b), pCB108 (c), and pCB149 (d) are either supercoiled or nicked as indicated

Table 1 LacI-induced Δ Lk in the absence or presence of IPTG

Plasmid ^a	Head-to-tail distance (bp)	IPTG	Δ Lk	
			Sc ^b	Nk ^b
pCB107	46	–	-3.5 ± 0.2	-1 ± 0.1
pCB107	46	+	-3.8 ± 0.1	-3.7 ± 0.7
pCB147	41	–	-1.1 ± 0.3	0.3 ± 0.1
pCB147	41	+	0 ± 0.1	0.2 ± 0.1
pCB108	46	–	-3.8 ± 0.5	-2.3 ± 0.5
pCB108	46	+	-4.6 ± 0.6	-4.8 ± 0.5
pCB149	41	–	-11 ± 0.3	0.3 ± 0.1
pCB149	41	+	0 ± 0.1	0 ± 0.1

^a All plasmids contain 16 *lac OI* in one or two different locations

^b The initial topological status of the DNA templates is either (–) supercoiled (Sc) or nicked (Nk)

Fig. 3 a A proposed model to show how DNA-looping proteins induce a Δ Lk to their recognition sites. The blue circle and red cylinder, respectively, represent the DNA recognition sequence for a site-specific DNA binding protein and the DNA-looping protein. This model has been adapted from Chen et al. (2010a). b An AFM image to demonstrate that LacI divided a supercoiled DNA molecule, plasmid pCB109 into two independent topological domains: a relaxed and a supercoiled domain. This AFM image has been adapted from Leng et al. (2011)

at their native positions and constrains three negative supercoils within the 401-bp DNA loop (Fulcrand et al. 2016a, b). Our bacterial genetic data demonstrated that the LacI-constrained DNA supercoiling plays an important role in regulating the basal expression of the *lac* operon in *E. coli* under various growth conditions (Fulcrand et al. 2016b). It is likely that LacI functions as a topological barrier to constrain free, unconstrained (–) supercoils within the 401-bp DNA loop of the *lac* promoter. These constrained (–) supercoils enhance LacI's DNA-binding affinity and thereby the repression of the promoter. Figure 5 shows a molecular model of the physical interactions between LacI and *lac* operators that play essential roles in the formation of the DNA topological barrier with three constrained (–) supercoils (Fulcrand et al. 2016b). The (–) supercoils constrained within LacI–*lacO*I complex and the DNA loop are important for the *lac* operon in vivo.

E. coli CRP

E. coli CRP is a 45-kDa homodimer (Kolb et al. 1983) and a general transcription factor that regulates more than 378 promoters and 500 genes in *E. coli* (Shimada et al. 2011). Genes modulated by CRP are generally involved in energy metabolism (Busby and Ebright 1999; Shimada et al. 2011). In the absence of glucose, cAMP binds to CRP and converts CRP into an active form that binds to the regulation sites and activates transcription of different operons. For instance, the CRP–cAMP complex binds to the CRP binding site centered at position –61.5 bp upstream from the transcription site of the

Fig. 4 **a** LacI stimulates TCDS. The standard in vitro transcription/supercoiling (T-S) reaction (the TCDS assay) was performed as previously described (Leng and McMacken 2002). The DNA samples were analyzed by electrophoresis at 1 V/cm for 16 h in a 1 % agarose gel in 1× TAE buffer, pH 7.8, containing 5 µg/ml chloroquine to assess the final topological status of the plasmid DNA templates. The DNA template is pYZX7. The T-S reaction mixtures, as indicated at the top of the image, also contained 40 nM of LacI-WT (lanes 3 and 4), Gly58+1 (lanes 5 and 6), Gly60+1 (lanes 7 and 8), Gly60+2 (lanes 9 and 10), Gly60+3 (lanes 11 and 12), K84A/-11 (lanes 13 and 14), and -11 aa (lanes 15 and 16). Lanes 4, 6, 8, 10, 12, 14, and 16 also contained 1 mM IPTG. **b** Map of pYZX7 derived from pRLM375 (Leng and McMacken 2002). Plasmid pYZX7 was constructed by inserting a 158-bp synthetic oligomer carrying two *lac* O1 and P1 sites into the unique XhoI site of pRLM375. Large filled arrowhead promoter for T7 RNA polymerase (T7P); winged triangles Rho-independent *E. coli* *rrnB* T1 or plasmid pBR322 P4 transcription terminators; open circles *lac* O1 sites; closed circles *lac* P1 site (CRP-binding sites). **c** CRP did not stimulate TCDS. Plasmid DNA samples (pYZX7) were analyzed by agarose gel electrophoresis in chloroquine after serving as templates in T-S reactions performed as described previously (Leng and McMacken 2002). As specified, the T-S reactions also contained 0 nM (lane 1), 25 nM (lane 2), 50 nM (lane 3), 100 nM (lane 4), and 200 nM (lane 5) CRP, respectively. Untreated pYZX7 was applied to lane 6

lac operon (Shanblatt and Revzin 1986), and strongly activates transcription initiation from the *E. coli* *lac* promoter (Liu et al. 2003).

The *E. coli* CRP–cAMP complex recognizes a 22-bp symmetric DNA sequence with a consensus sequence of 5'-AAAGTGATCTAGATCACATTT-3' (Busby and Ebright 1999). Upon binding, it sharply bends the DNA sequence in the CRP–DNA complex with a bending angle of ~90° (Kim et al. 1989; Lewis et al. 1996). There have been several attempts to determine CRP-induced ΔLk (Doucy-Rasy et al. 1989; Gang 2004; Kahn and Crothers 1998; Kolb and Buc

Fig. 5 LacI tetramer simultaneously binds to O1 and O2 operators of the *lac* promoter and forms a topological barrier that constrains three (–) supercoils in the 401-bp DNA loop. This molecular model has been adapted from Fulcrand et al. (2016b)

1982; Lutter et al. 1996; McKay and Steitz 1981). In 1981, McKay and Steitz proposed that CRP–cAMP complex might significantly unwind the DNA binding site according to a crystal structure of the CRP–cAMP complex (McKay and Steitz 1981). However, Kolb and Buc used gel electrophoresis to determine the CRP-induced ΔLk to be ~–0.1, a much smaller value (Doucy-Rasy et al. 1989). Later, Doucy-Rasy et al. (1989) and Kahn and Crothers (1998) used different approaches to confirm that the CRP–cAMP complex did not greatly unwind its binding sites. In a separate study, Gang utilized DNA molar cyclization factor by using DNA fragment carrying one CRP recognition site and showed that the CRP–cAMP complex did not unwind DNA at all (Gang 2004). On the other hand, Lutter et al. demonstrated that CRP was able to induce a substantial ΔLk to its binding site using plasmids carrying many tandem copies of a CRP recognition sequence if the CRP binding sites match the helical phase of the DNA bends (Lutter et al. 1996).

Recently, we constructed two plasmid DNA templates, pCB51 and pCB55, each carrying 24 CRP recognition sites (Chen et al. 2010a; fig. 1). As discussed above, if the CRP–cAMP complex unwinds its recognition site, the unwinding angle or ΔLk will be greatly amplified. The difference between pCB51 and pCB55 is the head-to-tail distance of the neighboring CRP binding sites and the phase match of the neighboring CRP sites. Our results clearly demonstrated that the CRP–cAMP complex could not induce a large ΔLk to its recognition sites (Chen et al. 2010a). Interestingly, the CRP–cAMP complex was not able to form DNA topological barriers and significantly promote TCDS in our protein-defined transcription-supercoiling systems (Fig. 4c). These results suggest that protein-induced ΔLk might be required for the formation of DNA topological barriers.

Bacterial DNA replication initiators: λ O and DnaA

The faithful replication of chromosomal DNA is an essential event for all organisms, which includes initiation, elongation, and termination. In bacteria, DNA replication usually initiates from a defined single site on the chromosome; the origin of DNA replication (OriC; Leonard and Grimwade 2009; Leonard and Grimwade 2015; Magnan and Bates 2015; Rajewska et al. 2012; Wolanski et al. 2014). OriC is composed of an AT-rich DNA unwinding element (DUE) and binding sites for DnaA or DnaA-like proteins (Leonard and Grimwade 2015; Rajewska et al. 2012; Wolanski et al. 2014). A critical step of DNA replication initiation is to unwind or open DUE and load DnaB helicase, DnaG primase and other replication proteins (Leonard and Grimwade 2009, 2015; Mott and Berger 2007). For instance, λ O is the replication initiator of bacterial phage λ (Struble et al. 2007; Zahn and Blattner 1985) and specifically binds to the four repeating sequences (iterons) of λ replication origin (Ori λ) (Scherer 1978). Upon O binding, Ori λ wraps around O protein and forms a nucleoprotein complex called “O-some” (Dodson et al. 1986; Schnos et al. 1989). As a result, the AT-rich DUE is unwound and ready for subsequent replication initiation (Dodson et al. 1989; Schnos et al. 1988). Wrapping DNA around the O protein should cause DNA unwinding or a ΔLk in the replication origin. Indeed, our previously published results demonstrated that binding the O protein to each iteron causes an induced unwinding angle of 80° or ΔLk of -0.222 (Chen et al. 2010a). Interestingly, O-induced ΔLk is independent of the distance between each O-binding site (Chen et al. 2010a). This result suggests that the O protein only introduces a ΔLk to its binding site and does not introduce significant amounts of ΔLk to the DNA sequences between each iteron. In other words, the protein–protein interaction between each O dimer does not significantly contribute to the induced ΔLk (Chen et al. 2010a).

Bacterial DNA replication initiator DnaA is a monomeric protein (Erzberger et al. 2002) and a member of the AAA+ protein superfamily (Erzberger and Berger 2006). Upon ATP binding, the monomeric DnaA transitions into a large oligomeric DnaA–ATP filament, binds to the DnaA boxes of OriC, and remodels OriC for the subsequent replisome assembly (Erzberger et al. 2006). Experimental evidence has shown that DnaA–ATP complexes allow OriC DNA wrapping around the right-handed DnaA filament (Erzberger et al. 2006) and stabilizes (+) DNA supercoils. This result suggests that the (+) supercoils around the DnaA filament may generate compensatory (–) writhe that could help open the neighboring DUE (Erzberger et al. 2006). However, recent studies have demonstrated that DnaA-mediated DNA wrapping serves as a nucleation site and directly melts DUE of OriC (Duderstadt and Berger 2013; Duderstadt et al. 2011). Nevertheless, DnaA-mediated DNA wrapping (winding or unwinding/ ΔLk) is critical for the opening of DUE (Duderstadt et al. 2011). Other factors such as (–) supercoiling and HU may also help melt/open DUE of OriC (Hwang and Kornberg 1992; Magnan and Bates 2015).

One intriguing feature of bacterial phage λ DNA replication is that the initiation of Ori λ in vivo and in vitro is strongly dependent on transcription at or near Ori λ (Chung 1996; Furth et al. 1982; Hase et al. 1989; Mensa-Wilmot et al. 1989). Our recent results showed that transcription-coupled DNA supercoiling is responsible for the activation of λ DNA replication (Leng et al. 2011). Specifically, the “O-some” (26) functioning as a DNA topological barrier (Leng et al. 2011) blocks, confines, and captures transcription-coupled DNA supercoiling, which unwinds DUE of Ori λ (30) and initiates DNA replication (Chung 1996). Transcription/promoters are also found near bacterial DNA replication origins (Leonard and Grimwade 2015; Wolanski et al. 2014). In many cases, promoters direct transcription away from DUE of bacterial origins (Leonard and Grimwade 2015; Wolanski et al. 2014). It is possible that transcription-coupled DNA supercoiling greatly facilitates opening/melting DUE of bacterial replication origins.

E. coli RNA polymerase

E. coli RNA polymerase holoenzyme $\sigma 70$ is a sequence-specific DNA binding protein and specifically recognizes the -35 and -10 sequences of *E. coli* promoters to form a closed complex and then an open complex (Harley and Reynolds 1987; Ruff et al. 2015). One critical event for RNA polymerases is to open or unwind the DNA molecule to initiate RNA synthesis de novo (Fisher 1982; Zuo and Steitz 2015). Early studies showed that *E. coli* RNA polymerase, upon binding to promoters, cause a 120 – 140° of DNA unwinding corresponding to a ΔLk of -0.33 to 0.39 per RNA polymerase molecule

(Saucier and Wang 1972; Wang et al. 1977). A more systematic study showed that *E. coli* RNA polymerase was able to unwind the DNA template $\sim 580^\circ \pm 30^\circ$ (ΔLk of -1.61 ± 0.08) for the initiation and elongation phases (Gamper and Hearst 1982). This unwinding angle corresponds to a 17 ± 1 -bp transcription bubble (Gamper and Hearst 1982) that is slightly larger than the 12- to 14-bp transcription bubble observed in various crystal structures (Bae et al. 2013; Murakami 2013; Zuo and Steitz 2015). Similar unwinding angles were obtained for *E. coli* RNA polymerases binding to promoters in other studies (Amouyal and Buc 1987; Bertrand-Burggraf et al. 1984; Chan et al. 1990; Schickor et al. 1990; Siebenlist 1979).

Other sequence-specific DNA-binding proteins

E. coli galactose repressor (GalR), a 74-kDa protein dimer, is a transcriptional factor that regulates the expression of galETKM in the *gal* operon (Majumdar et al. 1987; Weickert and Adhya 1993). It specifically binds to the O_E and O_I operators of the promoter region to inhibit transcription from two *gal* promoters P1 and P2 (Lewis and Adhya 2002). With the help of HU and (–) supercoiling, GalR is able to form a DNA loop upon binding to the O_E and O_I operators (Geanacopoulos et al. 2001; Lewis et al. 1999; Lia et al. 2003; Lyubchenko et al. 1997). Utilizing plasmid containing 18 tandem copies of O_E sites and methods described in Fig. 1, GalR-induced ΔLk was determined to be ~ -0.169 when relaxed; nicked DNA templates were used in the assay (Chen et al. 2010a). Interestingly, for plasmid pCB42 that contains 18 copies of O_E sites with a 20-bp space between the neighboring O_E sites, GalR-induced ΔLk was determined to be ~ -0.285 when (–) supercoiled DNA templates were used (Chen et al. 2010a). These results suggest that, similar to LacI, GalR is able to constrain superhelical energy within the GalR–*gal* O_E complexes. As expected, GalR failed to induce a ΔLk to its DNA templates when its inducer, D-galactose, was present (Chen et al. 2010a).

E. coli AraC, a 65-kDa homodimer, is a transcriptional factor that regulates the expression of araBAD of the L-arabinose operon (Carra and Schleif 1993; Schleif 2000). This protein has unique DNA-binding properties (Schleif 2000). In the absence of L-arabinose, AraC binds in trans to the I_1 and O_2 half-site of the L-arabinose operon in the promoter region and forms a DNA loop to inhibit transcription from the P_{BAD} promoter (Dunn et al. 1984; Lobell and Schleif 1990; Schleif 2000). However, in the presence of L-arabinose, AraC still tightly binds in cis to the neighboring I_1 and I_2 half-sites of the P_{BAD} promoter and does not form a DNA loop. As a result, it stimulates transcription from the P_{BAD} promoter (Dunn et al. 1984; Lobell and Schleif 1990; Schleif 2000). For both DNA-binding events, AraC sharply bends the DNA templates (Saviola et al. 1998). Using two unique

plasmid DNA templates pCB36 and pCB42 that carry 7 and 14 araI sites, we found that AraC-mediated DNA looping is required for AraC to induce a ΔLk to the DNA template (Chen et al. 2010a). For instance, AraC did not induce a ΔLk to pCB36 that contains araI sites in one location; it did not induce a ΔLk to plasmid pCB42 that contains 14 araI sites in two different locations in the presence of L-arabinose. Nevertheless, in the absence of L-arabinose, AraC introduced ~ 5 (–) supercoils to plasmid pCB42, which yielded a ΔLk of -0.333 per araI site (Chen et al. 2010a).

The restriction endonuclease EcoRI is another protein that causes DNA unwinding upon binding to its recognition site, 5'-GAATTC-3'. Using a plasmid DNA template that carries 18 EcoRI recognition site, Kim et al. determined the EcoRI-induced DNA unwinding angle to be 25° or ΔLk of -0.069 (Kim et al. 1984). Douc-Rasy used DNA minicircles and determined the EcoRI-induced unwinding angle to be $23 \pm 3^\circ$, which is consistent with the result of Kim et al. (Douc-Rasy et al. 1989). This small unwinding angle or ΔLk was confirmed by a crystal structural study (Frederick et al. 1984). Interestingly, RsrI, an isoschizomer of EcoRI also unwinds the recognition site of 25° (Aiken et al. 1991).

It was found that several eukaryotic Zinc Finger DNA-binding (ZFDB) proteins, such as Sp1, Sp1C, and ZF-QQR, were also able to unwind their DNA recognition sites (Shi and Berg 1996). Shi and Berg constructed several plasmid DNA templates that carry many tandem copies of a binding site for a ZFDB (Shi and Berg 1996). DNA unwinding assays similar to those methods described in Fig. 1 were used to determine ZFDB-induced DNA unwinding angles. The DNA unwinding angles for Sp1, Sp1C, and ZF-QQ were determined to be 17.8° (ΔLk of -0.049), 17.5° (ΔLk of -0.049), and 22.6° (ΔLk of -0.063), respectively. These ZFDB-induced ΔLk are consistent with the crystal structures of ZFDB–DNA complexes in which the DNA molecules are underwound (Shi and Berg 1996). Interestingly, these ZFDBs did not significantly bend their DNA recognition sequences (Shi and Berg 1996). According to these results, it was concluded that these ZFDBs induced the DNA unwinding rather than passively binding to the preexisting underwound DNA. It is also suggested that the ZFDB-induced DNA deformation is critical for their site specificity and overall DNA binding affinity (Shi and Berg 1996).

Conclusions and biological effects

Compared with other protein-induced DNA structural changes, such as bending and looping, protein-induced ΔLk is a “rarely” studied area for sequence-specific

DNA-binding proteins (Chen et al. 2010a). Since each DNA-binding protein may only induce a small ΔLk to their recognition sites, the best approach of studying protein-induced ΔLk is to construct plasmid DNA templates containing multiple tandem copies of a DNA-binding site for a sequence-specific DNA-binding protein. In this way, the protein-induced ΔLk should be greatly amplified. Nevertheless, constructing plasmid DNA templates carrying multiple tandem copies of a DNA-binding site is a time-consuming process, although the cloning strategy is straightforward. Additionally, a nicking endonuclease recognition site has to be cloned to the plasmid DNA templates in order to use the methods described in Fig. 1 to determine the protein-induced ΔLk . This lengthy cloning procedure at least partially contributes to the fact that protein-induced ΔLk has not been extensively studied. The commercial availability of different nicking endonucleases, however, makes this effort a little easier. An alternative strategy to study protein-induced ΔLk is to solve crystal structures of protein–DNA complexes. Indeed, many available crystal structures of protein–DNA complexes show DNA unwinding or untwisting (Chen et al. 2013; Duderstadt et al. 2011; Frederick et al. 1984; Zuo and Steitz 2015). Nevertheless, whether the observed DNA unwinding/untwisting causes a ΔLk has to be confirmed by the methods described in Fig. 1.

One important question for protein-induced ΔLk is why certain sequence-specific DNA binding proteins cause ΔLk to their DNA recognition sites. In other words, what are the biological effects of protein-induced ΔLk ? For most organisms, DNA is typically negatively supercoiled (Bates and Maxwell 2005). Free energy constrained in negative superhelicity greatly facilitates a number of essential DNA processes, such as DNA replication, recombination, and transcription in which sequence-specific DNA-binding proteins play a critical role (Champoux 2001; Cozzarelli and Wang 1990; Kornberg and Baker 1992; Wang 1996). It would be “wise” for certain DNA-binding proteins to recruit the free energy constrained in the negatively supercoiled DNA for different DNA transactions. For example, LacI may have evolved to utilize negative supercoils and help *E. coli* adapt rapidly to different environmental conditions (Fulcrand et al. 2016b). When the main carbon source, glucose, is abundant, *E. coli* cells are capable of generating sufficient amounts of ATP for their normal cellular functions. In this scenario, DNA gyrase drives the chromosomal DNA to more (–) supercoiled status (Jensen et al. 1995; van Workum et al. 1996). The excess negative supercoils constrained by LacI promote the formation of a highly stable repressosome that prevents the wasteful expression of *lacZYA*. However, when *E. coli* cells live in nutrient-deficient environments, the ATP/ADP ratio is

low, which significantly reduces the supercoiling activities of DNA gyrase (Hsieh et al. 1991; Westerhoff et al. 1988). In this case, the DNA around the *lac* promoter is relaxed (Jensen et al. 1995; van Workum et al. 1996), which significantly weakens the binding of LacI to the *lac* operators and therefore increases the basal level expression of *lacZYA*. This prepares the *E. coli* cells to respond rapidly to the presence of other carbon sources, such as lactose. In this way, a certain amount of lactose is transported inside the cells by lactose permease and converted to allolactose by β -galactosidase, the natural inducer of the *lac* operon to fully activate transcription of *lacZYA* of the *lac* operon. Other transcriptional factors may also use similar mechanisms to regulate their gene transcriptions.

A second biological effect of protein-induced ΔLk is to open/melt the DNA double helix for different biological process, such as DNA replication initiation. As discussed above, DnaA and λ O utilize the protein-induced ΔLk to open/melt the AT-rich DUE of replication origins to initiate DNA replication (Leonard and Grimwade 2009, 2015; Magnan and Bates 2015; Rajewska et al. 2012; Wolanski et al. 2014). Additionally, the nucleoprotein complexes by these replication initiators functioning as a DNA topological barrier (Leng et al. 2004, 2011; Leng and McMacken 2002) block, confine, and capture transcription-coupled DNA supercoiling, which helps open/melt DUE of replication origins and facilitates DNA replication initiation (Chung 1996). Another function of protein-induced ΔLk is to ensure binding specificity of a sequence-specific DNA binding protein. Upon binding, the DNA-binding protein unwinds or distorts the DNA sequence and brings different groups together to make further contacts between the DNA sequence and the protein. The binding free energy gained from the proper alignments between the protein and DNA-binding site should compensate the free energy loss by protein-induced unwinding/distortion and ensure binding specificity. A restriction enzyme such as EcoRI is an example for such protein–DNA interactions (Frederick et al. 1984).

Another potential effect of protein-induced ΔLk is to help the recognition of target DNA sequences by certain nucleoprotein complexes, such as CRISPR–Cas9 ((Gasiunas et al. 2012; Horvath and Barrangou 2010; Jinek et al. 2012; Sander and Joung 2014); CRISPR and Cas represent clustered regularly interspaced short palindromic repeats and CRISPR-associated protein, respectively). Recent crystal structural studies showed that the binding of CRISPR–Cas9 to its recognition sequences causes substantial DNA unwinding and forms an R-loop structure (Anders et al. 2014; Jiang et al. 2016; Nishimasu et al. 2014). It is likely that CRISPR–Cas9 induces a substantial ΔLk to its binding sites and negative supercoiling should help the binding of CRISPR–Cas9 to the target DNA sequences.

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Compliance with ethical standards

Conflicts of interest Fenfei Leng declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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