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DNA looping: the consequences and its control

Leonor Saiz and Jose MG Vilar

The formation of DNA loops by proteins and protein complexes is ubiquitous to many fundamental cellular processes, including transcription, recombination and replication. Recently, advances have been made in understanding the properties of DNA looping in its natural context and how they propagate to cellular behavior through gene regulation. The result of connecting the molecular properties of DNA looping with cellular physiology measurements indicates that looping of DNA *in vivo* is much more complex and easier than predicted from current models, and reveals a wealth of previously unappreciated details.

Addresses
Integrative Biological Modeling Laboratory, Computational Biology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 460, New York, NY 10021, USA

Corresponding author: Vilar, Jose MG (vilar@cbio.mskcc.org)

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Introduction

DNA looping is extensively involved in many cellular processes, such as transcription, recombination and replication [1–3,4••], enabling distal DNA regions to affect each other. It is especially prominent in the regulation of gene expression, wherein proteins bound far from the genes they control can be brought to the promoter region by looping the intervening DNA. The interplay between DNA looping and gene regulation was first identified in the *Escherichia coli ara* operon [5], although it was already suspected to occur in eukaryotic enhancers [6] and in prokaryotic transcription [7]. Since then, it has been identified in many other systems, such as the *gal*, *lac* and *deo* operons of *E. coli* [1,2], the lysogenic to lytic switch of phage λ [8] and the human β-goblin locus [9]. Recent examples show that it is present even in RXR (retinoid X receptor) [10] and p53 [11], two proteins widely implicated in cancer.

Full understanding of DNA looping integration in such a diversity of cellular processes requires quantitative approaches. A key quantity is the free energy of DNA looping, which determines how easily DNA can loop and therefore the extent to which distal DNA sites can affect each other [4••]. Through this quantity, DNA looping can easily be incorporated into thermodynamic models of the assembly of DNA–protein complexes that control different cellular processes. In this review, we discuss recent advances in the general understanding of the *in vivo* consequences of DNA looping and their implications for gene regulation. We consider first the *in vivo* molecular properties of the looping process, and examine their salient features, the differences compared with the *in vitro* data and the predictions of current elastic DNA models. We then sketch briefly the key thermodynamic concepts needed to develop quantitative models of DNA–protein complexes and explore the consequences of DNA looping for gene regulation.

Two types of DNA loops

DNA loops can be classified into two main categories with a fuzzy boundary: short or energetic (Figure 1a and b), and long or entropic (Figure 1c). This distinction comes from the physical forces that dominate their formation. For short loops, with lengths shorter than the DNA persistence length (~150 bp), the main determinant of looping is DNA elasticity. Thus, the bending and twisting of DNA, as well as the elastic properties of the molecules that tie the loop, play an important role. For long loops, in contrast, the limiting step is the erratic motion in the cell of the two DNA regions before they find each other. Thus, the main determinant is the lost of entropy that happens when two DNA regions are tied together.

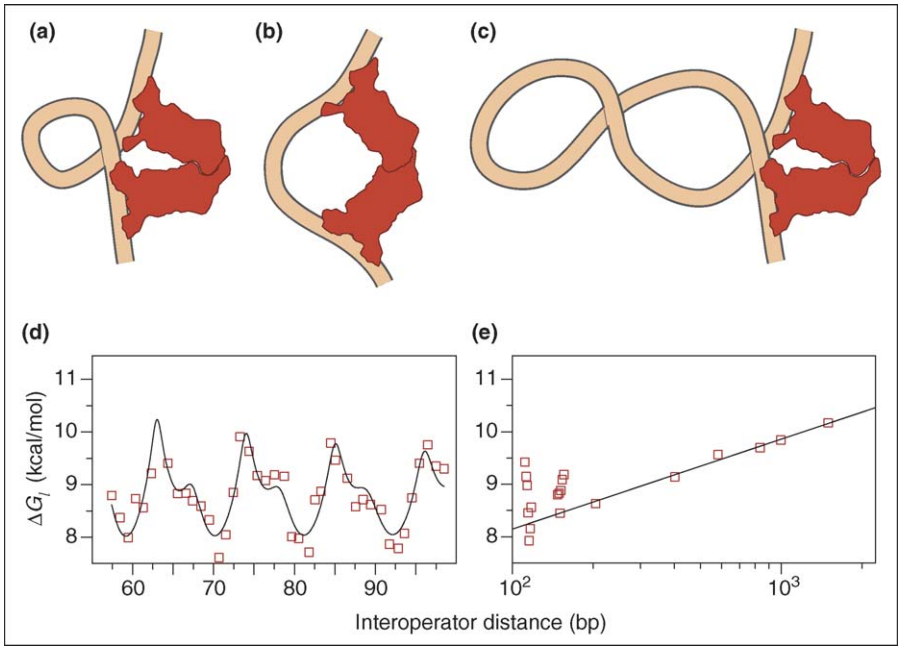
Current theories [12–14] and most *in vitro* experiments [14–16] indicate that the formation of short and long loops is extremely costly. And yet, short and long DNA loops are widely present *in vivo*. They can be as short as 60 bp in the *lac* operon [17] and 80 bp in nucleosome wrapping [18], and as long as 180 kb in mating type switching in yeast [19].

How does the intracellular environment mediate the formation of such loops? The first step to address this question is to obtain the properties of cellular components in their natural environment. The extreme complexity of the cell, however, poses a strong barrier to experimentally characterizing cellular components, not only because the properties of the components can change when studied *in vitro* but also because *in vivo* probing of the cell can perturb the process under study [20].

From cellular physiology to *in vivo* molecular properties

A combined computational and experimental approach has recently been used to infer the *in vivo* free energies of

Figure 1



Loop conformations and the *in vivo* free energy of DNA looping by the *lac* repressor. The bidentate *lac* repressor (shown in red) can loop DNA (orange thick line) in different ways: **(a)** short loop with repressor in a V-shape conformation; **(b)** short loop with repressor in an extended conformation; and **(c)** long loop with supercoiled DNA. The *in vivo* free energy of DNA looping [21••] as a function of the length of the loop for **(d)** short and **(e)** long loops has been obtained using a computational and experimental approach (red square symbols), as described in Saiz *et al*. [21••] (see text) based on the measured repression levels of Muller *et al*. [17]. For short loops, the black line represents the best fit to the looping free energy, ΔG_l, predicted by an elastic DNA model that considers the contributions of two loop conformations (Equations 1 and 2 of [28••]). The two alternative loop conformations of the *lac* repressor–DNA complex could involve two conformations of the *lac* repressor or two different binding motifs, as represented in the cartoons. For long loops, the black line represents the best fit using the theoretically predicted expression for an ideal flexible polymer: $1.24RT\ln(l) + 4.72$, where l is the length of the loop [4••].

DNA looping by the *lac* repressor [21••] from measurements of enzyme production in the *lac* operon [17] for different lengths of the loop. The key idea is to use a well-established mathematical model for the regulation of gene expression in the *lac* operon 'in reverse'. In this way, it is possible to go from the observed cellular behavior to the properties of the unperturbed cellular components. The free energy of looping by the *lac* repressor, under the specific experimental conditions analyzed using this approach [21••], follows from the concise expression:

$$\Delta G_l = -RT \ln \frac{R_{loop} - R_{noloop}}{R_{noloop} - 1}[N], \qquad (1)$$

wherewhere R_{loop} is the measured repression level, a dimensionless quantity used to quantify the extent of repression of a gene; R_{noloop} is the repression level in the absence of DNA looping; $[N]$ is the concentration of repressors; and RT is the gas constant times the absolute temperature ($RT \approx 0.6$ kcal/mol for typical experimental conditions). The results obtained differ markedly from the current *in vitro* view of DNA looping.

For short loops (Figure 1d), this analysis showed that the free energy of looping oscillates with the helical periodicity of DNA (~10.9 bp) as the length of the loop changes; this was expected because the two operators must have the right phase to bind simultaneously to the repressor. It also unexpectedly revealed that the free energy in a cycle behaves asymmetrically [21••]. This asymmetry is characterized by a second representative oscillatory component with a period of ~5.6 bp. Other striking features are that the amplitude of the oscillations is extremely small (~2.5 kcal/mol) and that the *in vivo* free energy does not seem to diverge for short loop lengths. These results indicate that the formation of *in vivo* DNA loops is much more complex and easier than expected from current theories, which predict symmetric oscillations that are, at least, twice as big [14,22].

For long loops (Figure 1e), the resulting *in vivo* free energy of looping nicely fits the theoretically predicted expression for a flexible polymer $\Delta G_{l_0} + \alpha RT \ln(l/l_0)$, where l is the length of the loop, l_0 is a reference length, and α is a constant [4••]. Intriguingly, theoretical estimates give $\alpha \approx 2.25$ [12,13], which is significantly different from the

inferred *in vivo* value $\alpha \approx 1.24$ ($1.24RT\ln(l) + 4.72$). This result is even more remarkable because the theoretical lower bound of this parameter for loop formation in three dimensions is $\alpha = 1.5$, the value for an ideal polymer without excluded volume effects. As in the case of short loops, the *in vivo* environment also seems to facilitate the formation of long DNA loops.

In vivo intricacies of DNA looping

The origin of the differences between predictions from continuum elastic models and the observed *in vivo* behavior remains far from being fully resolved. Recent structural and computational studies on DNA [18,23] indicate that the loop can be bent and twisted non-uniformly because of different contributions, such as, for instance, the anisotropic flexibility of DNA, local features resulting from the DNA sequence, and interactions with the *lac* repressor [24] and other DNA-binding proteins [25]. The formation of DNA loops is also tightly coupled to the molecular properties of the proteins and protein complexes that form the loop. Moreover, depending on the orientation of the two DNA binding sites and the properties of the looped DNA–protein complex, the DNA loop can be accomplished by following different trajectories [25–27].

Only very recently, it has become clear that the *in vivo* behavior of short loops (Figure 1d) can be accurately accounted for by the simultaneous presence of two distinct conformations of the looped DNA–protein complex [28••]. These two conformations have different bending and torsional properties. As the length of the loop changes, the less stable conformation becomes the most stable one. This alternating pattern is repeated periodically and different loop conformations are adopted to select the DNA configuration with the minimum free energy. It is also possible to use the formula for the free energy as a function of the repression level (Equation 1) with data from different mutants [29•] to infer the effects of key architectural proteins on DNA. When the HU protein, which helps bend DNA, is absent from the cell, the free energy of DNA looping increases and the oscillations become symmetric [28••]. Such facilitated bending is also present in eukaryotes, where nucleosomes in chromatin effectively increase the flexibility of DNA at short distances by a factor of two compared to naked DNA *in vitro* [30]. In all cases studied in [28••], two wild-type-like and one mutant strain, the contributions of at least two conformations are present.

The properties obtained by fitting the inferred *in vivo* data [28••] to an elastic model with two conformations are consistent with those obtained using a recent theory of sequence-dependent DNA elasticity for the *lac* repressor–DNA complex [31••]. This computational approach and the inferred *in vivo* data together highlight the need for more detailed models of DNA looping. The inferred high versatility of looped DNA–protein complexes in establishing different conformations in the intracellular environment seems to underlie the unanticipated behavior of the *in vivo* free energy of DNA looping for short loops, and could be responsible not only for asymmetric oscillations with decreased amplitude but also for plateaus and secondary maxima (Figure 1d). All these features indicate that the physical properties of DNA can actively be selected in order to control the cooperative binding of regulatory proteins and achieve different cellular behaviors.

Two modes of DNA looping

The study of the induction switches of phage λ and the *lac* operon led to the discovery of gene regulation [8,32]. As it turned out, both systems rely on DNA looping [33–35]. They exemplify two main modes of DNA loop formation. In the *lac* operon, DNA looping is mediated by the simultaneous binding of two DNA-binding domains of a single repressor molecule to two DNA sites known as operators [36]. In phage λ, in contrast, the loop is not formed by a single protein but rather by a protein complex that is assembled on DNA when the loop forms [34].

These two modes of looping are present in many systems. For instance, induced cooperativity similar to that of phage λ is observed for RXR, a nuclear hormone receptor [10]. In its tetrameric form, RXR has two DNA-binding domains and can loop DNA to bring transcription factors close to the promoter region. Retinoic acid controls whether or not the loop is formed, by preventing the assembly of the tetrameric complex from the constituent dimers, which can also bind DNA. On the other hand, the E2 transactivator protein of bovine papilloma virus loops DNA using the same looping mode as the *lac* repressor [37]. Remarkably, if more than two binding sites are present on the same strand of DNA, E2 can even form multiple simultaneous loops, which are visible by electron microscopy [37].

In general, multiple proteins are assembled to form functional complexes on looped DNA. In eukaryotic transcription, for instance, multiple DNA binding sites that are spread over long distances are involved in controlling the same localized DNA events. DNA looping in this case enables multiple proteins to affect the RNA polymerase in the promoter region. Enhancers, silencers or mediators bound at distal DNA sites are then brought to form part of, affect or interfere with the transcriptional complex. Understanding this type of molecular complexity requires quantitative approaches that extend beyond prototypical chemical reactions in a well-stirred reactor [4••].

A quantitative approach to the control of DNA looping

DNA looping is typically controlled by the interaction of proteins with DNA to form dynamic nucleoprotein

complexes. The most widely used quantitative approaches to study DNA–protein complex assembly are based on thermodynamics [38]. Thermodynamic approaches enable the straightforward connection of the molecular properties of the system with the effects that propagate to the cellular physiology. Each configuration of the DNA–protein complex, s, has an associated free energy, $\Delta G(s)$, which is connected to the equilibrium probability, P_s, of such a configuration through the statistical interpretation of thermodynamics; namely, $P_s = (1/Z)e^{-\Delta G(s)/RT}$, where $Z = \sum_s e^{-\Delta G(s)/RT}$ is the normalization factor [38].

The key quantities necessary to understand the control of DNA looping are positional, interaction, and conformational free energies [4••]. The positional free energy, p, accounts for the cost of bringing one component to the protein–DNA complex, for instance, bringing the *lac* repressor to its DNA binding site. Its dependence on the component concentration, [N], is given by $p = p_0 - RT\ln[N]$, where p_0 is the positional free energy at 1 M. This type of dependence indicates that it is easier to bring a component to the complex if its concentration is higher. Interaction free energies, e, arise from physical contact between components (e.g. electrostatic interactions) and conformational free energies, c, account for changes in conformation (e.g. looped versus unlooped states). Typical values (in kcal/mol) for the *in vivo* DNA–*lac* repressor complex are $p \approx 26$, $e \approx -28$ and $c \approx 23$. Two key points are that the different contributions can be positive or negative, and that typically their absolute values are much larger than the thermal energy (≈0.6). By collecting all the contributions to the free energy, it is possible to infer the dominant conformation of the protein–DNA complex for each specific cellular condition; this corresponds to the conformation with the lowest free energy.

To illustrate these concepts in more detail, we consider the binding of the bidentate *lac* repressor to two operators, *O*1 and *O*2 (Figure 2a). The *lac* repressor–DNA complex can be in five representative states [39]: (i) none of the operators is occupied; (ii) a repressor is bound to just *O*2, the auxiliary operator; (iii) a repressor is bound to just *O*1, the main operator; (iv) a repressor is bound to both *O*1 and *O*2 by looping the intervening DNA; and (v) two repressors are bound, one to each operator. The free energies of each of these states are $\Delta G_i = 0$, $\Delta G_{ii} = p + e_2$, $\Delta G_{iii} = p + e_1$, $\Delta G_{iv} = p + e_1 + e_2 + c_L$ and $\Delta G_v = 2p + e_1 + e_2$, respectively. Here, the quantity p is the positional free energy of the repressor and incorporates the dependence on the repressor concentration [N]; e_1 and e_2 are the interaction free energies between the repressor and *O*1 and *O*2, respectively; and c_L is the conformational free energy of DNA looping ($c_L \equiv p_0 + \Delta G_l$).

These free energies can be used to derive the probabilities of the different states (Figure 2b). For instance, the looped state (iv) is more probable than the one-repressor unlooped state (iii) if $e_2 < -c_L$; that is to say, looping will be favored whenever establishing a second binding contact overcompensates the cost of looping the DNA. In this case, DNA looping increases the occupancy of the DNA binding sites. If $p > c_L$, the looped state (iv) is more probable than the two-repressor unlooped state (v). This inequality is remarkable because it also indicates that the looped state is not favored at sufficiently high repressor concentrations. Thus, the repressor is responsible for forming the loop at low to moderate concentrations, and for preventing it at high concentrations (Figure 2b).

Straightforward application of the standard thermodynamic approach [40] in a general framework is of limited use because the number of states that must be considered typically increases exponentially with the number of components. It has become clear recently that it is possible to overcome this limitation and express the free energy of all these states in a compact form using binary variables [41••]. In the case of the *lac* operon, this new approach leads to:

$$\Delta G(s) = (p + e_1)s_1 + (p + e_2)s_2 + (c_L - ps_1s_2)s_L, \qquad (2)$$

where s_1 and s_2 are binary variables that indicate whether ($s_i = 1$; for $i = 1,2$) or not ($s_i = 0$; for $i = 1,2$) the repressor is bound to *O*1 and *O*2, respectively; and s_L is a variable that indicates the conformational state of DNA, either looped ($s_L = 1$) or unlooped ($s_L = 0$). Thus, it is possible to write a global concise expression, instead of one for each of the five states, to specify the thermodynamic properties of the system. This expression can be used to compute different static and dynamic quantities without having to represent explicitly all the potential states [41••].

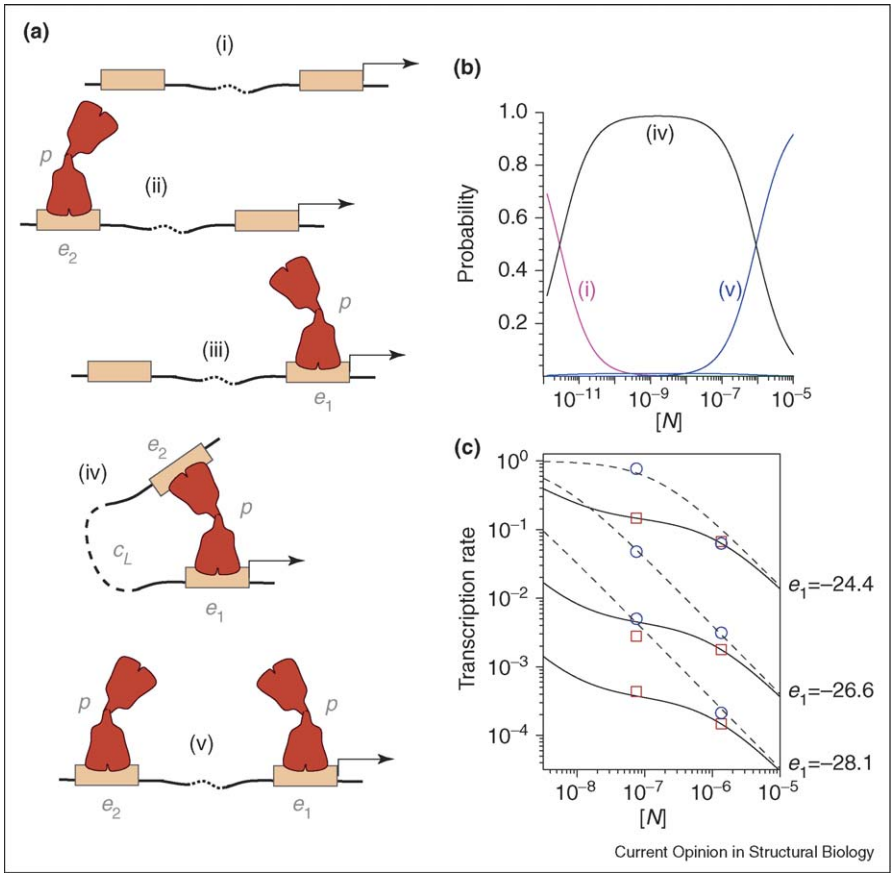
How fast is DNA loop formation?

The dynamic properties of DNA are also important in many processes, for instance, in controlling transcriptional noise [4••]. The relationship between kinetic and thermodynamic properties, known as the principle of detailed balance, can be exploited to infer the rate of loop formation, k_{loop} [39]. Assuming that the rate of dissociation of one repressor domain from DNA does not depend on whether the other domain is bound to DNA, it leads to:

$$k_{loop} = k_a e^{-\Delta G_l/RT}, \qquad (3)$$

where $k_a = 8.8 \times 10^7\ \mathrm{M^{-1}\ s^{-1}}$ is the association rate constant for the binding of the repressor to the operator, which, for $\Delta G_l = 8.4$ kcal/mol, results in $k_{loop} = 74\ \mathrm{s^{-1}}$ [39]. Thus, unlooped DNA with a repressor bound to one operator reloops within 10–20 ms. This timescale is similar to that of the wrapping of DNA around nucleosomes, wherein unwrapped DNA rewraps within ~10–50 ms [42••].

Figure 2



Relevant states of the *lac* repressor bound to two operators, their probabilities and their effect on transcription regulation. **(a)** The *lac* repressor–DNA complex has five representative states. The promoter (arrow), downstream of the main operator, is repressed when the *lac* repressor (shown in red) is bound to the main operator (states iii, iv and v) and unrepressed when the main operator is unoccupied (states i and ii). Binding to the auxiliary operator does not affect transcription. The black line represents DNA and the two *lac* operators are shown as orange boxes. Here, p is the positional free energy of the repressor, e_1 and e_2 are the interaction free energy between the repressor and the main and auxiliary operators, respectively; and $c_L \equiv p_0 + \Delta G_l$ is the conformational free energy of DNA looping. **(b)** The probability of the different states as a function of the repressor molar concentration [N] has been obtained using a statistical thermodynamics approach, as described in the text. The values used for the different contributions to the free energy (in kcal/mol) are $e_1 = -28.1$, $e_2 = -26.6$, $p = 15 - 0.6\ln[N]$ and $c_L = 23.35$. Only the states with relevant populations are labeled. The looped state (iv) is the most abundant, except at low and high repressor concentrations. **(c)** The normalized transcription rate as a function of the *lac* repressor concentration for one (blue circles and black dashed lines) and two (red squares and continuous black lines) operators shows excellent agreement with the available experimental data [45]. The computed values of the normalized transcription rate $\tau = (1/Z)\sum_s (1 - s_1)e^{-\Delta G(s)/RT}$ (lines) are compared with the experimental data (symbols) from [45] at two repressor concentrations for three different strengths of the main operator.

The effects of DNA looping

DNA looping has many obvious effects because of its role in mediating long-range interactions on DNA. It allows two, or more, DNA regions that are far apart to come close to each other, which is needed, for instance, to allow the transfer of genetic information that happens during recombination [19,43]. DNA loops are also used to tie the end of chromosomes and regulate the length of telomeres [44]. Beyond these systems, in which it is strictly required, DNA looping is also used to increase the strength of binding of regulatory molecules to their cognate sites. The thermodynamic approach we have discussed shows how such an increase is achieved for the *lac* operon, wherein the looped state is always more stable than both unlooped states with one repressor bound (Figure 2b). DNA looping also has other more subtle roles, which are strongly inter-related with the inherent stochastic nature of cellular processes.

Computational modeling of the *lac* operon [39], together with experimental data [45], suggests that DNA looping can be used to decrease the sensitivity of transcription to changes in the number of regulatory proteins. The transcription rate of the *lac* operon with DNA looping shows a plateau-like behavior, centered at 50 nM, which does not occur for just a single operator (Figure 2c). The low

sensitivity obtained with DNA looping in this region can be used to achieve fairly constant transcription rates among cells in a population, irrespective of fluctuations in the number of *lac* repressor molecules. In contrast, using a single operator just propagates the fluctuations proportionally.

DNA looping can also reduce the intrinsic fluctuations of transcription [39]. If transcription switches slowly between active and inactive, there are long periods of time in which proteins are produced constantly and long periods without any production. Therefore, the number of molecules would fluctuate strongly between high and low values. In contrast, if switching is very fast, protein production happens in short and frequent bursts. The absence of long periods of time with either full or null production gives a narrower distribution of the number of protein molecules. DNA looping naturally introduces a fast timescale for the switching of transcription: the time it takes for the repressor to be recaptured by the main operator before unbinding the auxiliary operator, which, as we have shown above, is much shorter than the time needed for a new repressor to bind from solution. Therefore, DNA properties are also important for controlling transcriptional noise.

Conclusions

DNA looping is an extremely important process for the functioning of even the simplest types of cells. Besides providing a backbone for fundamental long-range interactions, DNA looping can be used to simultaneously increase specificity and affinity, and, at the same time, to control the intrinsic stochastic nature of cellular processes. In particular, it can buffer molecular variability to produce phenotypically homogeneous populations and decrease transcriptional noise [4••].

It is becoming increasingly clear that the cell has found ways to loop DNA that extend beyond the classical view of an extremely stiff polymer at short length scales. Recent approaches connecting cellular physiology measurements with the *in vivo* free energy of DNA looping by the *lac* repressor indicate that DNA loops can form extremely easily in the intracellular environment: the *in vivo* free energy of DNA looping changes within a very narrow window of about 2.5 kcal/mol for loop lengths that range from 50 bp to 1.5 kb (Figure 1d and e). These changes in the free energy are much smaller than predicted from current DNA elastic models and lie within typical values of the free energy of interaction between regulatory molecules [46].

The properties of *in vivo* DNA looping seem to have been tuned so that the effects of regulatory molecules are strongly dependent on their precise DNA positioning and, at the same time, are easily tunable and modifiable by their cooperative interactions. At the intracellular level, the looping properties of DNA are affected, among other factors, by the sequence dependence of DNA elasticity, the presence of alternative loop conformations, interactions with different proteins, and DNA supercoiling [14]. Understanding how all these factors are combined to obtain the observed behavior is one of the main challenges that lies ahead.

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