Sequence-independent and reversible photocontrol of transcription/expression systems using a photosensitive nucleic acid binder

André Estévez-Torres[a,b], Cécile Crozatier[a], Antoine Diguet[c], Tomoaki Hara[d], Hirohide Saito[d], Kenichi Yoshikawa[a,b], and Damien Baigl[b,c,1]

[a]Departments of Physics and [d]Gene Mechanisms, Kyoto University, Kyoto 606-8502, Japan; [b]Spatio-Temporal Order Project, ICORP (International Cooperative Research Project), JST (Japan Science and Technology Agency), Kyoto 606-8502, Japan; and [c]Department of Chemistry, Ecole Normale Supérieure, 75005 Paris, France

To understand non-trivial biological functions, it is crucial to develop minimal synthetic models that capture their basic features. Here, we demonstrate a sequence-independent, reversible control of transcription and gene expression using a photosensitive nucleic acid binder (pNAB). By introducing a pNAB whose affinity for nucleic acids is tuned by light, in vitro RNA production, EGFP translation, and GFP expression (a set of reactions including both transcription and translation) were successfully inhibited in the dark and recovered after a short illumination at 365 nm. Our results indicate that the accessibility of the protein machinery to one or several nucleic acid binding sites can be efficiently regulated by changing the conformational/condensation state of the nucleic acid (DNA conformation or mRNA aggregation), thus regulating gene activity in an efficient, reversible, and sequence-independent manner. The possibility offered by our approach to use light to trigger various gene expression systems in a system-independent way opens interesting perspectives to study gene expression dynamics as well as to develop photocontrolled biotechnological procedures.

DNA conformation | gene regulation | RNA | light | synthetic biology

The regulation of gene activity in an organism is a complex and intricate process that involves the precise control of thousands of proteins. An important part of this regulation takes place at the transcription level, where 2 basic strategies are observed (1). First, a transcription factor (*trans* factor) binds to a gene regulatory sequence (*cis* element) and regulates the transcription activity of a single gene or a small set of genes. The understanding of this strategy has been one of the landmarks of molecular and cellular biology (2) and has recently led to the fields of systems (3) and synthetic biology (4), allowing the engineering and control of gene networks that support genetic oscillators (5) or respond to light (6). However, a transcription factor can only bind to DNA if its sequence is physically accessible and, thus, the higher-order structure of the chromosome is also expected to regulate gene activity, presumably of larger sets of genes. This second regulatory strategy has long ago been observed in eukaryotes (7), where 2 types of chromatin structures are distinguished: heterochromatin (8), which remains condensed and contains few genes being expressed, and euchromatin, that displays a looser structure and contains highly expressed genes. Recent studies show that the spatial organization of the bacterial chromosome is dynamically regulated, mainly through supercoiling and condensation state (9), and this affects gene expression (10).

To help in understanding the features needed to regulate gene activity through changes in chromatin structure, we first explored a minimal synthetic regulatory strategy: DNA conformational state is controlled by an external light stimulus and, as a result, transcription activity is regulated in the presence of an RNA polymerase (Fig. 1*A*). To achieve this goal, we simply added a photosensitive nucleic acid binder (pNAB) to the transcription medium. As a pNAB, we used an azobenzene trimethylammonium bromide surfactant (AzoTAB) recently demonstrated by Le Ny and Lee to be a photosensitive DNA condensing agent (11). AzoTAB is a cationic surfactant which apolar tail undergoes a *trans* to *cis* isomerization at 365 nm. Consequently, there exists an AzoTAB concentration range for which genomic DNA is compacted under dark conditions but unfolded under UV illumination at 365 nm (11, 12). This can be interpreted as a stronger DNA binding affinity of the *trans* form due to its more hydrophobic apolar tail. We hypothesized that the light-dependent compaction of DNA could be used to tune RNA production by controlling the accessibility of the template sequence for the transcription enzyme. We further demonstrate the ability of AzoTAB to efficiently switch on and off protein translation and expression in a complex in vitro system involving more than 20 proteins, ribosomes, and different nucleic acids.

Results

DNA Conformation and Transcription Activity of T4 DNA and *E. coli* RNA Polymerase in the Presence of AzoTAB. We first studied the effect of AzoTAB in the transcription of T4 DNA by *E. coli* RNA polymerase (RNAP). T4 DNA is a 166-kb duplex DNA carrying approximately 40 promoters for this enzyme (13). Transcription activity was measured as a function of [AzoTAB] in the absence and in the presence of UV illumination (Fig. 1*B*). Transcription activity is defined as the amount of produced RNA divided by the amount produced in a reference reaction ([AzoTAB] = 0 mM, -UV). In parallel, we characterized the conformation of individual DNA molecules under the conditions of transcription by fluorescence microscopy (FM) using YOYO-1 as a DNA dye (Fig. 1*B Insets* and Movie S1). Complementary to FM, static light scattering (SLS) experiments (14) were also used to follow the conformational changes of T4 DNA in the transcription medium as a function of [AzoTAB] and UV illumination (Fig. S1). The curve of transcription activity follows remarkably well the evolution of DNA conformation. At low [AzoTAB] (≤ 1.3 mM), that is, when DNA is in a coil state, transcription slightly decreases but remains high regardless of UV illumination. For [AzoTAB] > 1.3 mM, a significant decrease of transcription activity is observed until total inhibition for [AzoTAB] ≥ 2 mM, which also corresponds to compaction of all DNA molecules (Fig. 1*B Insets* and Fig. S1). In this concentration range, UV

CHEMISTRY

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

Fig. 1. The conformation and transcription activity of genomic DNA is controlled using UV illumination in the presence of the photosensitive condensing agent AzoTAB. (*A*) After addition of *trans*-AzoTAB, DNA is compact and transcription is inhibited. Upon UV illumination, AzoTAB isomerizes to *cis*, which triggers DNA unfolding and switches on transcription. The process is reversible upon VIS (>400 nm) illumination which compacts DNA and switches transcription back to the off state. (*B*) Transcription activity of T4 DNA as a function of AzoTAB concentration in the dark (red squares) and after UV illumination for 10 min (blue triangles). The *Insets* show representative fluorescence microscopy images of T4 DNA for 1.3, 2.0, and 3.0 mM AzoTAB demonstrating a good correlation between DNA condensation state and transcription activity. Each image is 6 × 6 µm. The dashed line represents the onset of increase of static light scattering intensity (-UV) (Fig. S1). All experiments were performed under the same conditions: 1 µg/mL T4 DNA, 0.02 U/µL *E. coli* RNA polymerase, 40 mM Tris-HCl, pH 7.5, 150 mM KCl, 10 mM MgCl$_2$, 0.5 mM NTPs, and 10 mM DTT, T = 37°C.

illumination induces DNA unfolding accompanied by a strong increase of transcription activity up to 80%. At high [AzoTAB] ($\geq$ 2.6 mM) the recovery of transcription activity by UV light is less efficient. It can be interpreted by the incomplete unfolding of DNA molecules as observed by FM (Fig. 1*B Inset*, [AzoTAB] = 3 mM). Finally, the activation of transcription activity upon UV illumination does not depend whether UV is applied before or after RNAP introduction (Fig. S2), indicating that UV has no direct effect on the polymerase. All these results show that the presence of a photosensitive DNA condensing agent in the transcription medium enables the control by light of the transcription activity of a genomic DNA through its conformational changes.

Dependence of Transcription Photocontrol on Template Length and Structure. To demonstrate the universal character of the present approach we performed similar experiments using various DNA templates containing a single T7 promoter and T7 RNAP as a polymerase (Fig. 2). When a linearized 5-kb plasmid was used as a template, the photoswitch of RNA production was remarkably efficient (Fig. 2*A*). With an increase in [AzoTAB], after a first

plateau around 100%, transcription activity strongly decreases to reach 0% for [AzoTAB] $\geq$ 2 mM, which corresponds to full compaction according to SLS data (dashed line and Fig. S3*A*). In the latter region, UV illumination induces DNA unfolding accompanied by RNA production up to amounts significantly larger than that of the reference reaction ([AzoTAB] = 0 mM, -UV). The on/off control and enhancement of RNA production by UV light was confirmed by gel electrophoresis of the RNA transcripts (Fig. 2*B*): no effect of AzoTAB and UV light for [AzoTAB] $\leq$ 1.3 mM; partial inhibition and recovery by UV for [AzoTAB] = 1.7 mM; total inhibition and enhanced recovery by UV for [AzoTAB] $\geq$ 2 mM. The lower and upper bands correspond to the 900-mer RNA coding for GFP and the full length 5-kb transcript, respectively (Fig. S4), as the T7 promoter is located at one extremity of the linearized plasmid and the terminator sequence is not fully effective in stopping RNA synthesis by T7 RNAP (15). It is interesting to note that RNA transcripts are identical in length and relative concentration regardless of UV illumination conditions and [AzoTAB], which shows that the methodology allows controlling the amount of transcripts without modifying their nature. The accessibility of the promoter is thus essentially affected and not the processivity of the transcription enzyme. For the same promoter, we studied the effect of the template structure (Fig. 2). Three DNA templates containing a single T7 promoter were used: a 5-kb linearized plasmid (Fig. 2*A*), a 140-bp linear fragment (Fig. 2*C*), and the same plasmid in a circular, supercoiled state (Fig. 2*D*). The transcription activity of the short fragment as a function of [AzoTAB] is similar to that of the linearized plasmid, both in -UV and +UV conditions, which shows that the photocontrol process is almost independent of the DNA template length. The only difference is observed at low AzoTAB concentration where the short fragment displays a gradual decrease of transcription activity while the linearized plasmid behaves in a nearly all-or-none fashion. In the case of the circular plasmid, -UV transcription activity gradually decreases to reach total inhibition at [AzoTAB] = 2 mM, which also corresponds to DNA compaction according to SLS data (Fig. S3*B*). However, in this case transcription activity is only partially recovered (between 20% and 50%) upon UV illumination. For the 3 templates, transcription activity is fully inhibited at the AzoTAB concentration required for DNA compaction (dashed lines) and recovered upon UV illumination. However, significant differences are observed in the profile of transcription inhibition and in the extent of recovery after UV. We can interpret these trends in terms of the physico-chemical nature of the DNA folding transition. According to the literature, we expect that (*i*) the linearized 5-kb plasmid condenses in an all-or-none fashion, as it has been established for linear DNA molecules larger than a few thousands base pairs (16); (*ii*) short 140-bp DNA fragments autoassemble into multimolecular condensates (17); and (*iii*) circular, supercoiled plasmid fold in a continuous manner upon addition of a condensing agent (18). Inhibition of transcription activity follows this trend (Fig. 2, red squares), that is, a steep decrease for the linearized plasmid and a more progressive one for the 2 other templates. The marked different recovery of transcription activity between linear and circular template suggests that, in the first case, the folding transition is reversible upon UV illumination in agreement with observations with T4 DNA (Fig. 1*B Insets* and Movie S1), while the unfolding of the circular supercoiled plasmid might lead to kinetically trapped conformations hindering transcription. Finally, the enhancement over 100% of transcription activity in the presence of *cis*-AzoTAB has been reproducibly observed for the 2 linear templates (Fig. 2 *A*–*C*), with 2 independent techniques for the linearized plasmid: RiboGreen fluorescence and RNA gel electrophoresis. This is probably due to the conformation of DNA, which is slightly more compact than in the absence of AzoTAB but is not fully condensed. Similar increase of transcriptional activity has already been reported for T7 RNAP in the presence of multications (19).

Fig. 2. The photoresponse of various T7 promoter systems demonstrates enhanced on/off switching of RNA production triggered by light, regardless of DNA template length but depending on its structure. Transcription activity as a function of AzoTAB concentration in the absence (-UV, red squares) and in the presence (+UV, blue triangles) of 10 min UV illumination light for different DNA templates carrying a T7 promoter: 5-kb linearized plasmid (*A*), 140-bp linear fragment (*C*), and 5-kb circular supercoiled plasmid (*D*). (*B*) Scheme of the sequence (*Top*) and non-denaturant agarose gel of the transcripts produced by the 5-kbp linearized plasmid (*Bottom*). The dashed lines represent the onsets of increase of static light scattering intensity (Fig. S3). All experiments were performed under the same conditions: 2 μg/mL DNA template, 5 U/μL T7 RNA polymerase, 40 mM Tris-HCl, pH 7.5, 25 mM NaCl, 8 mM MgCl$_2$, 0.5 mM NTPs, and 5 mM DTT, T = 37°C.

Kinetics of the Photocontrol Process. After the thermodynamic characterization of the photocontrol process, we explored the kinetic aspects and the possibility to dynamically trigger RNA synthesis for the T7 promoter system with the 140-bp fragment at 2 mM AzoTAB. The *trans* to *cis* isomerization of AzoTAB as well as DNA unfolding under UV have characteristic times of approx. 3 min (Fig. 3*A*, *Top* and *Middle*). The amount of produced RNA after 20 min of transcription reaches a plateau after a similar characteristic time of UV exposure (Fig. 3*A*, *Bottom*). This shows that, under the illumination conditions used

here, the kinetically determinant step for transcription recovery is the photoisomerization of AzoTAB and that the production of RNA is activated within 3 min of UV exposure. This relatively fast response time allows one to envision dynamic control of RNA synthesis. To this end we followed RNA production kinetics from several identical solutions illuminated with UV (10 min) and visible (3 min) light pulses along the course of the transcription reaction (Fig. 3*B*). In all cases, no transcription is observed until application of UV light ('+UV' arrows). Right after UV exposure, a strong activation of RNA synthesis is

Fig. 3. The fast response of DNA conformation and transcription activity to UV illumination allows one to dynamically photoswitch on and off RNA production along the course of transcription reaction. (*A*) Concentration of *trans*-AzoTAB determined by UV/VIS spectrophotometry, static light scattering intensity, and amount of produced RNA for the 140-bp fragment bearing a T7 promoter after 20 min of transcription as a function of UV illumination time. UV was applied at the beginning of the transcription. (*B*) RNA production kinetics from reactions of identical composition under different illumination conditions (1 color per reaction). t = 0 corresponds to the addition of T7 RNA polymerase. Arrows indicate the time of application of 10 min of UV (+UV) or 3 min of visible illumination (+Vis).

CHEMISTRY

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

Fig. 4. Translation and expression of a fluorescent protein is photocontrolled using AzoTAB in a cell-free expression system. (*A*) Translation activity of a mRNA coding for EGFP and (*B*) expression activity of a plasmid coding for GFP protein as a function of AzoTAB concentration in the absence (red squares) and in the presence (blue triangles) of 1.5 min of 365 nm UV illumination. The experiments were performed in PURE System, with mRNA and DNA templates both at a final concentration of 1 μg/mL. (*C*) Representative fluorescence microscopy images of mRNA (50 nM) labeled by Ribogreen in PURE System buffer without tRNA and protein factors as a function of AzoTAB concentration, with or without UV exposure (365 nm). Images were focused on the glass substrate. (Scale bar, 10 μm.)

observed. Interestingly, all UV-induced activations display similar kinetics regardless of the time of application of UV light. Transcription can also be efficiently stopped by visible light exposure ('+Vis' arrow). FM observations (Fig. S5) indicate that multimolecular DNA condensates appearing upon addition of 2 mM AzoTAB are disrupted after 10 min of UV illumination and appear again after further exposure to 3 min of visible light. This sequence of condensation/unfolding/recondensation perfectly correlates with the inhibition/activation/inhibition of RNA synthesis observed in the kinetic experiments (Fig. 3*B*).

Photocontrol of Translation/Expression. The systems studied until here were relatively simple, involving only a template DNA and a single protein. One might expect that the low affinity and millimolar concentration of AzoTAB would preclude its application in a more complex system. We thus performed translation and expression experiments where EGFP or GFP production was monitored in the presence of the same pNAB (AzoTAB) using an mRNA fragment or a DNA plasmid as a template, respectively. These experiments were carried out using PURE System, a cell-free, in vitro, translation/expression system composed of purified components such as translation factors, aminoacyl-tRNA synthetases and other proteins, ribosomes, aminoacids, and tRNAs (20, 21). Fig. 4*A* displays the translation activity of an EGFP-coding, 759-b mRNA as a function of [AzoTAB] and UV illumination. In comparison with transcription experiments, UV exposure time in the translation/expression system was decreased to 1.5 min. For longer exposure times translation activity for [AzoTAB] = 0 mM decreased

significantly below 100%, indicating that the cell-free expression system is photodamaged (Fig. S6). In the absence of UV light, increasing AzoTAB concentration gradually reduces EGFP production until complete inhibition is reached for [AzoTAB] = 3 mM. In the presence of UV light for 1.5 min, EGFP translation activity remains constant and around 90% up to [AzoTAB] = 1 mM. From [AzoTAB] = 1 mM to [AzoTAB] = 3 mM, the activity after irradiation slightly decreases to reach 50 ± 13% at [AzoTAB] = 3 mM. The recovery of translation activity is particularly efficient in this concentration range where UV exposure results in a 2-, 3.6-, and 5.5-fold increase of EGFP production for [AzoTAB] = 1, 2, and 3 mM, respectively. Fig. 4*B* shows GFP expression activity for the plasmid used in the previous transcription experiments as a function of [AzoTAB] and UV illumination. Again, in the absence of UV, increasing concentrations of AzoTAB gradually decreases expression activity until complete inhibition at [AzoTAB] ≥ 2.5 mM. In the presence of UV light for 1.5 min, expression activity is significantly enhanced. For instance, at [AzoTAB] = 2 mM, UV illumination results in a 4.8-fold increase of GFP expression activity, from 11 ± 0.3% (-UV) to 53 ± 3% (+UV). At [AzoTAB] = 2.5 mM, the amount of expressed GFP was below the detection limit in the absence of UV but increased to 26 ± 13% after UV exposure. With a linearized plasmid, efficient photocontrol of GFP expression was also obtained (Fig. S7). Compared with the photocontrol of transcription activity (Fig. 2), the results obtained in the translation/expression system show 2 main differences: (*i*) translation and expression activities both decrease more gradually as a function of [AzoTAB] and (*ii*) the onset of activity inhibition is observed at a lower [AzoTAB]. To check whether the conformational state of the mRNA could explain these observations, we recorded FM images of the 759-b mRNA stained with RiboGreen in the conditions of translation (but without tRNA and protein factors) for different [AzoTAB] and illumination conditions (Fig. 4*C*). Due to the short length of the mRNA fragment, aggregates of mRNA are observed as bright spots while non-aggregated mRNA appears as an intense fluorescent background. To be able to quantify the amount and size of these aggregates, FM images were focused at the surface of the glass slide holding the mRNA solution. The gain of the camera and excitation intensity were kept constant for all images (except for 0 mM, -/+ UV where the fluorescent background was too intense), so that the intensity can be compared for the different conditions. No aggregates were observed at [AzoTAB] = 0 mM, regardless of the illumination. In the absence of UV illumination some aggregates appear at [AzoTAB] = 1 mM, in agreement with the loss of translation activity observed in Fig. 4*A*. At [AzoTAB] = 2 mM the number of aggregates significantly increases while the background fluorescence, signature of free mRNA, decreases. Further addition of AzoTAB up to 3 mM results in larger and brighter aggregates. Upon UV illumination, the number and/or average size of the aggregates significantly decreases for [AzoTAB] ≥ 1 mM. Comparing Fig. 4 *A* and *C*, all these results are compatible with a description in which the degree of aggregation of the mRNA controls translation activity (Fig. 4*A*). Interestingly, under the conditions of translation, the minimal concentration of AzoTAB that induces mRNA aggregation (1 mM) is lower than the minimal concentration to induce DNA compaction (2 mM) (Fig. S8), which indicates a greater specificity of this nucleic acid binder toward RNA. Again, simply adding a photosensitive nucleic acid binder allows one to efficiently control by light the output of a complex sequence of biochemical reactions involving transcription and translation.

Discussion

We have described a sequence-independent approach to control, using light, RNA and protein synthesis using a photosensitive nucleic acid binder (pNAB). By introducing a pNAB that

controls DNA conformation by light in various transcription systems (different templates and different enzymes), we could reversibly switch on and off RNA synthesis by simple light pulses (365 nm: on/visible light: off) along the course of the transcription reaction, without affecting the nature of the RNA transcripts. Two experimental facts strongly indicate that the control of transcription activity is due to the changes in the conformational state of DNA rather than the protein-AzoTAB interactions. First, we have shown a strong correlation between transcription activity and DNA condensation (Fig. 1 *B* and *F*ig. S5). Transcription activity was totally inhibited only when DNA condensates were observed and recovered back only when they were disrupted. Second, the observed differences in the photocontrol of transcription between the linear and the circular template can only be explained if the structure of the DNA plays a dominant role. Although AzoTAB and the RNAP may interact at millimolar concentrations, this interaction could not explain the experimental observations but only account for effects of second order.

Certainly, the millimolar concentrations of AzoTAB needed to observe the phenomena described above are high compared to the nanomolar to micromolar concentrations of active molecules in vivo. But let us recall that the effect observed here is sequence-independent and, as a result, one may expect low affinity constants (and thus high concentrations). Indeed, in nature, the specificity of a transcription factor toward DNA is negatively correlated with the number of genes it regulates (22). Local *trans* factors have higher specificity than global *trans* factors. Interestingly, proteins that are identified in bacteria to play a role in controlling chromatin structure display low sequence specificity and DNA binding affinities (23). For instance, Fis is a DNA-binding protein suspected to regulate nucleoid condensation in *Escherichia coli*. Fis binds to DNA in a largely sequence-independent manner with an affinity constant in the nanomolar range but it only induces DNA condensation for concentrations higher than 1 μM (24). If a 15 kDa protein optimized through millions of years of evolution to regulate chromatin structure bear such low affinities toward DNA binding, it is not surprising that our synthetic 300-Da analogue needs millimolar concentrations to be active. Additionally, a low affinity is usually necessary to ensure the reversibility of DNA conformational changes (25).

As simple as the AzoTAB molecule might be, it has proved to be remarkably efficient at controlling protein translation and expression in a complex in vitro system involving tens of different proteins, as well as ribosomes. Although non-specific interactions between AzoTAB, proteins and ribosomes cannot be ruled out, our results qualitatively indicate that the source of the photocontrol effect is the aggregation/condensation state of the mRNA.

Conclusion

The use of a photosensitive nucleic acid binder (pNAB) as presented here combines a great simplicity with a universal physicochemical approach of controlling gene expression at 2 different stages: transcription and translation. This approach only requires the addition of an adequate pNAB to the reaction system, with the possibility to activate various kinds of gene expression systems by light in an efficient and reversible manner. In all of the cases studied here, the condensation state of the nucleic acid was shown to control genetic activity: the conformation of the DNA template regulates transcription while the aggregation of mRNA controls the outcome of translation. We thus expect these investigations to be relevant for the engineering of controllable gene expression systems and draw attention into the importance of DNA conformational changes as a regulatory mechanism in gene expression.

Methods

For all transcription experiments, buffer, ribonucleotidetriphosphates (NTPs), AzoTAB, DNA, and the corresponding RNA polymerase (RNAP) were assembled in this order and the transcription reaction was carried out at 37°C. Except for kinetic experiments, all reactions were performed for 20 min either in the dark (-UV) or exposed to 365 nm UV illumination during the first 10 min of transcription (+UV). After 20 min, the RNA concentration of the reaction medium was quantified by RiboGreen fluorescence at 532 nm.

In translation and expression experiments, water, PURE System (PURE System classic II, Post Genome Institute) solution containing salts and tRNAs, RNA or DNA template, AzoTAB, and PURE System solution containing enzymes and ribosomes were assembled in this order and the translation/expression reaction was carried out at 37°C for 75 min. When necessary, UV exposure at 365 nm was applied during the first 1.5 min of incubation. After 75 min, EGFP or GFP fluorescence was measured in a microplate reader. (Full method in *SI Materials and Methods*).

1. Balleza E, et al. (2009) Regulation by transcription factors in bacteria: Beyond description. *FEMS Microbiol Rev* 33:133–151.
2. Monod J, Jacob F (1961) On the regulation of gene activity. *Cold Spring Harb Symp Quant Biol* 26:193–211.
3. Alon U (2007) Network motifs: Theory and experimental approaches. *Nat Rev Gene* 8:450–461.
4. Forster AC, Church GM (2006) Towards synthesis of a minimal cell. *Mol Syst Biol* 2:45.
5. Elowitz MB, Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* 403:335–338.
6. Levskaya A, et al. (2005) Engineering *Escherichia coli* to see light. *Nature* 438:441–442.
7. Wasylyk B, Thevenin G, Oudet P, Chambon P (1979) Transcription of *in vitro* assembled chromatin by *Escherichia coli* RNA polymerase. *J Mol Biol* 128:411–440.
8. Djupedal I, Ekwall K (2008) The paradox of silent heterochromatin. *Science* 320:624–625.
9. Gitai Z, Thanbichler M, Shapiro L (2005) The choreographed dynamics of bacterial chromosomes. *Trends Microbiol* 13:221–228.
10. Willenbrock H, Ussery DW (2004) Chromatin architecture and gene expression in *Escherichia coli. Gen Biol* 5:252.
11. Le Ny A-LM, Lee CT Jr (2006) Photoreversible DNA condensation using light-responsive surfactants. *J Am Chem Soc* 128:6400–6408.
12. Sollogoub M, et al. (2008) Photocontrol of single-chain DNA conformation in cell-mimicking microcompartments. *ChemBioChem* 9:1201–1206.
13. Miller ES, et al. (2003) Bacteriophage T4 genome. *Microb Mol Biol Rev* 67:86–156.
14. Wilson RW, Bloomfield VA (1979) Counter-ion-induced condensation of deoxyribonucleic acid. A light-scattering study. *Biochemistry* 18:2192–2196.
15. Hartvig L, Christiansen J (1996) Intrinsic termination of T7 RNA polymerase mediated by either RNA or DNA. *EMBO J* 15:4767–4774.
16. Yoshikawa K, Takahashi M, Vasilevskaya VV, Khokhlov AR (1996) Large discrete transition in a single DNA molecule appears continuous in the ensemble. *Phys Rev Lett* 76:3029–3031.
17. Pelta J, Livolant F, Sikorav J-L (1996) DNA aggregation induced by polyamines and cobalthexamine. *J Biol Chem* 271:5656–5662.
18. Sato YT, et al. (2005) Folding transition into a loosely collapsed state in plasmid DNA as revealed by single-molecule observation. *FEBS Lett* 579:3095–3099.
19. Tsumoto K, Luckel F, Yoshikawa K (2003) Giant DNA molecules exhibit on/off switching of transcriptional activity through conformational transition. *Biophys Chem* 106:23–29.
20. Shimizu Y, et al. (2001) Cell-free translation reconstituted with purified components. *Nat Biotechnol* 19:751–755.
21. Shimizu Y, Kanamori T, Ueda T (2005) Protein synthesis by pure translation systems. *Methods* 36:299–304.
22. Lozada-Chávez I, Espinosa Angarica V, Collado-Vides J, Contreras-Moreira B (2008) The role of DNA-binding specificity in the evolution of bacterial regulatory networks. *J Mol Biol* 379:627–643.
23. Dame RT (2005) The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin. *Mol Microbiol* 56:858–870.
24. Skoko D, et al. (2006) Mechanism of chromosome compaction and looping by the *Escherichia coli* nucleoid protein Fis. *J Mol Biol* 364:777–798.
25. Geoffroy M, Faure D, Oda R, Bassani DM, Baigl D (2008) Photocontrol of genomic DNA conformation using a photosensitive gemini surfactant: Binding affinity versus reversibility. *ChemBioChem* 9:2382–2385.

CHEMISTRY

BIOPHYSICS AND COMPUTATIONAL BIOLOGY