Communications

constraint imposed by the DNA duplex was incompatible with the known RNA tertiary structure,[2] then a competition was established in which either the DNA constraint or the RNA conformation must be compromised (Figure 1).[1] These
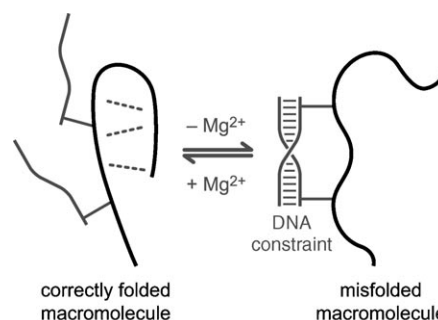

Figure 1. Use of a DNA constraint to control the folding of a macromolecule such as RNA. In the absence of the DNA constraint, RNA folding is characterized by its $[Mg^{2+}]_{1/2}$ value. The DNA constraint suppresses native folding even at $[Mg^{2+}] > [Mg^{2+}]_{1/2}$. Under these conditions, modulation of the constraint, as described herein, switches the RNA folding state from misfolded to correctly folded.

experiments demonstrated a new approach to DNA nanotechnology[3] in which the conformations of other macromolecules are controlled by DNA acting as a dynamic structural element. A fundamental feature of this approach is that the effect of the DNA constraint on macromolecular structure should be modulated by external stimuli. Herein, we show that DNA constraints on macromolecular conformation can indeed be modulated in several ways. The DNA constraint can be destroyed irreversibly by degradation with a nuclease or restriction enzyme. The constraint can also be destroyed with a reducing agent that cleaves a disulfide linkage between the RNA and DNA. The DNA constraint can be modulated reversibly by sequential binding and removal of oligonucleotides. Finally, when one of the DNA constraint strands incorporates an aptamer sequence, the cognate small-molecule ligand can modulate the DNA constraint. This wide range of methods for the modulation of DNA constraints upon macromolecular conformation will expand greatly the applicability of our new approach to DNA nanotechnology.

The stability of RNA tertiary structure depends much more strongly on the presence of Mg^{2+} than does the formation of DNA duplexes.[4] Under typical in vitro folding conditions, such as Tris-borate buffer solution (Tris = tris(hydroxymethyl)aminomethane; pH 8.3) and 35°C, the structured P4–P6 RNA has an empirical Mg^{2+} concentration termed the $[Mg^{2+}]_{1/2}$ value, which characterizes its folding. A shift in the $[Mg^{2+}]_{1/2}$ value acts as a sensitive probe for thermodynamic perturbations upon RNA structures,[5] including effects due to DNA constraints.[1] A DNA duplex that is structurally compatible with the folded RNA conformation does not affect the $[Mg^{2+}]_{1/2}$ value; in contrast, an incompatible DNA constraint increases $[Mg^{2+}]_{1/2}$ because the DNA duplex must be disrupted for the RNA to fold.[6] The Mg^{2+}-dependent folding of P4–P6 is monitored quantitatively by nondenaturing polyacrylamide gel electrophoresis (native

Modulation of DNA Constraints That Control Macromolecular Folding**

Chandrasekhar V. Miduturu and Scott K. Silverman

We recently reported double-stranded DNA as a constraint to control macromolecular conformation.[1] In these experiments, two short 10- to 20-nucleotide DNA strands were covalently attached through their 5′-termini to specific nucleotides within the autonomously folding 160-nucleotide P4–P6 RNA domain (molecular weight $\approx 51\,000$). When the attached DNA strands were complementary, a duplex was formed by Watson–Crick base pairing. If the geometric

[*] C. V. Miduturu, Prof. S. K. Silverman
Department of Chemistry
University of Illinois at Urbana-Champaign
600 South Mathews Avenue, Urbana, IL 61801 (USA)
Fax: (+1) 217-244-8024
E-mail: scott@scs.uiuc.edu

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

PAGE).[5,7,8] The $[Mg^{2+}]_{1/2}$ value for unconstrained wild-type P4–P6 is ≈ 0.7 mM. Owing to the highly cooperative folding transition that has a Hill coefficient of $n \approx 4$ on native PAGE, wild-type P4–P6 is almost completely folded when $[Mg^{2+}] > 1.0$ mM. However, when an incompatible DNA constraint is attached, the $[Mg^{2+}]_{1/2}$ value shifts considerably to the right; for example, to ≈ 12 mM when a 10-bp constraint is attached at P4–P6 nucleotides U107 and C240, which are too far apart for the DNA constraint to span and yet allow the RNA conformation to be maintained.[1] At a fixed intermediate Mg^{2+} concentration such as 1.5–2.0 mM, at which the wild-type (unconstrained) RNA is entirely folded, the DNA duplex prevents folding of the constrained P4–P6. Modulation of the DNA constraint at the fixed intermediate Mg^{2+} concentration should then switch the unfolded or folded status of the RNA. We took advantage of the native PAGE folding assay and the strong dependence of RNA folding on Mg^{2+} concentration to demonstrate modulation of the DNA-constraint effect in several ways.

First, we established that a DNA constraint could be destroyed irreversibly with enzymes or chemical reagents. This was performed with either deoxyribonuclease (DNase) or exonuclease III, both of which nonspecifically degrade the 20 bp duplex DNA (Figure 2a).[9] Alternatively, the DNA constraint was cleaved sequence specifically with the restriction enzyme BsrBI. In all these cases, the RNA was misfolded at $[Mg^{2+}] = 1.5$ mM with the DNA constraint intact; removal of the constraint by the nuclease allowed the RNA to fold
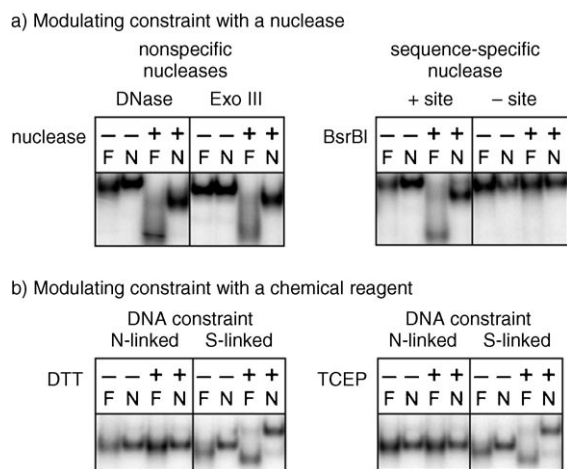

a) Modulating constraint with a nuclease

b) Modulating constraint with a chemical reagent

Figure 2. Modulation of the DNA constraint with a nuclease or a chemical reagent, as monitored by native PAGE. a) Modulation of the constraint with nonspecific deoxyribonucleases or with the BsrBI restriction enzyme. The lanes labeled "−site" represent BsrBI treatment of a constraint sequence that lacks the BsrBI recognition site. The $[Mg^{2+}]$ was constant at 1.5 mM ($T = 25\,°C$). F and N denote foldable and nonfoldable versions of P4–P6 RNA, respectively. Without nuclease treatment, both F and N migrate equivalently. With nuclease treatment, F migrates much faster than N, which is the signature of P4–P6 folding.[5] b) Modulation of the constraint by reduction with DTT or TCEP (10 mM). Only after reductive cleavage of the S-linked constraint does F migrate much faster than N.[11] For the N-linked constraint, both DNA strands were joined to RNA by reductive amination.[1] For the S-linked constraint, one strand was joined by reductive amination and the other through a disulfide linkage.

properly. We also modulated the DNA constraint by using the reducing agent dithiothreitol (DTT). For this purpose, one of the two DNA strands was attached to RNA not through reductive amination (N-linked)[1] but instead through disulfide formation (S-linked), by using a DNA strand with a 5′-SH group and an RNA nucleotide with a 2′-OCH$_2$CH$_2$SH moiety.[10] Disruption of the S-linked constraint with DTT led to native RNA folding, and the reducing agent tris(2-carboxyethyl)phosphine (TCEP) gave equivalent results (Figure 2b).[11,12]

We sought to modulate the DNA constraint reversibly by adding a complementary (C) DNA oligonucleotide that competes for one of the two duplex strands (Figure 3a).[13] The DNA-constraint strand targeted by the complement was extended at its 3′-end to provide an initiation site for strand invasion. Modulation of the DNA constraint was entirely reversible because subsequent addition of a rescue (R) strand restored the initial constrained state. Full restoration of the constraint required tuning of the relative DNA:DNA binding affinities through strategic mutations in the C strand such that R complemented C perfectly, whereas the affinity of C for the constraint strand was weakened slightly.[13c,e]

Integration of fluorescence response into the reversible DNA-constraint modulation would allow simple and rapid detection of macromolecular conformation changes by spectroscopic rather than electrophoretic assays. For this purpose, we appended a pyrene chromophore to the RNA; pyrene is known to report well on Mg^{2+}-dependent P4–P6 tertiary folding.[8,14] The RNA folding state was readily monitored through repeated cycles of C and R strand addition (Figure 3b) in accord with the native PAGE experiments.[15]

Modulation of the DNA constraint with a small-molecule ligand instead of an oligonucleotide would be a potentially general method to transduce ligand–aptamer interactions into changes in macromolecular conformation (Figure 4a). Towards this goal, one of the DNA-constraint strands was redesigned to incorporate the aptamer sequence for the ligand hemin (molecular weight 652),[16] such that binding of hemin would compete with formation of the duplex constraint. Exposure of the DNA-constrained RNA to hemin indeed led to modulation of the constraint, as shown by return of the $[Mg^{2+}]_{1/2}$ value to very near to that found for unconstrained P4–P6 (Figure 4b). Importantly, restoration of normal RNA folding upon hemin addition was suppressed by specific DNA mutations[17] that are known to disable aptamer–hemin interactions but do not alter the integrity of the constraint.

In summary, we have demonstrated a variety of approaches for the modulation of duplex DNA constraints on the macromolecular folding of RNA. These experiments provide the fundamental control elements of a new direction in DNA nanotechnology in which the conformations of other macromolecules are dynamically controlled by DNA. Our most generalizable approach is the use of a ligand–aptamer interaction as the control element (Figure 4). Although the present experiments used the previously reported hemin–aptamer combination, in principle it should be feasible to identify aptamers for nearly any small molecules of interest[18] and to integrate these ligand–aptamer combinations into
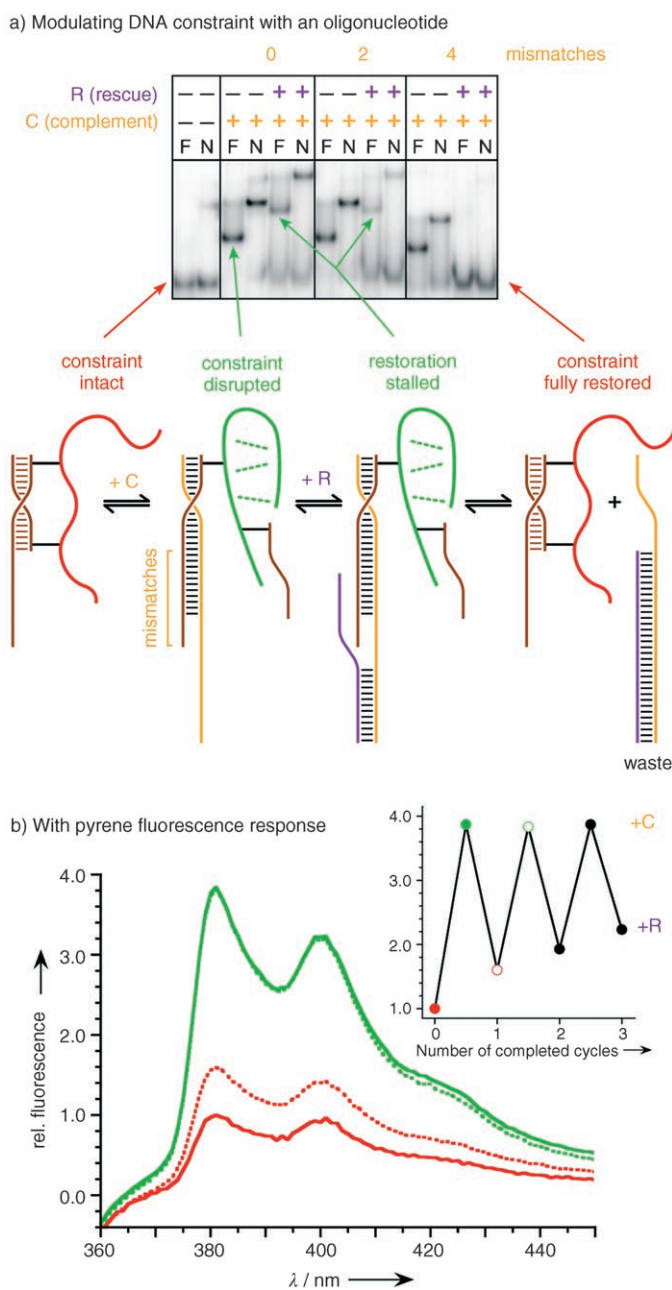
a) Modulating DNA constraint with an oligonucleotide

b) With pyrene fluorescence response

Figure 3. Modulation of the DNA constraint by added oligonucleotides, as monitored by native PAGE or pyrene fluorescence. a) Native PAGE. The [Mg^{2+}] was constant at 1.5 mM ($T=35\,°C$). b) Pyrene fluorescence under similar conditions. The inset shows reversibility over several cycles of complement (C) and rescue (R) strand addition.[15] After each addition, samples were incubated for 1 h, which was sufficient for completion of the fluorescence changes. Other groups have also described duplex exchanges that require minutes or longer.[13]

DNA nanotechnology. The present experiments used RNA as the macromolecule whose structure is controlled,[1] although it should be possible to control the structures of other macromolecules,[19] including nonbiological foldamers.[20] In future investigations, the combination of DNA-mediated conformational control with fluorescence detection of structural changes or with changes in RNA catalytic activity should
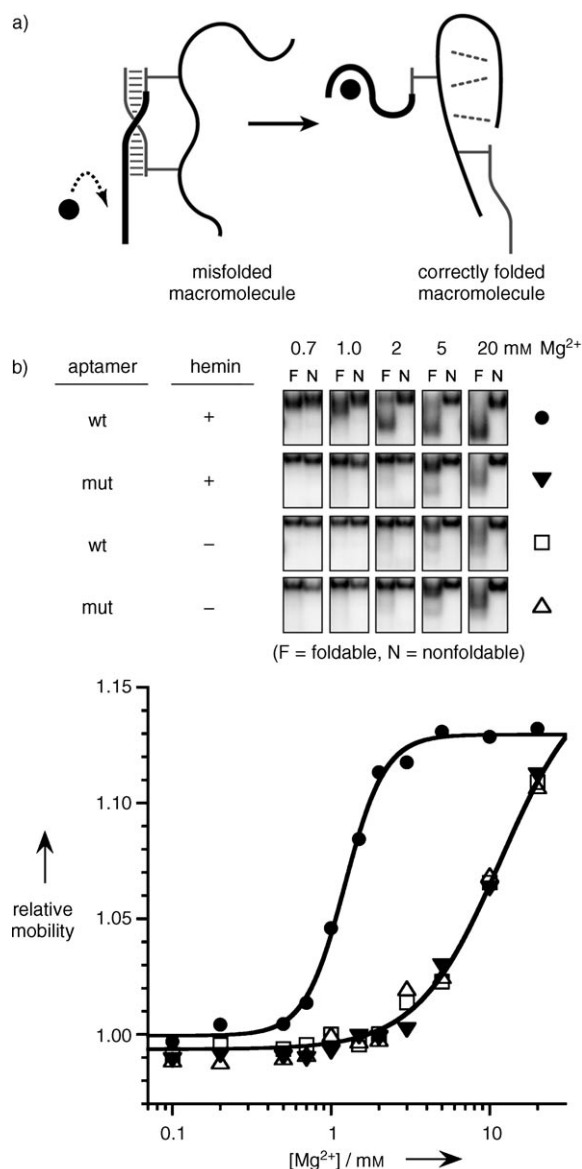
a)

b)

(F = foldable, N = nonfoldable)

Figure 4. Modulation of the DNA constraint by competing ligand–aptamer interactions. a) Design of the competition. b) Native PAGE data demonstrating experimental validation of the design by using the hemin aptamer ($T=25\,°C$).[16] Inclusion of hemin decreases the [Mg^{2+}]$_{1/2}$ value from ≈ 12 mM to 1.2 mM; the latter value is comparable to that of unconstrained wild-type P4–P6 under the same conditions (data not shown). In contrast, when the known single-nucleotide G9A mutation that prevents hemin binding[17] is present in the aptamer sequence, addition of hemin does not affect the [Mg^{2+}]$_{1/2}$ value. wt=wild-type, mut=mutant.

allow creation of sensors whose functions are governed by formation and modulation of DNA constraints.

[1] C. V. Miduturu, S. K. Silverman, *J. Am. Chem. Soc.* **2005**, *127*, 10144–10145.

[2] J. H. Cate, A. R. Gooding, E. Podell, K. Zhou, B. L. Golden, C. E. Kundrot, T. R. Cech, J. A. Doudna, *Science* **1996**, *273*, 1678–1685.

[3] a) F. C. Simmel, W. U. Dittmer, *Small* **2005**, *1*, 284–299; b) S. H. Lee, C. Mao, *BioTechniques* **2004**, *37*, 517–519; c) N. C. Seeman, *Nature* **2003**, *421*, 427–431.

[4] a) I. Tinoco, Jr., C. Bustamante, *J. Mol. Biol.* **1999**, *293*, 271–281; b) S. Nakano, M. Fujimoto, H. Hara, N. Sugimoto, *Nucleic Acids Res.* **1999**, *27*, 2957–2965.

[5] S. K. Silverman, T. R. Cech, *Biochemistry* **1999**, *38*, 8691–8702.

[6] A compatible DNA duplex does not decrease the Mg^{2+} requirement for RNA folding, because the duplex is intact in both the unfolded and folded RNA conformations. Therefore, the free-energy change for the unfolded-to-folded RNA interconversion is unchanged by attachment of the DNA duplex.

[7] a) B. T. Young, S. K. Silverman, *Biochemistry* **2002**, *41*, 12271–12276; b) S. K. Silverman, M. Zheng, M. Wu, I. Tinoco, Jr., T. R. Cech, *RNA* **1999**, *5*, 1665–1674; c) S. K. Silverman, M. L. Deras, S. A. Woodson, S. A. Scaringe, T. R. Cech, *Biochemistry* **2000**, *39*, 12465–12475; d) S. K. Silverman, T. R. Cech, *RNA* **2001**, *7*, 161–166; e) E. A. Doherty, R. T. Batey, B. Masquida, J. A. Doudna, *Nat. Struct. Biol.* **2001**, *8*, 339–343; f) S. Matsumura, Y. Ikawa, T. Inoue, *Nucleic Acids Res.* **2003**, *31*, 5544–5551.

[8] S. K. Silverman, T. R. Cech, *Biochemistry* **1999**, *38*, 14224–14237.

[9] The DNA sequences are provided in the Supporting Information.

[10] S. Jin, C. V. Miduturu, D. C. McKinney, S. K. Silverman, *J. Org. Chem.* **2005**, *70*, 4284–4299.

[11] The altered mobilities observed upon removing the S-linked constraint are due to changes in compactness. See the Supporting Information for full details of all native PAGE experiments and for denaturing PAGE experiments that verify DNA cleavage by the nuclease or reducing agent.

[12] In principle, it should be possible to reverse the removal of the DNA constraint by oxidative restoration of the disulfide linkage, but we have not attempted this process.

[13] a) F. C. Simmel, B. Yurke, *Phys. Rev. E* **2001**, *63*, 041913; b) F. C. Simmel, B. Yurke, *Appl. Phys. Lett.* **2002**, *80*, 883–885; c) J. J. Li, W. Tan, *Nano Lett.* **2002**, *2*, 315–318; d) A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, Jr., M. I. Blakey, F. C. Simmel, *Phys. Rev. Lett.* **2003**, *90*, 118102; e) P. Alberti, J. L. Mergny, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1569–1573; f) W. U. Dittmer, A. Reuter, F. C. Simmel, *Angew. Chem.* **2004**, *116*, 3634–3637; *Angew. Chem. Int. Ed.* **2004**, *43*, 3550–3553; g) W. U. Dittmer, F. C. Simmel, *Nano Lett.* **2004**, *4*, 689–691.

[14] M. K. Smalley, S. K. Silverman, *Nucleic Acids Res.* **2006**, *34*, 152–166.

[15] The imperfect efficiency observed in the Figure 3b inset is similar to inefficiencies observed in independent studies that also rely on DNA oligonucleotides as "fuel" strands.[13c,e,f]

[16] a) Y. Li, C. R. Geyer, D. Sen, *Biochemistry* **1996**, *35*, 6911–6922; b) P. Travascio, Y. Li, D. Sen, *Chem. Biol.* **1998**, *5*, 505–517.

[17] H.-W. Lee, D. J.-F. Chinnapen, D. Sen, *Pure Appl. Chem.* **2004**, *76*, 1537–1545.

[18] a) T. Hermann, D. J. Patel, *Science* **2000**, *287*, 820–825; b) J. Liu, Y. Lu, *J. Fluoresc.* **2004**, *14*, 343–354.

[19] a) B. Choi, G. Zocchi, S. Canale, Y. Wu, S. Chan, L. J. Perry, *Phys. Rev. Lett.* **2005**, *94*, 038103; b) B. Choi, G. Zocchi, Y. Wu, S. Chan, L. J. Perry, *Phys. Rev. Lett.* **2005**, *95*, 078102.

[20] D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893–4012.