provides strong support for the idea that the splicing event is coupled to the stability of the RNA. Accordingly, defined intron sequences seem less likely to be involved in a positive transcriptional regulation event. Thus, while the polarity of the intron is critical to its function, the species of origin and the relative position in the mRNA are not. These observations suggest that introns represent functional elements in the generation of certain stable mRNAs.

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1. Tilghman, S. M., Curtis, P. J., Tiemeier, D. C., Leder, P. & Weissmann, C. *Proc. natn. Acad. Sci U.S.A.* **75,** 1309–1313 (1978).
2. Lai, C.-J., Dhar, R. & Khoury, G. *Cell* **14,** 971–982 (1978).
3. Blanchard, J. M., Weber, J., Jelinek, W. & Darnell, J. E. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5344–5348 (1978).
4. Lindenmaier, W. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 6196–6200 (1979).
5. Cordell, B. *et al. Cell* **18,** 533–543 (1979).
6. Dugaiczyk, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 2253–2257 (1979).
7. Lomedico, P. *et al. Cell* **18,** 545–558 (1979).
8. Busslinger, P., Portman, R. & Birnstiel, M. *Nucleic Acids Res.* **6,** 2997–3008 (1979).
9. Cochet, M. *et al. Nature* **282,** 567–574 (1979).
10. Van Ooyen, A., Vanden Berg, J., Mantei, N. & Weissmann, C. *Science* **206,** 337–344 (1979).
11. Gannon, F. *et al. Nature* **278,** 428–434 (1979).
12. Lai, E. C. *et al. Cell* **18,** 829–842 (1979).
13. Tiemeier, D. C. *et al. Cell* **14,** 237–245 (1978).
14. Leder, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 6185–6191 (1978).
15. Tilghman, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 4406–4410 (1977).
16. Berget, S. M., Moore, C. & Sharp, P. C. *Proc. natn. Acad. Sci. U.S.A.* **74,** 3171–3175 (1977).
17. Lai, C.-J. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **76,** 71–75 (1979).
18. Khoury, G., Gruss, P., Dhar, R. & Lai, C.-J. *Cell* **18,** 85–92 (1979).
19. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, D. *Proc. natn. Acad. Sci. U.S.A.* **75,** 4853–5847 (1978).
20. Seif, I., Khoury, G. & Dhar, R. *Nucleic Acids Res.* **6,** 3387–3398 (1979).
21. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8,** 127–142 (1980).
22. Gruss, P., Lai, C.-J., Dhar, R. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4317–4321 (1979).
23. Hamer, D. H. & Leder, P. *Cell* **18,** 1299–1302 (1979).
24. Hamer, D. H. & Leder, P. *Nature* **281,** 35–40 (1979).
25. Konkel, D. A., Tilghman, S. M. & Leder, P. *Cell* **15,** 1125–1132 (1978).
26. Ghosh, P. K. *et al. J. biol. Chem.* **253,** 3643–3647 (1978).
27. Maniatis, T. *et al. Cell,* **15,** 687–701 (1978).
28. McCutchon, J. H. & Pagano, J. S. *J. natn. Cancer Inst.* **41,** 351–357 (1968).
29. Lai, C.-J. & Nathans, D. *Virology* **60,** 466–475 (1974).
30. Tegtmeyer, P. & Ozer, H. L. *J. Virol.* **8,** 516–524 (1971).
31. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
32. Reddy, V. B. *et al. Science* **200,** 494–502 (1978).
33. Fiers, W. *et al. Nature* **273,** 113–120 (1978).
34. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5350–5354 (1977).
35. Murray, V. & Holliday, R. *FEBS Lett.* **106,** 5–7 (1979).
36. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L. & Steitz, J. A. *Nature* **283,** 220–224 (1980).
37. Kozak, M. *Cell,* **15,** 1109–1123 (1978).
38. Brockman, W. W. & Nathans, D. *Proc. natn. Acad. Sci. U.S.A.* **71,** 942–946 (1974).
39. Penman, S. *J. molec. Biol.* **17,** 117–130 (1966).
40. Schaffner, W., Kunz, Daetwyler, H., Telford, J., Smith, H. O. & Birnstiel, M. L. *Cell* **14,** 655–671 (1978).
41. Aleström, P., Akusjärvi, G., Perricaudet, M., Mathews, M. B., Klessign, D. F. & Pettersson, U. *Cell* **19,** 671–681 (1980).

Theoretical analysis of transitions between B- and Z-conformations in torsionally stressed DNA

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Recently, a new structure called Z-DNA has been proposed for alternating poly(dG-dC)·poly(dG-dC) sequences based on crystallographic analysis of the hexanucleotide[1]. The Z-form is a left-handed double helix containing 12 base pairs per turn. In contrast, the Watson–Crick B-form helix is thought to have 10.4 pairs per turn of right-handed helix[2,3,8]. A cooperative, salt-induced conformational transition has been observed in poly(dG-dC)·poly(dG-dC)[4], which has been interpreted as being between the B-form (low salt) and the Z-form (high salt)[1]. We now analyse the possibility that such transitions could occur in susceptible sequences in physiological conditions as a consequence of the torsional stresses imposed by superhelicity. As *in vivo* DNA commonly occurs in a negatively supercoiled, hence underwound, state, these transitions could serve important biological functions. Both thermodynamic and statistical mechanical theories of stress-induced two-state transitions have been developed previously[5,6]. Here we apply these theories to transitions between the B-form and the Z-form in regions of appropriate base sequence. Assuming that the unstressed duplex is entirely B-form, we show that when the molecule is constrained to be underwound, susceptible regions may transform to the Z-form, thereby absorbing most of the torsional deformation. Interestingly, this transition is relatively independent of temperature.

Consider a linear segment containing N base pairs, of which N_Z sequential pairs may undergo the B–Z transition. Let this segment be twisted by q turns away from its unstressed (hence totally B-form) shape. This torsional deformation will be partitioned between local transitions to the Z-form and smooth twisting in both the B- and Z-form regions so as to minimize the total conformational free energy involved. Denote the number of base pairs per helix repeat in the unstressed B-form by $A_B = 10.4$ and in the Z-form by $A_Z = -12$ (the negative sign deriving from the presumed opposite handedness of the two forms). Let τ_B (and τ_Z) denote the twist rate at which the B-form (and Z-form) regions are deformed away from their unstressed shape. If n bases occur in the Z-form, then

$$\tau_B = 2\pi[q + (n/A) - (n\tau_Z/2\pi)]/(N-n) \quad (1)$$

where $1/A = 1/A_B - 1/A_Z$. The conformational free energy associated to this torsionally deformed structure is given by

$$F(n, \tau_Z) = a + bn + \frac{nC_Z\tau_Z^2}{2} + \frac{C_B}{2(N-n)}\left[2\pi\left(q + \frac{n}{A}\right) - n\tau_Z\right]^2 \quad (2)$$

Here, C_Z and C_B are the torsional stiffnesses of the Z- and the B-form structures, b is the free energy required to change one base pair from B to Z and a is the extra free energy needed to initiate the transition in a region. Although C_Z is not known, it is reasonable to assume that $C_Z \simeq C_B$. Stable conformations occur at those values of n and τ_Z which minimize the free energy of equation (2)[5]. It follows that the segment must be underwound

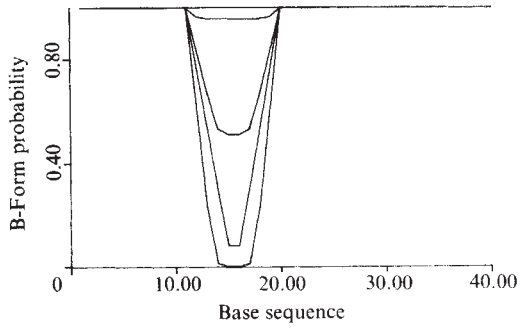

Fig. 1 The probability of being in the B-form is computed for a sequence of $N = 80$ base pairs, of which $N_Z = 8$ consecutive pairs are susceptible to transition to the Z-form. For clarity, the transition profiles of a sequence of 40 base pairs containing the susceptible region are plotted at various values of the torsional deformation q. First, when $q = 0$ all base pairs are in the B-form with probability 1. The other four curves are for $q = -0.38m$, $m = 1, 4$. As the amount of negative torsional deformation increases, so does the probability of susceptible pairs transforming to Z-form. The parameters used in this calculation are $T = 298$ K, $\sigma = 1.36 \times 10^{-6}$, $s = 3.86$. For intermediate values of q (before the transition is complete) the statistical mechanical calculation gives $\tau_B = \tau_Z = -0.4$ deg per base pair. The transition profiles calculated at $T = 353$ K are virtually identical to those shown here. These results agree well with those from the thermodynamic analysis.

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by $q < -bNA/4\pi^2 C_B$ before stable regions of Z-structure occur. When q satisfies this condition, unique non-zero values of n and τ_Z exist which minimize the conformational free energy[5]. These are

$$n = -qA - (bNA^2/4\pi^2 C_B) \quad (3)$$

$$\tau_B = \tau_Z = -bA/2\pi C_B \quad (4)$$

where $0 < n < N_Z$. (If the segment is sufficiently underwound that $n = N_Z$, then $\tau_z = \tau_B$ is found from equation (1). To illustrate these results we use values $C_B = 8.5 \times 10^{-12}$ erg base pair rad^{-2} and $b = 0.8$ kcal mol^{-1} from the literature[4,7]. Then a segment must be underwound by 0.33 deg per base pair before the onset of transition to structures containing stable Z-form regions. When deformed beyond this threshold, enough susceptible base pairs switch to Z-structure for the twist rates to be maintained at $\tau_B = \tau_Z = -0.33$ deg per base pair. Only when the segment is so underwound that all susceptible sequences are in the Z-form do τ_B and τ_Z decrease below -0.33 deg per base pair.

To augment the above treatment we apply the statistical mechanical theory of two state transitions in torsionally stressed DNA[6] to the present problem. This renders computable the transition probability profiles of specific base sequences, which more accurately reflect the fluctuational character of these transitions.

The partition function for this transition is[6]

$$Z = \sum_{n=0}^{N_Z} \left[I_n \sum_{r_n} (s^n \sigma^\gamma) \right] \quad (5)$$

where r_n enumerates all states containing n Z-form base pairs, s is the equilibrium constant for a susceptible base pair, σ is the cooperativity factor and γ is the number of beginnings of sequences of base pairs in the Z-form. Also,

$$I_0 = \exp(-2\pi^2 q^2 C_B \beta/N), \quad (6a)$$

$$I_n = \left(\frac{2\pi(N-n)}{NnC_B\beta}\right)^{1/2} \exp\left(\frac{-2\pi^2 C_B \beta (q + n/A)^2}{N}\right), \quad 0 < n \leq N_Z \quad (6b)$$

From this partition function one can determine transition profiles, the expected value of τ_B and other equilibrium properties of the system as described in ref. 6. Figure 1 shows the results of a sample calculation carried out on a sequence of 80 base pairs, of which 8 were susceptible to the transition. Note that because the enthalpy of the B–Z transformation is zero[4], this two-state transition is not thermal in character, but rather depends primarily on the imposed torsional deformation q.

We have presented two theoretical analyses of transitions between B-form and Z-form in torsionally stressed DNA. In this initial formulation the possible role of local denaturation (perhaps necessary at the junction between B-form and Z-form regions, for example) has not been considered. A three-state treatment of this problem will be presented elsewhere. Despite lack of precise knowledge of several important parameters (torsional stiffness C_Z, cooperativity factor σ), the present analysis predicts that B–Z transitions could occur in response to the torsional deformations consequent on (negative) DNA superhelicity. In principle, it is possible that similar transitions also occur between other conformational states of the DNA. It has been suggested that stress-induced conformational transitions at defined sequences may serve diverse regulatory functions by altering the accessibility of the bases[5].

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1. Wang, A. *et al. Nature* **282,** 680–686 (1979).
2. Wang, J. *Proc. natn. Acad. Sci. U.S.A.* **76,** 200–204 (1979).
3. Levitt, M. *Proc. natn. Acad. Sci. U.S.A.* **75,** 640–644 (1978).
4. Pohl, F. & Jovin, T. *J. molec. Biol.* **67,** 375–396 (1972).
5. Benham, C. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3870–3874 (1979).
6. Benham, C. *J. chem. Phys.* **72,** 3633–3639 (1980).
7. Barkley, M. & Zimm, B. *J. chem. Phys.* **70,** 2991–3007 (1979).
8. Crick, F., Wang, J. & Bauer, W. *J. molec. Biol.* **129,** 449–461 (1979).

Metarhodopsin I/metarhodopsin II transition triggers light-induced change in calcium binding at rod disk membranes

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The hypothesis of Yoshikami and Hagins[1] that calcium ions act as diffusible transmitter molecules between the photochemistry of rhodopsin and the subsequent electrical events at the outer plasma membrane of rods initiated many investigations on light-stimulated calcium release in vertebrate photoreceptor cells (see refs 2, 3). Although it now seems firmly established that light has some effect on the redistribution of calcium in various disk preparations[2,4,5], reconstituted systems[6,7] and intact rod outer segments[3,8], the physiological significance remained unclear. We previously reported a rapid, light-triggered calcium release from binding sites at the disk membrane in the presence of calcium ionophore A23187 (refs 3, 8). However, there is no evidence for rapid calcium release into the cytosol in the absence of ionophore. On fragmentation of intact rod outer segments, calcium release due to a light-regulated change of calcium binding appeared almost completely abolished[3,8]. We describe here experiments with sonicated rod outer segments in which the previously observed loss of the calcium release capacity has been prevented. Calcium release in sonicated disks in the presence of A23187 kinetically follows the metarhodopsin I/metarhodopsin II transition ($\tau_{1/2} = 10$ ms, activation energy $E_A = 34$ kcal mol^{-1}), suggesting that calcium release is triggered by this photochemical transition.

Intact cattle rod outer segments have been isolated according to Schnetkamp *et al.*[9]. Disk vesicles were obtained by sonication of intact rod outer segments in a medium containing sucrose, 600 mM; Ficoll 400, 5% v/v; HEPES, *p*H 7.0 2 mM, for 30 s at 40 W. The suspension also contained about 3–4 μM of free calcium introduced by the stock suspension of rod outer segments.

Inhibition of calcium release can be prevented by keeping the ionic strength of the final suspension medium below 5 mM of 1:1 electrolyte, irrespective of the fragmentation procedure (lysis or sonication) and the fragmentation conditions (for example, high or low ionic strength). We conclude that the apparent abolition of the release capacity in fragmented rods is caused by regulation of calcium binding by cations other than calcium which had no access to the site of calcium release in both rods with a leaky and an intact plasma membrane.

Rapid calcium release was followed by means of kinetic flash spectrophotometry with the calcium indicator arsenazo (III). Details on working with arsenazo (III) and the principle and instrumentation of flash spectrophotometry have been reviewed elsewhere[3,10]. In a suspension of sonicated disks buffered with only 2 mM HEPES at *p*H 6.8, *p*H measurements with the *p*H indicators bromcresol purple and Mg-arsenazo (III) (ref. 11) demonstrated that the total proton buffering capacity (membranes and HEPES) is sufficient to suppress light-induced *p*H changes which might give rise to *p*H-induced absorption changes of arsenazo (III) (not shown). To obtain kinetically undistorted recordings of calcium release in sonicated material at higher time resolution (Fig. 1*a*), signals due to the intrinsic photochemistry of arsenazo (III) (ref. 3) have been recorded separately and subtracted from the superimposed calcium-indicating signals by means of a signal averager with a data reduction program (Nicolet 1072). The signal-to-noise ratio was improved by sampling of signals over several repetitions from one cuvette.

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