

production, and per capita traditional fuel consumption together explained 42% (all tropical countries), 33% (Africa), 96% (Asia) and 48% (Latin America) of variance in deforestation rate.

On the other hand, a forward stepwise regression analysis using independent variables that were significantly correlated ($P < 0.05$) with deforestation in tropical countries showed that population density ($\beta = 0.57$), external debt ($\beta = 0.52$) and cattle density ($\beta = 0.32$) are important in that order when all countries are considered together (Fig. 1). In Africa, population density has greatest effect on deforestation rate, in Asia crop-land area, and in Latin America cattle density. Again, the most significant results of our analyses are the differences between regions, also revealed by other analyses^{1,9}.

Our analyses point to several possible causes of deforestation and have important policy and management implications. The availability of remote-sensing imagery and local data on socioeconomic variables, give us the potential to identify accurately the causes of deforestation at smaller scales; as we have done for the Sarapiquí region in Costa Rica (A. Sanchez-Azofeifa, S. D. and K. S. B., unpublished results) and the western Ghats, India (S. Menon & K. S. B., unpublished results). Policies to stem deforestation must take into account regional as well as country-wide differences. More emphasis is needed on land distribution and agrarian reform in Latin America, where much of the land is used inefficiently for cattle ranching, and in Asia, alternatives must be found for subsistence-level agriculture, which drives agricultural frontiers onto marginal lands occupied by forests.

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Kinked DNA

Kinks are thought to be important in DNA–protein interactions¹ but evidence for their existence in free DNA has previously been indirect. The atomic force microscope (AFM) allows direct visualization of molecular structure *in situ*^{2,3}, and using our newly developed AFM (magnetic a.c. mode or MacMode)⁴ we have observed small DNA circles change from smoothly bent to abruptly kinked shapes. This effect was dependent on the presence of specific divalent cations. The DNA was bent smoothly in the presence of Mg^{2+} , but consisted of nearly straight segments connected by kinks in the presence of Zn^{2+} .

Tandem sequence repeats of $d(A)_5$ and $d(GGGCC[C])$ bend DNA⁵. We ligated the following oligomer:

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CCCCAAAAGGGCCAAAAGGGCCAAAAGGGCCAAAAGGG
TTTTTCCCGGTTTTTCCCGGTTTTTCCCGGTTTTTCCCGGG
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to produce DNA circles. Maximum formation of circles occurs when the ligated oligomers are 126-base-pairs long. DNA longer than this forms superhelices, and thermal fluctuations are needed to bring the ends together. As a result, the larger closed circles are axially strained on ligation. We extracted circles and purified them as described elsewhere⁶. We placed DNA solutions (0.5 mg ml^{-1} in 1 mM Mg^{2+} or Zn^{2+}) into the sample cell of a scanning probe microscope (PicoSPM; Molecular Imaging Corp., Phoenix, Arizona) where the molecules adsorbed spontaneously onto a mica substrate. Images were obtained *in situ* and remained stable on repeated scanning.

DNA images obtained in the MacMode had an average full-width at half-height of 3.5 nm, so the instrumental broadening is little more than 1 nm, a significant improvement in resolution over previous AFM technologies. DNA circles of 168 base pairs in 1 mM MgCl_2 are smoothly curved, with less than one abrupt bend or kink per molecule (Fig. 1a).

When molecules from the same sample are imaged in 1 mM ZnBr_2 , their appearance changes dramatically (Fig. 1b). The DNA consists of nearly straight segments connected by abrupt kinks. This phenomenon was not observed in 126-base-pair circles, so we conclude that axial strain is required for this conformational change. The relative lack of kinks in the presence of Mg^{2+} and the absence of kinks in smaller circles under all ionic conditions demonstrate that single-strand breaks did not cause the kinks.

The strong enhancement of kinking observed here in the presence of Zn^{2+} is qualitatively consistent with earlier electron microscopy⁷ and electro-optical⁸ studies. If

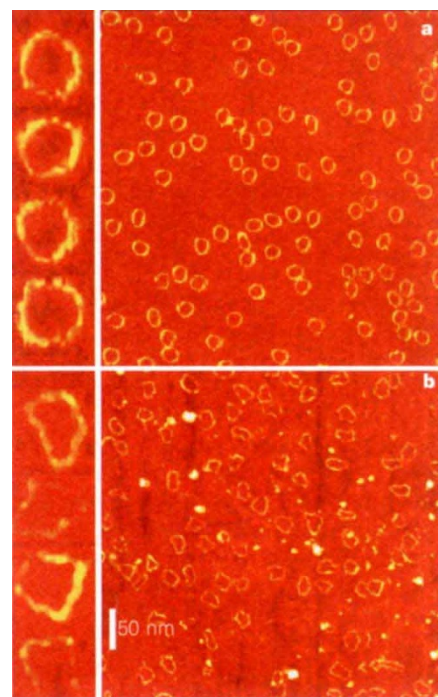


Figure 1 Images of 168-base-pair DNA minicircles in 1 mM MgCl_2 (a) and 1 mM ZnBr_2 (b), showing a fourfold increase in kink density in Zn^{2+} . Selected molecules are displayed magnified by a factor of four to the left of each image. The tip was oscillated at 25 kHz with an amplitude of 5 nm and the image was acquired in five minutes.

kinks are readily generated *in vivo* through a combination of axial strain and appropriate ionic conditions, then one might speculate that local writhing stress, to which DNA is subjected during various regulatory events, may promote the formation of localized kinks at specific DNA sequences. These could serve as signals for the assembly and/or localization of nucleoprotein complexes.

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