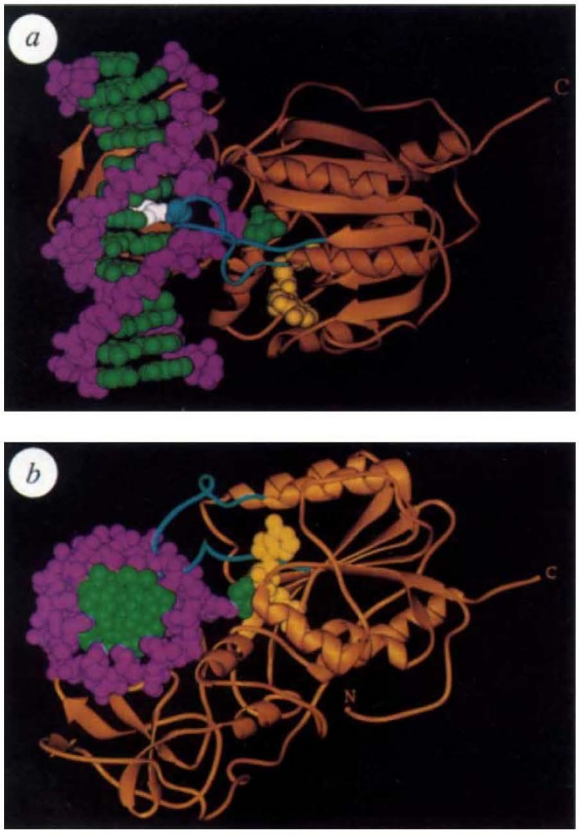

FIG. 2 Two views of the ternary complex formed by the M.HhaI m5C-MTase, a 13-mer DNA duplex containing its recognition sequence, and AdoHcy, the end product of the reaction. (DNA bases, green; sugar–phosphate backbones, magenta. Protein, brown; active-site loop and Ser 87, blue; Gln 237 on one of the two glycine-rich recognition loops, white. Cofactor AdoHcy, yellow). *a*, View normal to the helix axis, with the large domain on the right and the small domain positioned behind the DNA. *b*, View down the helix axis with the large domain on the right and the small domain below the helix. The DNA nestles in a cleft between the large and small domains.

large domain, as are most of the invariant residues which are positioned in the loop regions facing the cleft. The small domain contains the variable regions postulated to recognize the target sequence. The catalytic Cys 81 residue is in a loop region in the cleft adjacent to the AdoMet-binding pocket. However, the 10 Å separation between the donor methyl of AdoMet and the sulphur atom of Cys 81 in the binary complex suggested that a conformational transition must occur to bring these atoms closer together on formation of the ternary complex with DNA.

Now that we have a ternary structure[3], with a resolution of 2.8 Å, what does it tell us? Two views of the complex appear in Fig. 2 and show its unique recognition features. The m5C-MTase interacts with the DNA using both the active-loop site on the large domain (residues 80–99; blue in Fig. 2) which contacts the minor groove, and two glycine-rich loops on the small domain (residues 233–240 and 250–257) which contact the major groove. This results in the d(G-f^{5}C-G-C) binding site on the DNA duplex being enveloped by loop segments of the enzyme on opposite faces of the helix (Fig. 2). The active-site loop containing the catalytic nucleophilic Cys 81 residue on the large domain shifts by 25 Å towards the DNA-binding cleft on proceeding from the binary to the ternary complex. The target cytosine has looped out of the helix and become embedded in the large domain of the enzyme, while its space in the helix is occupied by Gln 237 from one of the glycine-rich recognition loops originating in the small domain of the enzyme (Fig. 2).

The base-specific interactions are restricted to the central d(G-f^{5}C-G-C) segment of the duplex, and include several intermolecular hydrogen bonds involving main-chain atoms on the enzyme. The m5C-MTase contacts six adjacent phosphates on the methylated strand and four phosphates on the complementary strand, the outermost phosphates on the complementary strand spanning a footprint of ten base pairs. Most of the intermolecular contacts involve direct interactions, but also include one base-specific and one phosphate contact bridged by water molecules. This complex represents a reaching out between extended segments on the enzyme and the DNA, and the resulting intermolecular interactions constitute a handshake at the molecular level.

The DNA oligomer in the ternary complex consists of a dodecanucleotide duplex with single-base overhangs, and adopts helical parameters characteristic of a B-DNA helix, with standard Watson–Crick base pairs, except for the segment centred about the target cytosine site. The dihydrocytosine intermediate (**2** in Fig. 1) that results following Michel addition and methyl transfer adopts a C4′-*exo* sugar pucker and a *syn* glycosidic torsion angle. Both the base and sugar of the target cytosine are looped out of the helix, and this is accompanied by large distortions of the flanking phosphates that propagate in the 5′ direction along the phosphodiester backbone. The resulting increase in the interstrand phosphorus–phosphorus distance provides a pathway for looping the cytosine out of the helix. The Cys 81 is covalently linked to the C6 position of the targeted cytosine, with the

RÉSUMÉ

Whirls apart

THE feeling of sitting in the midst of chaos is not an uncommon one. But, say Richard L. Kautz and Bret M. Huggard, anyone who really wants to experience chaos should try the nearest amusement park (*Am. J. Phys.* **62**, 59–66; 1994). Their subject is a fiendish ride known as the 'Tilt-a-Whirl' with seven cars each spinning freely about the centre of its own platform, hurled around an undulating track by radial arms. Kautz and Huggard's mathematical description of the ride shows it to be a fine example of deterministic chaos, characterized by apparently random dynamics and extreme sensitivity to initial conditions. Shifting position in the car, for instance, can turn a tame ascent into a frenzied whirl. Not recommended reading for the queasy.

Gutter press

THERE's no need to scour the Amazon jungles or the depths of the sea in search of species as yet innocent of scientific description — R. Bertolani and I. M. Kinchin found theirs in the sediment clogging a rain gutter in Guildford, as remote a place as any within a short train ride from London. Bertolani and Kinchin were looking for tardigrades (water bears), microscopic creatures thought to be akin to arthropods. Among five species recovered, one was distinguishable from known tardigrades by its ornate egg-shell architecture in particular, and it now rejoices in the name *Ramazzottius varieornatus* (*Zool. J. Linn. Soc., Lond.* **109**, 327–333; 1993).

Piece work

As all children know, one way to find out how something works is to take it to pieces and try the components in different combinations. Such is the tack taken by H. Stenmark *et al.* (*EMBO J.* **13**, 575–583; 1994), who have created hybrids of two members of the rab family of GTP-binding proteins. These proteins are found in distinct organelles in the cell, and each seems to have a highly specific job in directing particular vesicles to a particular destination. Stenmark *et al.* find that to make one of the rabs carry out the other's function not only did they have to transfer the C-terminal domain known to be important in targeting, but also the N terminus and two other domains involved in nucleotide-dependent conformational changes. Hybrids such as these will be of great help in identifying accessory proteins which interact with rabs and assist in taking vesicles to their correct destination.

Second thoughts

THIS year there will be a leap second on 30 June between 23:59:59 and 00:00:00 Universal Time, by decree of the International Earth Rotation Service (*IAU Circ.* No. 5929). Plan now to make the most of the extra time.