

Looping Out the DNA of *E. coli*

from our Molecular Genetics Correspondent

THE enormous degree of condensation of the eukaryotic chromosome is well known; and its structure seems to be maintained by interactions between DNA and its protein components. But in bacteria, no nuclear membrane confines the genome and no proteins seem to be required to maintain its structure. Yet the DNA of *Escherichia coli* is some thousand times longer than the bacterial cell and has the compact appearance of a "nuclear body".

Many attempts to isolate bacterial DNA have concentrated on preparing long threads of the unbroken genetic material, but the bacterial DNA typically seems to be highly folded within the cell. Stonington and Pettijohn (*Proc. US Nat. Acad. Sci.*, **68**, 6; 1971) first developed a method in which cells are lysed so that their genetic material can be obtained as a very rapidly sedimenting complex, about 80 per cent DNA by weight and containing some 10 per cent each of protein and RNA. The protein seems to be mostly RNA polymerase and much of the RNA is mRNA and rRNA. But the compact structure of the complex is lost when the RNA is degraded by ribonuclease, which argues that the RNA may play some structural part in holding the chromosome together.

Worcel and Burgi, using this method of preparation (*J. Mol. Biol.*, **71**, 127; 1972), report that they have prepared folded chromosomes from *E. coli* and find that they seem to have a definite organized tertiary structure. The complex sediments rapidly, but within an extensive range of S values, from 1,300S to 2,200S. One possible explanation for the breadth of this range is that the 2,200S structures represent chromosomes near the end of their replication cycles, with almost twice as much DNA content as the 1,300S structures derived from chromosomes at the beginning of the cycle. This idea is supported by an experiment in which bacteria were starved of amino-acids; this treatment causes chromosomes to accumulate at the initiation step of replication. Cells treated in this way have a much sharper peak when the folded chromosomes are centrifuged, with a value of about 1,300S.

The dye ethidium bromide intercalates between the base pairs of DNA and causes a change in the pitch of the double helix—this can be detected because it creates superhelical turns in a circular DNA molecule such as that of *E. coli*. Worcel and Burgi found that all the chromosomes in the preparation of folded DNA genomes behaved under this treatment as though comprising

perfect circular double helices, with no nicks at all in the chromosome. But most of the DNA single strands obtained by denaturation have about two breaks each, making four breaks in the double-stranded chromosome. And, of course, there must be structural discontinuities in DNA at the site of replication. The chromosome is not immediately unwound when nicks are introduced by treating the folded preparation with DNAase, but sediments more slowly as more nicks are made, until reaching a minimum value of 900S when six and forty nicks have been introduced into the complete duplex molecule. These nicked complexes behave as linear duplex molecules when ethidium bromide is added.

The complex can also be unfolded by treatment with RNAase, SDS, or mechanical shear. The effect of RNAase is quite unlike the effect of DNAase, for it is all or none—some chromosomes are completely unfolded by RNAase whereas others are completely unaltered. This implies that a single RNA species, perhaps one molecule in each chromosome, may be implicated in maintaining the structure of the genome.

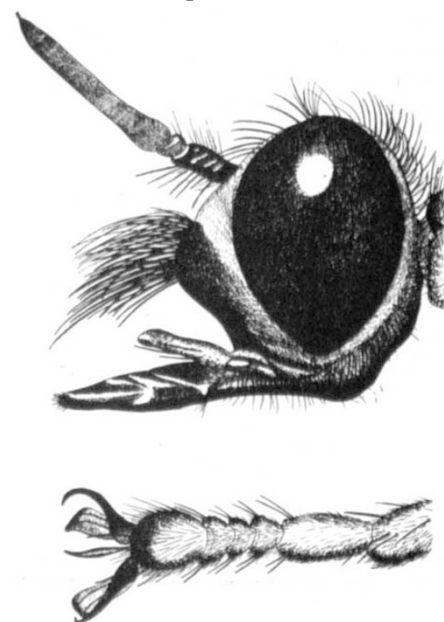
The paradoxes in these results are reconciled by a model in which Worcel and Burgi suggest that the *E. coli* chromosome consists of a large number of loops, about fifty, each of the same superhelical concentration. Rotational events must be unable to propagate from one loop to the next. Each nick (either made spontaneously during preparation or by treatment with RNAase) must relax the coiled structure of only one loop. The loops are held together by a core which may be an RNA molecule; any disruption of this core unfolds the DNA. One can only speculate about whatever interactions may exist between DNA loops and the RNA core. If there are fifty loops in a chromosome, each loop if extended would still be twenty times the diameter of the nuclear body of the bacterium. Worcel and Burgi therefore suggest that each loop contains about 200 turns of a DNA coil, with about 400 base pairs per turn.

Topological questions about the state of DNA must now therefore be asked about bacterial genomes as well as eukaryotic chromosomes. The most important problem is the nature of the forces which act between DNA and RNA to hold the loops to the core. Another point to be resolved about the structure itself is how much specificity exists in the constitution of the loops. Replication overall can be accounted

for by treating the chromosome as containing three sites of nucleation, two for the daughter chromosomes under synthesis which expand at the expense of that of the parental chromosome. This leaves unanswered, however, more local problems such as how DNA passes through the replication apparatus and how genes are always available for transcription. The conventional pictures of extended DNA as the active form of the *E. coli* chromosome will clearly prove to be a great oversimplification.

ENTOMOLOGY

A Grasping Robber



ROBBER flies (Asilidae), as their name suggests, are well adapted for a predatory way of life; they have not only powerful clawed legs for catching and holding their prey, but also a strong proboscis with which to pierce their victims.

One of the special features of robber flies is a stout bristle, or empodium, between the two claws of each foot. In an asilid from South Africa this structure is so well developed, particularly on the middle and hind legs, that a new genus and species, *Empodiodes greatheadi*, to accommodate the fly has been created by H. Oldroyd of the British Museum (Natural History) (*J. Nat. Hist.*, **6**, 635; 1972). The figures show a profile of the head of the new asilid, and its fore leg which displays the empodium between the two claws and the pulvilli or pads.