tion of higher conformational energy. Reasonable conjectures can then be made from Perutz's structural data about the segments associated with the responsive groups of protons. The method clearly has great potential for wider exploitation, based as it is on the amplification of energetically minuscule events.

DNA SYNTHESIS

Filling the Gaps

from our Molecular Genetics Correspondent

THE idea first proposed by Okazaki that DNA chains are synthesized in short pieces which are only later joined together explains many of the problems of replications, in particular how two chains of opposite polarity are synthesized by an enzyme which moves in only one direction. But no DNA polymerase yet discovered is able, *in vitro* at any rate, to start synthesis of a chain. A solution to the problem of how each Okazaki fragment is started was first proposed by Okazaki and his colleagues last year and is now expanded in more detail by Hirose, Okazaki and Tamanoi (*J. molec. Biol.*, **77**, 501; 1973).

Each fragment of DNA starts off with a length of RNA according to this model; the RNA is removed before synthesis of the fragment is completed. The RNA-DNA nascent fragments can be isolated by their buoyant density, greater than that of single strands of DNA, in CsCl gradients. The proportion of RNA can be estimated from the extent of the buoyant density shift and Hirose *et al.* report that pulse-labelled Okazaki fragments of 400–2,000 nucleotides contain an RNA segment of 50–100 nucleotides. The same length is estimated by measuring the decrease in the length of the fragments when the RNA moiety is degraded by alkali or RNase; and RNA chains of this length can be isolated after degradation of the DNA moiety with DNase.

If the RNA chain initiates each fragment, it must be located at the 5' end. The small shift in size of the fragment when RNA is removed shows that it must be located at one end; labelling the 5' base by removing its phosphate and replacing it with ^{32}P confirms that it is part of RNA. After the RNA has been degraded, similar experiments can be used to label the first base of the remaining DNA chain. The number of 5' RNA and DNA termini labelled in this way is always identical, confirming that one RNA sequence lies at the beginning of each DNA fragment. The first base of the DNA chain is always cytidine and the last base of the RNA is always a pyrimidine, so that it is possible that a specific sequence—presumably repeated very many times in the *Escherichia coli* genome—provides the point of switch from RNA to DNA.

The ineluctable progress of Okazaki's research therefore makes inescapable the conclusion that DNA chains must start with RNA, leaving for resolution the important questions of the sequences (if any) which dictate where RNA synthesis begins and ends and the nature of the RNA polymerase which transcribes them.

While or after the RNA is removed by an unknown mechanism, the 3' end of one Okazaki fragment must be extended to the 5' end of the next fragment. But this leaves a break in the integrity of the phosphodiester chain between the two fragments. The existence and making good of this nick has been one of the more controversial aspects of the Okazaki model for discontinuous growth of DNA chains. The enzyme polynucleotide ligase has been implicated in this action during phage T4 replication, but mutations in the ligase of *E. coli* have produced more equivocal results, taken to cast some doubt on the idea that ligase has this function in bacterial replication.

Mutations in the ligase gene of *E. coli* have now been placed in a standard genetic background by Gottesman, Hicks and Gellert (*ibid.*, 531) and Konrad, Modrich and Lehman (*ibid.*, 519). One ligase mutant, *lig-4*, was originally isolated as a double mutant, *lig-4 lop-8*. The *lig* mutation produces a ligase enzyme defective at high temperature; *lop* increases the amount of enzyme in the cell without altering its sequence and is therefore a control mutation of some kind. Both map close together. Another ligase mutant, *lig ts-7*, was isolated in a genetic background containing many other mutations and has now been separated from them; *lig-4*

has been separated from *lop-8*, so that two strains of ligase mutant are available for analysis.

Both strains have at high temperature a much impaired ability to join together Okazaki fragments and therefore accumulate 10S pieces of DNA. Although the *lig ts-7* mutant is a conditional lethal which dies at high temperature, the *lig-4* mutant can grow in these conditions. The cells of *lig ts-7* are less active in ligase function at high temperature than those of *lig-4*, so that the continued viability of the latter mutant may be due to the persistence of some ligase activity even at high temperature.

Ligase is clearly identified by these experiments as the enzyme responsible for the final step in joining together the Okazaki fragments. Because double mutants of *polA* and *lig-4* are inviable at high temperature, it is possible that the Kornberg polymerase I may also be involved in this step and perhaps in the removal of RNA and extension of Okazaki fragments until they are adjacent. The *lig-4* cells have only 1% of the wild type ligase activity at high temperature; *lig-4 lop-8* double mutants have about 5%. Both cell types can grow, although defective in sealing Okazaki fragments together; this points to the conclusion that cells contain far more ligase activity than they need, explaining the reason for mutations producing equivocal results when higher levels of ligase activity are possible. It is necessary to achieve a very great reduction in the ligase activity to prevent its action in replication. The purpose of the excess ligase activity is a matter for speculation at present, but it may be implicated in repair and recombination pathways.

X-Ray Flux from the Vela Pulsar

IN next Monday's *Nature Physical Science* (August 20) Cruise and Newton report X-ray observations of the vicinity of PSR0833-45 (the Vela pulsar) and two other pulsars. Because there is a strong dependence of pulsar luminosity on pulsar period, it is not surprising that no X rays were detected from MP0833-40 (period 0.766 s) or from MP0736-40 (period 0.375 s), but they were observed because of their proximity on the sky to the Vela pulsar (period 0.089 s). The observations of the latter, however, help to provide constraints on the nature of the pulsar's spectrum (see figure).

The observations were made using a proportional counter sensitive in the range 2.8 to 9.9 keV, mounted on a Skylark rocket flown from Woomera on November 11, 1970. Together with two positive observations by Harnden (*Astrophys. J. Lett.*, **172**, L91; 1972) and upper limits due to Bunner (*God-*

ard Space Flight Center Report X-683-71-375), the latest upper limits require that the spectrum is either a power law with exponent -1 or an unusual spectrum with a double peak in the X-ray region. A power law spectrum of injected electron energies, with exponent -2 , would produce the required X-ray spectrum, according to Cruise and Newton.

Pulsed energy spectrum of the Vela pulsar, PSR0833-45.