provide a model for studies of nitrogen-fixation genes, but may also enable one to produce a range of plasmids carrying mutations in known Nif genes that can be used for complementation studies of these genes in other bacteria. Furthermore, studies of the transfer and expression of the *Klebsiella* Nif genes in a range of nitrogen-fixing and non-fixing bacteria should help to answer some of the simplest questions that can be asked about the feasibility of performing wide intergeneric transfers of nitrogen-fixation genes. In this respect the observation that transfer to *Pseudomonas aeruginosa* did not occur is slightly discouraging, since RP4 (the parent plasmid) was isolated in this organism and a derivative of it carrying the *E. coli* histidine operon produced by Olsen and Gonzalez (*Biochem. biophys. Res. Commun.*, **59**, 377; 1974) is both transmissible to, and stable in, *P. aeruginosa*. The other point concerns the formation of the RP4-Nif plasmid. This was made in *E. coli* as the result of recombination between an F′ Nif previously produced by Cannon, Dixon and Postgate (*J. gen. Microbiol.*, in the press) and the R factor RP4. In the present report Dixon, Cannon and Kondorosi suggest that this recombination occurred as a result of an interaction between identical translocating sequences carried on both plasmids. These sequences, corresponding to the carbenicillin resistance determinant on RP4 (TnA) and the same resistance determinant on the F′, are capable of translocating on to a wide range of plasmids (Hedges and Jacob, *Molec. gen. Genet.*, **132**, 31; 1974). If the authors' suggestion that these translocating sequences can indeed facilitate recombination between plasmids that carry them, then they may well have demonstrated a general procedure for producing recombinant plasmids *in vivo*.

These studies would not have been possible without the availability of a group of R factors having the apparently unique property of being transferable to a very wide range of bacteria. The R factors, which all belong to the incompatibility group P (Datta *et al.*, *J. Bact.*, **108**, 1244; 1971), first appeared in the late 1960s in isolates of *Pseudomonas aeruginosa* found to be highly resistant to carbenicillin, a particularly efficient semi-synthetic penicillin for use in pseudomonas infections. Studies of these pseudomonads soon showed that in many of the isolates resistance to carbenicillin was plasmid borne (that is, R-factor mediated) and linked to determinants for resistance to tetracycline and kanamycin. Although at the time a number of plasmids were known in *Pseudomonas*, R factor mediated drug resistance in these exceptionally antibiotic-resistant bacteria had not been reported. The most unusual property of these R factors, however, was their ability to transfer from *P. aeruginosa* to *E. coli* (Sykes and Richmond, *Nature*, **226**, 952; 1970). Although the publications that have followed this original observation suggest that all Gram-negative bacteria can act as recipients, as yet no report of transfer by conjugation or transformation to a

Membrane nearest neighbours

from Howard Goldfine and Alan F. Horwitz

ALTHOUGH the series of twelve papers describing the total synthesis of the structural gene for a tRNA precursor in the February 10th issue of the *Journal of Biological Chemistry* is undoubtedly the more impressive achievement, two other papers from the laboratory of H. Gobind Khorana may be of even greater interest to those concerned with membrane structure and function. These papers describe the synthesis of various photosensitive fatty acids and phospholipids and provide evidence that these compounds can be incorporated into natural biological membranes. These new analogues should prove useful as membrane probes to study previously inaccessible interactions between lipid and protein in the membrane.

Chakrabarti and Khorana (*Biochemistry*, **14**, 5021–5033; 1975) describe the relatively easy four-step synthesis of azidostearic acids from commercially available hydroxystearate isomers. By standard synthetic methods they have put these and other photosensitive fatty acids on the middle carbon of the glycerophosphate backbones of phosphatidylcholine and phosphatidylethanolamine, both with palmitate esterified at the C-1 position. When the phospholipids were dispersed in an aqueous medium as sonicated vesicles and irradiated at 253 nm for 1 hour, extensive photolysis and cross linking occurred.

Greenberg, Chakrabarti and Khorana (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 86–90; 1976) now report that an unsaturated fatty acid requiring mutant of *Escherichia coli* utilises the series of azidostearic acids with the azido group at various positions along the hydrocarbon chain. Most of these compounds were capable of supporting growth to the extent observed with the common unsaturated fatty acid, oleic acid. 12-Azido-oleic acid, a 16-azido *trans*-hexadecenoic acid, and two fatty acids with a bulkier 4-azido-2-nitrophenoxy group were also active, but the azidonitrophenoxy group on oleic acid did not permit growth at or above 25 °C. These studies show that the azido fatty acids are incorporated into cellular phospholipids to the extent usually observed with *cis*-monounsaturated fatty acids. Furthermore, they were incorporated largely at the middle carbon of the glycerophosphate backbone as is usually the case with unsaturated fatty acids. Since the photosensitive fatty acids behave like unsaturated fatty acids, the MIT group looks forward to being able to study the interactions of lipids with functional membrane proteins.

When irradiated the azido groups form highly reactive intermediates, which attach covalently to nearest neighbours. This cross linking should provide information on the amino acid residues in close apposition to phospholipids, and since the photosensitive group can be placed near either the carboxyl or methyl terminus of the fatty acid chains, it should be possible to determine the depth of penetration of proteins into the bilayer and to map the exposed amino acid residues as well.

Reconstitution of membrane proteins with specific photosensitive phospholipids provides an alternative means of determining such spatial relationships. The polar head group of the lipids is another potential site for lipid–protein interaction, and two affinity labels have already been synthesised by Chakrabarti and Khorana, in which a polar photoactivable group replaces ethanolamine.

Extension of their work with an *E. coli* mutant to a similar mutant of a *Pseudomonas* species is already in progress, and they visualise future work with *Neurospora* and yeast mutants and even with mammalian cells grown in fatty acid deficient media. Although not specifically mentioned, questions of lipid–lipid "nearest neighbours" and membrane lipid asymmetry can also be approached with these compounds in either natural membranes or artificial systems. One caution, however, is noted. The azido group absorbs weakly in the ultraviolet and there is obvious overlap with nucleic acid and protein absorption. The use of radioactively tagged azido fatty acids may permit shorter irradiation times but other more photoreactive groups are being sought.