Irradiation for a period of 20 min reduced the survival of conidia to 20%. The growth of irradiated conidia in the presence of bleomycin, mitomycin C or Esidrex is associated with a 2- to 3-fold increase in the frequency of gene convertants, but was not accompanied by an increase in point mutants. When conidia were grown on cellophane but otherwise treated as before the frequency of gene convertants was increased 8-fold, but induction of point mutants was negligible. This effect was the same for irradiated and un-irradiated conidia. The environment created by the cellophane in contact with the medium appears to affect the action of each of the three compounds synergistically.

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Use of [3H]tetrahydrocerulenin to assay condensing enzyme activity in Streptomyces erythreus

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Erythronolide synthase from Streptomyces erythreus catalyses the successive head-to-tail addition of six methylmalonyl-CoA units to a propionyl-CoA primer, to form the 14-membered lactone ring of 6-deoxyerythronolide b, a key intermediate in the biosynthesis of erythromycins. Analogous enzymes are involved in the formation of a wide range of polyketide natural products (Omura & Nakagawa, 1981). Because of the very low activity of erythronolide synthase in cell-free extracts (Wawzskiewicz & Lynen, 1964) and its apparent instability upon fractionation (for a review see Corcoran, 1981), almost nothing is known about its structure and mechanism. However, the reaction it catalyses bears a strong resemblance to some of the reactions involved in fatty acid biosynthesis.

We have synthesized [3H]tetrahydrocerulenin (Roberts & Leadlay, 1983), a radiolabelled analogue of cerulenin (2R,3S-epoxy-4-oxo-7,10-trans,trans-dodecanoic acid amide), an antibiotic from Cephalosporium caerulens, which is a potent site-specific inhibitor of the β-keto-acyl-ACP synthase from almost all sources tested (Omura, 1981). Cerulenin inhibits the formation of erythromycin in resting cells of S. erythreus and it also prevents the synthesis of 6-deoxyerythronolide b in cell-free extracts (G. Roberts, unpublished work). We have therefore examined extracts from cells at the onset of erythromycin production for the presence of [3H]tetrahydrocerulenin-binding proteins.

Extracts of S. erythreus (CA340, Abbott Laboratories, Chicago, IL, U.S.A.) were prepared, at 4°C, as described previously (Roberts & Leadlay, 1983). Labelling with [3H]tetrahydrocerulenin revealed a major band whose mobility on sodium dodecyl sulphate/gel electrophoresis suggested a subunit M, of about 37000. These extracts were chromatographed on a column of ω-aminohexylagarose, from which 80-85% of the protein emerged unretarded. A single major peak of condensing enzyme activity was then eluted with a salt gradient (Fig. 1). After further chromatography on a column of Sephadex G-150, calibrated with proteins of known size, most of the apparent condensing enzyme activity eluted at the position appropriate for a protein of M, about 75000 (Fig. 1). At this stage, the activity, after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, co-migrated with a distinct Coomassie-stained band, which represented about 10% of the protein present in the track.

Further purification of this material was attempted by ion-exchange chromatography, but the yield of the original

Abbreviation used: ACP, acyl carrier protein.
polypeptide was poor, and fluorography revealed two new radiolabelled bands, of approx. M, 20000 and 15000 respectively, the latter band containing almost all of the tritium label. S. erythreus produces trypsin (Nagata & Yoshida, 1981), and, although the extraction buffer contained soya bean trypsin inhibitor (20µg/ml), 2mM-EDTA and 0.1 mM-phenylmethanesulphonyl fluoride, the radiolabelled polypeptide of M, 15000 is present as a minor component even in cell-free extracts. However, when the extraction buffer was supplemented with 1mM-benzamidine and 1mM-aminobenzamidine, this component was no longer detectable. The polypeptide with an M, of 37000 was still the major radiolabelled species, but another detectably labelled species was now seen, with an M, of approx. 43000.

These results show that, using the ability to bind [3H]-tetrahydrocerulenin as an assay, an apparent condensing enzyme activity can be monitored through several purification steps. However, stringent precautions are required to prevent loss through proteolysis. Further work will be required to confirm the identity of the 37 kDa polypeptide as a condensing enzyme, and to distinguish between two possibilities. First, this polypeptide might be an active fragment of a multifunctional protein (a Type I system). The partial purification of fatty acid synthase from Streptomyces coelicolor by Flatman & Packter (1983) has shown it to be a system of this type, with an M, of approx. 350000 in its dimeric form. An alternative possibility finds its precedent in the fatty acid synthase of Escherichia coli and the polyketide flavonone synthase from parsley, which consist of discrete components of low M, (Type II systems). Thus β-ketoacyl-ACP synthase I from E. coli has a subunit M, of 40000 (Garwin et al., 1980), and the condensing enzyme of flavonone synthase a subunit M, of 42000 (Kreuzaler et al., 1979), and both exist as dimers. The condensing enzyme of erythronolide synthase could well be of this type.

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Recent advances in the methodology for recombinant DNA experiments with Streptomyces (reviewed by Hopwood et al., 1983) have enabled the cloning of structural genes for drug-resistances, auxotrophic markers and enzymes involved in the biosynthesis of secondary metabolites. The majority of cloning experiments have been done with Streptomyces lividans, which has a high efficiency of transformation (>10⁶/µg of DNA), as recipient. S. lividans also behaves phenotypically as a 'restrictionless' host: in our hands, efficiency of transformation into S. lividans was independent of the streptomycele host in which the plasmid DNA had been grown previously. However, not all Streptomyces behave in this way. The industrial strains in particular have elevated restriction activity. Presumably, they have been selected adventitiously in strain improvement programmes for this characteristic by being resistant to infection by actinophages, which resulted in more consistent/better performance in the fermenter. When these strains were used as recipients for interspecific DNA manipulations, or when Escherichia coli was the source of transforming DNA, efficiency of transformation was reduced significantly by at least a factor of 10³ and often no transformants were obtained at all. A method has been developed to select for mutants which are phenotypically 'restriction-deficient'. The mutants have good efficiencies of transformation, irrespective of the source of transforming DNA.

The methodology for construction and selection of a restriction-deficient mutant of an industrial strain of Streptomyces rimosus is summarized in Fig. 1. Nitrosoguanidine mutagenesis with 1% survival proved efficient. The source of DNA was plasmid pFZ24, a variant of the

‘Restriction-deficient’ mutants of industrial Streptomyces

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