The pathway of fatty acid synthesis is carried out by all organisms that have been examined except the archaea, bacteria, which do not contain fatty acids in their membranes (Fulco, 1983). The first seven reactions of the fatty acid synthesis pathway are identical in all eubacteria and in plant plastids such as the chloroplast (which are the exclusive site of fatty acid synthesis in higher plants) the proteins of the complex consisting of separate monofunctional enzymes, are referred to as type I1 systems. We will now consider these type I systems. We will now consider these type I systems.

Despite the basic similarity of the reaction pathway in all organisms, a remarkable variety of structural forms are seen in the enzymes of the fatty acid synthase (FAS) complex. In most eubacteria and in plant plastids such as the chloroplast (which are the exclusive site of fatty acid synthesis in higher plants) the proteins of the complex exist as six or more separate enzymes plus a small non-enzymic protein which bears the phosphopantetheine prosthetic group, the acyl-carrier protein (Ohlrogge, 1982, Fulco, 1983). In fungi, all of the six activities and the acyl-carrier function are distributed between only two non-identical polypeptide chains, with the native enzyme having six copies of each subunit (Schweizer et al., 1978) A small group of eubacteria typified by Mycobacterium spp. have all functions on one very large polypeptide chain, with the native enzyme being a hexamer similar in size to the fungal enzyme (Fulco, 1983). Finally, in vertebrates the enzyme is a dimer of identical polypeptide chains containing all seven functions (Stoops et al., 1975; Guy et al., 1978; McCarthy & Hardie, 1983)

The FAS systems found in most bacteria and in plants, consisting of separate monofunctional enzymes, are referred to as type II systems, while those comprising multifunctional enzymes are referred to as type I systems. We will now consider these type II systems. We will now consider these type II systems.

Of these possibilities, the third seems intuitively the most attractive. However, it is, of course, necessary to provide evidence in favour of this hypothesis. If the type I systems arose from type II systems by gene fusion, one can envisage three possibilities:

(1) that the different systems arose independently;
(2) that the type I systems are the ancestral type, and that the type II systems developed from them by gene splitting;
(3) that the type II systems are the ancestral type, and that the type I systems developed by gene fusion.

Of these possibilities, the third seems intuitively the most attractive. However, it is, of course, necessary to provide evidence in favour of this hypothesis. If the type I systems arose from type II systems by gene fusion, one would expect the active sites on the type I systems to still exist as relatively independent domains which would be folded in a similar manner to their monofunctional relatives. This in turn leads to two experimentally testable predictions:

(1) there should be amino acid sequence similarities between the domains of type I systems and the equivalent type II proteins;
(2) it should be possible to generate active fragments containing single functions by limited proteolysis of type I systems. We will now consider these predictions in turn and discuss the extent to which they have been fulfilled. Since the fungal type I system is the subject of another contribution to this Colloquium (Schweizer et al., 1986), I will concentrate on the vertebrate type I system which we have studied.
Sequence homologies between multifunctional and monofunctional FAS systems

The most convincing evidence for the gene fusion hypothesis has come from studies of the acyl-carrier domain. In vertebrate type I FAS systems the 4'-phosphopantetheine prosthetic group is esterified to the 250 kDa multifunctional polypeptide and not to a separate acyl-carrier protein (Stoops et al., 1975; McCarthy & Hardie, 1983). The terminal thiol of the prosthetic group can be alkylated with absolute specificity using the reactive substrate analogue, chloro-[^14C]acetyl-CoA (McCarthy & Hardie, 1982a). Using this radioactive tag we were able to purify a 10 kDa fragment derived by limited digestion of native rabbit FAS with pancreatic elastase (McCarthy et al., 1983a). This fragment is very similar in size to the acyl-carrier proteins of type II FAS systems, and shows around 30% identity in amino acid sequence with acyl-carrier protein from Escherichia coli or barley (McCarthy et al., 1983a; McCarthy & Hardie, 1984). This sequence similarity is highly significant. Fig. 1 shows a dot matrix plot comparison of the sequences of E. coli acyl-carrier protein and the rabbit acyl-carrier fragment: a strong diagonal with very little background noise is evident. The sequence around the phosphopantetheine attachment site is also highly conserved between goose and rabbit FAS, with 17 identities and one conservative replacement out of 18 residues (Poulouse et al., 1984).

The high degree of conservation of acyl-carrier proteins/domains is perhaps not surprising, since this region must interact with all of the six or seven catalytic centres of the FAS complex. It remains to be seen whether the other domains will be sufficiently conserved to allow for definitive assessment of evolutionary relationships between multifunctional and monofunctional systems. However, short amino acid sequences available at three other sites in vertebrate type I fatty acid synthases are at least suggestive of such relationships:

1. Sequences around the reactive serine residue of the single acetyltransferase domain of vertebrate FAS show some similarity with the equivalent regions of the acetyltransferase and the malonyl-palmityltransferase domains of FAS from Saccharomyces cerevisiae (McCarthy et al., 1983b; Mikkelsen et al., 1984; Schweizer, 1984; McCarthy & Hardie, 1986);

2. Short sequences around the reactive serine residue of the thioesterase domain of goose and rabbit FAS show some similarity with those around the reactive serine residue of serine proteases (Pouflage et al., 1981; Hardie et al., 1985).

3. An 11 residue sequence believed to be at or near the NADP-binding site of goose FAS enoyl reductase shows statistically significant homology with sequences in the NAD-binding domains of several dehydrogenases (Pouflage & Kolattukudy, 1983).

Isolation of active fragments from multifunctional FAS systems

Although there is elegant genetic evidence that the various activities of yeast FAS exist as independent domains (see Schweizer et al., 1986), the only success that has been achieved in isolating active fragments of yeast FAS is a 56 kDa fragment of the β-subunit that has acetyltransferase activity (Lynen, 1980). Rather more success has been obtained for vertebrate FAS systems. Several groups have isolated active thioesterase fragments (Smith et al., 1976; Guy et al., 1978; Lin & Smith, 1978; Bedord et al., 1978; Puri & Porter, 1982; Mattick et al., 1983). The active fragments have molecular masses in the region of 32–36 kDa. It is very interesting that the mammary glands of some mammals, and the uroepigal (preen) glands of waterfowl, contain distinct monofunctional medium-chain thioesterases with molecular masses of 29–33 kDa (Knudsen et al., 1976; Libertini & Smith, 1978; Rogers et al., 1982). It seems likely that these will turn out to be homologous with the integral long-chain thioesterase domains of vertebrate type I FAS systems.

If the acyl-carrier domain of rabbit FAS is isolated after limited proteolysis without prior chemical modification, the 10 kDa fragment obtained is functional, at least in the acetyltransferase reaction (McCarthy & Hardie, 1982b). Finally, the 3-oxoacyl reductase domain of chicken liver FAS has been isolated as a 36 kDa fragment (which also contains the 10 kDa acyl-carrier domain) by combined digestion with kallikrein and subtilisin (Wong et al., 1983).

Mapping of the domains of type I FAS systems

The order of the functional domains of the multifunctional polypeptide chains of type I vertebrate FAS systems has been partially established by limited proteolysis of enzyme labelled at different active sites (McCarthy & Hardie, 1983; Mattick et al., 1983; Tsukamoto et al., 1983). The three domains which have been isolated as active fragments (see above) are the three C-terminal domains, suggesting that this region is particularly flexible and contains extensive regions which may be necessary to allow the acyl carrier to interact with the other six active sites.

An interesting conclusion to emerge from these domain mapping studies is that the domains on the single polypeptide of vertebrate FAS (which occur in identical locations in mammalian and avian FAS) must be in a
different order on the two polypeptides of yeast FAS

(McCarthy et al., 1983c; McCarthy & Hardie, 1984, 1986). This suggests that the gene fusion events leading to yeast and vertebrate type I systems may have occurred independently. We have however argued, admittedly on limited evidence, that the two-chain yeast FAS and the unusual single-chain FAS of the eubacterium Myco-

bacterium smegmatis may lie on the same evolutionary pathway.

Conclusions and future prospects

There now seems little doubt that vertebrate and fungal type I FAS systems have arisen by gene fusions, probably along independent pathways. These systems now provide excellent models for studying the evolution of proteins by fusion of pre-existing genetic elements, a mechanism which may be of general applicability to other proteins. Further understanding of the evolution-
ary history of type I FAS systems will come from analysis of DNA sequences at both the cDNA and genomic levels, already well under way in the yeast system. FAS cDNA clones have already been obtained from goose uropygial gland (Morris et al., 1982) and rat liver (Nepokroeff et al., 1984). Current evidence suggests that vertebrate FAS mRNA is extremely large (9–12 kb; 6.6 kb would be required to code for the protein), and it appears that existing cloned sequences are derived from the extensive 5′-untranslated region of the mRNA. We have recently obtained the rat liver clones from the labor-
atory of the late John Porter, and are using these, and synthetic oligonucleotides based on the acyl-carrier domain amino acid sequence, to screen libraries which have been generated from cDNA selected for maximum length. When the sequence of the coding region is available, it will be very interesting to compare its sequence with those of components of type II FAS systems and other monofunctional proteins.

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The components of the plant fatty acid synthetase system

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It is now apparent that the principal, if not the only, site of fatty acid biosynthesis in higher plant cells is the plastid (Zikley & Canvin, 1972; Nakamura & Yamada, 1974; Weaire & Keckwick, 1975; Vick & Beevers, 1978, Ohlrogge et al., 1979). Those systems for which the reduntant has been examined have been found to respond to both NADH and NADPH. The reduced pyridine nucleotides have been found to act synergistically in both the Euglena (White et al., 1971) and the avocado plastid (Caughey & Keckwick, 1982) multi-enzyme systems, NADH reducing the level of NADPH at which maxi-

mum activity is obtained. The soluble synthase from

Abbreviation used: ACP, acyl-carrier protein.

Safflower seeds has been reported to require both NADH and NADPH (Jaworski et al., 1974).

Fatty acid synthetase systems from avocado (Persea americana), mesocarp plastids (Harwood & Stumpf, 1972; Caughey & Keckwick, 1982), Euglena (Hendren & Bloch, 1980), Safflower seeds (Carthamus tinctorius) (Shimakata & Stumpf, 1982a) and spinach leaves (Spin-

acea oleracea) (Shimakata & Stumpf, 1982a-d) are eluted from gel filtration columns in fractions corresponding to proteins of molecular mass less than 100 kDa and can be resolved into component enzymes whose elution volumes overlap to give an overall synthesis of fatty acids from malonyl-CoA by their co-operative action.

The components forming saturated fatty acids

All the plant fatty acid synthetase systems so far reported require the 4′-phosphopantetheine-containing

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