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The Structure of Mammalian Fatty Acid Synthase Turned Back to Front

On page 1667 of this issue, Stuart Smith and colleagues [1] demonstrate that the animal fatty acid synthase is a head-to-head dimer rather than the head-to-tail dimer depicted in textbooks. This has important ramifications for the mechanisms of other multifunctional enzymes such as polyketide synthases [2].

Fatty acid synthesis in vertebrates is catalyzed by three distinct enzyme systems. Two organellar systems exist, one for the synthesis of lipoic acid and one for synthesis of very long chain sphingolipid fatty acids from shorter acids made in the cytosol. The third system, that responsible for synthesis of the great bulk of the fatty acid moieties of the membrane lipids, is the cytosolic fatty acid synthase (FAS). The animal FAS is a very large protein composed of two identical multifunctional polypeptides of 272 kDa each structured into discrete domains that together possess all seven of the active sites required to synthesize fatty acids from malonyl-CoA [3]. Despite the fact that each subunit contains all the required catalytic sites, the monomeric protein is not proficient in fatty acid synthesis—only the dimeric protein is active. Early biochemical work suggested that the two subunits lay side-by-side in a fully extended antiparallel orientation [4–6]. However, later studies with wild-type and mutant proteins began to erode confidence in the head-to-tail model. Now, Smith and coworkers use molecular modeling, crosslinking, and mass spectrometry to provide compelling evidence that the monomers actually lie in a head-to-head configuration [1].

In the early 1980s, Salih Wakil and his coworkers set out to understand why activity resided solely in the dimeric protein [7, 8]. By that time, model substrate enzymatic assays of proteolytic fragments and covalent labeling of the fragments with intermediates and inhibitors led the idea of the sites being arrayed as beads on a string. The first bead is the 3-ketoacyl synthase (KS) domain and the next to last is the acyl carrier protein (ACP) domain, which carries a 4′-phosphopantheteine (4′-PP) prosthetic group [3, 4]. In the course of fatty acid synthesis, the ACP domain must interact with each of the active sites since the growing acyl chain is attached to the thiol of the 4′-PP moiety [3, 4]. In most of the active sites the ACP domain acts only to position the substrate and to facilitate chemistry. However, the interaction between the KS and ACP domains is more intimate. The first step in the elongation cycle is transfer of the acyl chain from the 4′-PP thiol to that of an unusually reactive cysteine residue (pKa 6.5) in the KS active site to form an acyl-enzyme intermediate [3, 4]. Early studies revealed crosslinks formed between the reactive KS cysteine and cystamine formed from the thiol end of 4′-PP at a ratio that was consistent with a dimer composed from two different monomers [5, 7, 8]. In this head-to-tail configuration, the dimeric enzyme, although organized horizontally, was vertically bisected in function. The left half of the dimer would comprise one functional fatty acid synthesis catalytic center whereas the right half would comprise the second catalytic center. This picture provided a tidy explanation for the dependence of activity on dimerization and the head-to-tail model therefore became generally accepted [4–6].

In the mid 1990s, however, in vitro mutagenesis techniques coupled with improved methods of protein expression and manipulation were brought to bear on FAS structure and mechanism by Smith and coworkers [9, 10]. The early experiments were not targeted at testing the model. Indeed, the Smith group had interpreted their prior data in terms of the head-to-tail dimer model [5]. The first technical advance was high-level expression of the human FAS in the baculovirus system, which gave a source of high-quality protein [10]. The baculovirus system also allowed the use of multiple N-terminal affinity tags in purification. By using two different tags, heterodimers composed of two monomers that differed in the activities of their catalytic centers could be assembled and isolated [9]. Because only the heterodimers bound to both affinity columns, homodimers of one or the other monomer could be readily removed by successive affinity chromatography steps [9]. This approach allowed complementation analyses [11, 12]. Complementation was observed between monomers that were mutant in what were different fatty acid synthetic centers according to the head-to-tail model [11, 12]. In the extreme case, a dimer composed of a monomer lacking the nucleophilic thiol of the KS domain paired with a monomer lacking the essential 4′-PP prosthetic group had significant activity [12]. The head-to-tail model could clearly not accommodate these results.

Last year in pounding a penultimate nail into the coffin of the 1980s model, the Smith laboratory reported construction of a dimeric FAS composed of a wild-type monomer and a seven-knock-out mutant monomer in which function of each of the active sites had been destroyed by mutation [13]. This dimer had a very respectable FAS activity. The possibility that the activity was due to reassortment of the monomers to form an active enzyme was meticulously ruled out. Although the heterodimer results were incompatible with the head-to-tail model, they failed to provide positive evidence for the arrangement of the monomers in the active dimeric FAS. The Smith group has done that now by focusing on the N-terminal KS domain [1]. It was first found that the N-terminal 40% of the protein had association-dissociation properties identical to those of the intact FAS. Smith and coworkers then took advantage of the fact that the active site of the KS domain could be readily modeled on the crystal structures of FabF, a dimeric protein that is the generic KS of bacterial fatty acid synthesis [2]. Using this model, they introduced specific cysteine residues within the N-terminal region of the full-length protein and found rapid and efficient crosslinking with bifunctional reagents. Construction of heterodimers in which only one of the two subunits carried an introduced cysteine showed that both introduced cysteine residues were required for crosslinking. The standard of proof was moved beyond convincing to compelling by mass spectroscopic documentation of

the crosslinked termini. This was cleverly done by introduction of a cleavage site for TEV protease downstream of the introduced cysteine residues. The crosslinked proteins were then digested with TEV to liberate the crosslinked peptides for purification followed by characterization by mass spectroscopy. The mass spectral analyses demonstrated that indeed the designed crosslinks between the KS domains had been formed, and thus the dimer must be composed of monomers in a head-to-head configuration. These experiments provide a platinum standard of crosslinking analysis.

The detailed FAS structure remains to be established, but the complementation data argue that the regions of the two subunits downstream of the KS domain are somehow coiled together and this coiling is required to hold each monomer in the proper conformation for catalysis. Recent cryo-electron microscopy data suggest that the FAS dimer has an extremely flexible H-shaped structure [14–16]. The present data argue that the KS domain makes up the cross-stroke of the H. Why did the head-to-tail model seem so reasonable (and why was head-to-head evidence missed) in the 1980s? I believe this was largely due to the weakness of the tools then available compared to the size and complexity of FAS. SDS gel resolution of large proteins was poor and mass spectroscopy of large molecules would have been only a dream. Despite this wrong turn, it seems unlikely to me that the incorrect head-to-tail model seriously impeded progress with FAS. The difficulties have been technical, rather than conceptual, and working with such large enzymes remains a formidable challenge.

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Selected Reading

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