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Taking a Closer Look at Fatty Acid Biosynthesis

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Saturated fatty acids, one of the central players in energy metabolism,[1] are relatively uncomplicated molecules. However, this structural simplicity belies a complex assembly process carried out by enzymes called fatty acid synthases (FASs).[2] Starting from the C_2 unit acetate, building a typical fatty acid such as palmitate (C_{16}) requires fourteen rounds of chain extension with malonate, a carboxylated form of the same building block. Each of these cycles involves five catalytic domains (Figure 1 A): an acyl transferase (MAT), which recruits the malonate to the FAS, a ketosynthase (KS) to join the building block to the growing chain, and three reductive activities, ketoreductase (KR), dehydratase (DH) and enoyl reductase (ER), which together furnish the saturated acyl product. To facilitate these sequential reactions, Nature has developed a "solid-phase strategy" for fatty acid construction. Throughout the biosynthesis, the growing chain is covalently tethered to a noncatalytic domain, called an acyl carrier protein (ACP), that keeps it from diffusing away into the cell. Once the fatty acid is complete, a sixth domain called a thioesterase (TE) releases the chain from its linkage to the ACP. In bacteria, mitochondria and plants, each of these catalytic functions is present as a discrete, monofunctional protein (so-called "type II" organization).[3] In contrast, in fungi and animals, the enzymatic domains are strung together like beads on a string, to form gigantic multienzyme polypeptides (type I architecture).[2]

FAS has recently emerged as a target for chemotherapy in many human cancers, as the enzyme is highly over-expressed in tumor cells.[1] Efforts to design specific inhibitors of the FAS require a detailed understanding of the biosynthetic machinery, motivating efforts to image representative FAS multienzymes by using cryoelectron microscopy[4] and X-ray crystallography.[5] The modular polyketide synthases (type I PKSs), which construct dozens of secondary metabolites of medicinal value, appear to be close evolutionary cousins of the animal FAS:[6] each PKS is formed from a succession of FAS-like modules, linked together like an assembly line (Figure 2 A, below).[2] Thus, it is hoped that high-resolution structural information on FAS will illuminate the inner workings of PKS systems, facilitating attempts to redirect polyketide biosynthesis by genetic engineering.[7] X-ray and NMR structures of several individual domains excised from the FAS (including the ACP[8] and TE[9]) have been available for several years. Recently, Nenad Ban and colleagues achieved a further breakthrough in the field, publishing the first medium-resolution (4.5 Å) picture of the entire FAS multienzyme from the pig.[5] This remarkable feat allowed all of the domains, with the notable exception of the ACP and TE, to be localized within the structure. The same authors have now gone one better, improving the resolution of the structure to 3.2 Å (Figure 1 B).[10]

FAS retains the overall shape discerned at lower resolution, in which the twin polypeptides wrap around each other to form an X-shaped homodimer. As before, a lateral asymmetry is apparent, with the chains adopting slightly different conformations. Newly visible at this resolution, however, are the protein backbones, which trace an extraordinarily circuitous route through the complex, weaving outwards towards the periphery of the structure, and then back inwards several times (Figure 1 C). This seemingly uneconomic design might reflect the evolutionary history of the animal FAS, in which it was cobbled together from individual type II components.[6] Despite this nonlinear path, all of the domains of each subunit end up on one side or the other of the central axis of pseudosymmetry, forming two independent reaction chambers. Unambiguous identification of the domains reveals that the FAS is divided into two parts, a lower chain-extension region consisting of the KS and the dual-function MAT domain, and an upper portion comprising the domains that perform the reductive tailoring steps (KR, DH and ER). The connection between the two regions, a portion of the linker between the MAT and DH domains, is surprisingly tenuous, accounting for its susceptibility to proteolytic cleavage.[11]

The enhanced view of the structure exposes two previously unknown, nonenzymatic functions that occupy the fringes of the complex. On a sequence level, the domains sit adjacent to each other within the "central core" of the FAS (Figure 1 A), a region thought previously to help the FAS to dimerize.[12] The first domain is a pseudoketoreductase (ψKR), which weighs in at about half the size of the active KR. Purged of its catalytic activity, it apparently serves to support the catalytic function of the adjacent KR through heterodimer formation. The second domain, whose existence was predicted earlier,[2] has been designated as a pseudomethyltransferase (ψME), reflecting its conserved fold but inability to bind cofactor *S*-adenosylmethionine (SAM). This discovery suggests that the ancestor of the modern FAS incorporated a methylation reaction, an activity that is retained to this day in some modular PKSs.[6,13] Identifying these pseudodomains, which both make intimate contact with the KR, helps to explain an earlier, puzzling result that mutations to the "core" region disrupted the ability of the KR domain to bind NADPH.[14]

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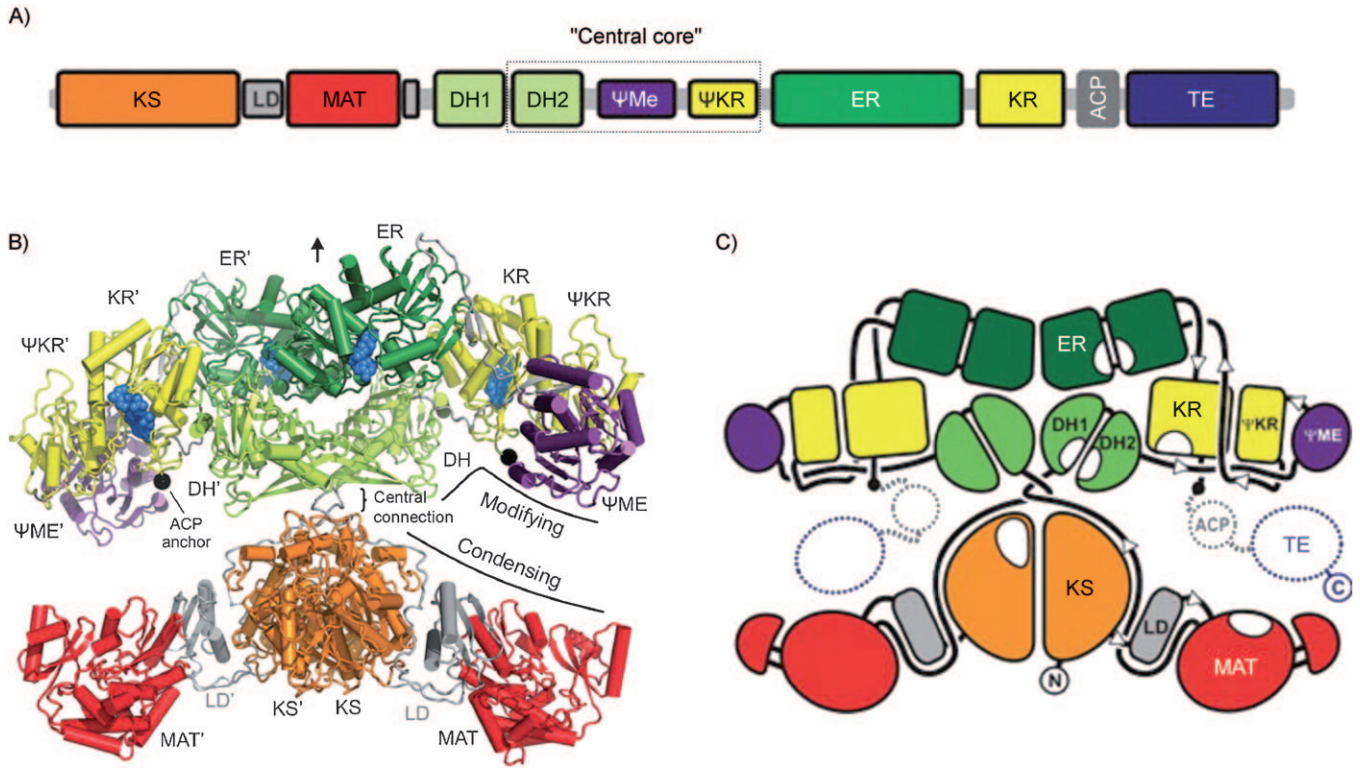

Figure 1. Structure and organization of the animal fatty acid synthase (FAS). A) Linear sequence of domains in the FAS, drawn approximately to scale. The region originally designated as the "central core" is boxed. The labels DH1 and DH2 identify the two halves of the pseudodimeric DH domain "double hot-dog" fold, while the gray boxes designate linker regions that together form a folded structural domain. B) Solved structure of the animal FAS, colored according to the domains shown in A. Structured and unstructured linker regions are shown in gray. Bound $NADP^+$ cofactors and the attachment sites for the C-terminal ACP–TE didomain are shown as blue and black spheres, respectively. The pseudo-twofold rotational axis of the dimer is indicated by an arrow. Domains within the second polypeptide of the dimer are designated with a prime. The modifying and condensing regions of the FAS are joined together through a short linker, as indicated. C) Schematic representation of the structure shown in (B). The hypothesized positions of the ACP and TE domains are indicated. Reprinted with permission from ref. [10], Copyright AAAS, 2008.

Unfortunately, even at higher resolution, the critical ACP domain and its attached TE remain invisible. This result is nonetheless informative, as it strongly suggests that the ACP–TE didomain is a highly mobile region within the structure. These data add further weight to a model for FAS operation in which the ACP moves in order to ferry the growing chain to its reaction partners. They also underline the inability of a single crystallographic snapshot to reveal the large-scale protein motions that are characteristic of these mega-multienzymes. Clearly, many more static frames of the FAS biosynthetic "movie" will be required to fully understand how the protein accomplishes its complex biosynthesis.

Nonetheless, the site of anchoring of the ACP underneath the catalytic KR domain is apparent (Figures 1B and C). Attachment of the ACP to the KR by a 12–14 residue linker (maximum length 40 Å), along with the steric constraints imposed by the protruding ψME, appear to confine the ACP to a single reaction chamber within the structure, formed by the domains within its own polypeptide. Thus, to access each of the catalytic domains in turn during a typical chain-extension cycle, the ACP must shuttle back and forth several times through the active-site cleft. As with the extensive tangling of the backbone, this inelegant trajectory might reflect the "just good enough" nature of this solution to fatty acid biosynthesis.

A potentially more significant issue is that chemical cross-linking studies on animal FAS have shown that the ACP can interact with the KS on the opposite subunit, and in fact prefers this domain to the KS on its own polypeptide.[2] However, such a partnership is visibly excluded in the present structure. To address this discrepancy, the authors propose that the flexible connector between the two regions of the FAS can unwind, allowing the clusters of domains to rotate with respect to one another.[2] A 180° twist would drag the ACP into the second reaction chamber, providing it with access to the KS and MAT domains in the mirror subunit. This model also accounts for data obtained from mutant complementation experiments[2] which showed that the ACP is unable to cooperate with any of the reductive domains or the TE on the opposite polypeptide—in the crystal structure, this set of domains is located on the same side of the pivot point, and so moves together with the ACP.

It is tempting, as the authors have done,[10] to propose a model for modular PKS architecture based on the FAS structure (Figure 2B). Indeed, several structural elements—the entire KS–AT didomain region,[15] and the ΨKR[16]—are shared, supporting a common evolutionary origin for the two systems.[6] In addition, the processing region of FAS exhibits a modular organization, in which domains alternate with flexible linker regions. This architectural arrangement would appear

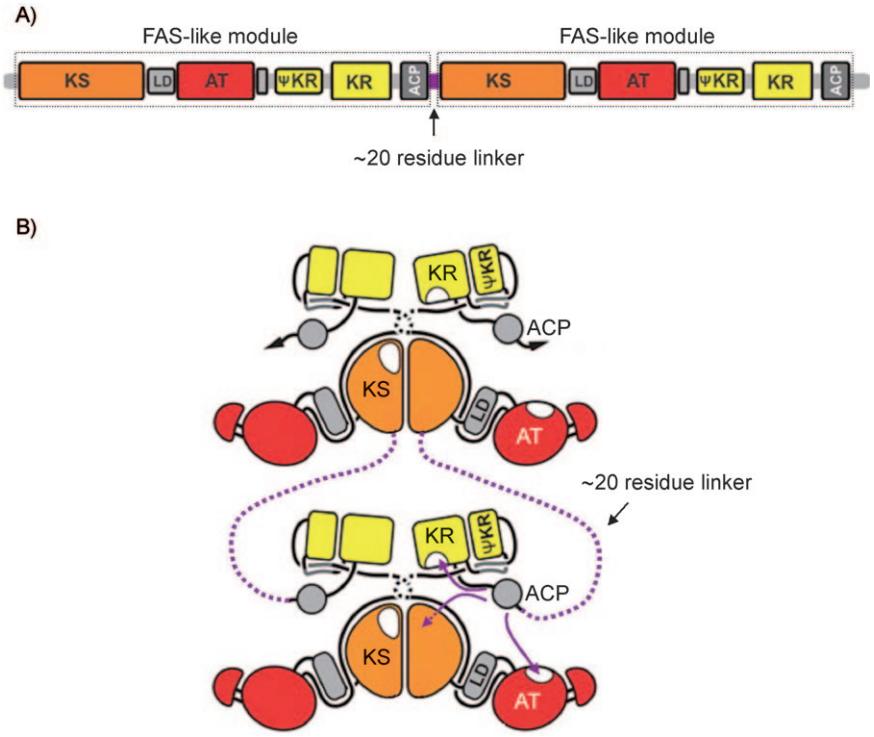

Figure 2. Proposed structural model for modular polyketide synthases (PKSs). A) Linear sequence of domains in a representative PKS. Each of the modules resembles an animal FAS that has lost several domains from its modifying region (i.e., DH1, DH2 and ER). The modules are joined together into a single polypeptide by a short linker region. B) Architectural model for the modular PKS proposed on the basis of the animal FAS structure. The ca. 20 residue linkers (purple) joining the ACP domains of the first module to the homodimeric KS of the subsequent module must also allow the ACPs to interact with all of their partners within the reaction centers (shown for one module with purple arrows). Adapted with permission from ref. [10], copyright AAAS, 2008.

to accommodate deletion of individual activities, an appealing mechanism for the evolution of modular PKS from a FAS-like ancestor. Nonetheless, there are several fundamental features of PKS operation that are tricky to reconcile with the observed domain topology.

The defining characteristic of modular PKS is the presence of multiple, consecutively acting modules (Figure 2A). In many cases, the modules are housed within a single polypeptide: the ACP of one module (ACP_n) is joined directly to its partner KS in the following module (KS_{n+1}), by a short linker (typically 20, but as few as 17 residues (about 48 Å)). The length of this connector seems insufficient to allow the ACP to reach all of the widely spaced active sites within its reaction center—some separated by as much as 72 Å[5]—and to span the distance to the next homodimeric KS by looping outside of the reductive region of the complex (Figure 2B). The same issue arises for termination modules, in which the ACP is linked directly to a dimeric thioesterase.[17] In the alternative situation, in which successive modules are located on separate polypeptides, the ACP_n/KS_{n+1} interface is reconstituted with the aid of sequence elements called "docking domains".[18] These recognition units are situated at the extreme termini of both interacting proteins and are homodimeric. This docking domain self-association, if long-lived, also appears incompatible with the segregation of the ACP domains into isolated reaction chambers. In addition, in direct contrast to what the proposed structural model would predict, PKS ACP domains have been shown to ignore the KS domain on their own polypeptide, and to instead collaborate exclusively with the KS on the opposing subunit.[19] Taken together, these observations suggest that it might be premature to assume that the architecture of the modular PKSs closely resembles that of the animal FAS. Nonetheless, the Ban group's success at producing the first high-resolution image of the FAS should encourage the members of the PKS community in their attempts to obtain the initial frames of a much longer and more complicated molecular movie.

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