present. At high pressures and low temperatures, Ice I transforms to Ice II, which has a rhombohedral crystal lattice structure of hexagonal tubes of oxygen ions with an ordered proton sublattice. Higher pressure ice polymorphs Ices V, VI, and VII may also be present in the interiors of the larger moons.

What Kubo and his colleagues have now shown is that a high-pressure polymorph of ice, Ice II, also deforms by grain size–sensitive creep at low stresses. They have done this by fabricating fine-grained Ice II by transformation from Ice I in a cryogenic high-pressure cell and then deforming the ice in the cell to measure the flow stress at which it creeps. By cycling an Ice II sample back to Ice I and then transforming it to Ice II again, they created still finer grained Ice II, measured its flow stress, and so on. They found that triply transformed Ice II flows at less than half the stress of a single-transformation sample. The grain sizes of their singly, doubly, and triply transformed samples were revealed by scanning electron microscope (SEM) analyses of the indium metal sleeves that jacket the ice samples, as well as by direct cryogenic SEM analyses of Ice II grains partially decorated with Ice I. They found a correlation between the number of transformation cycles and the flow stresses, demonstrating that the rheology of Ice II is grain size–sensitive at low stresses.

This finding has direct planetary implications because stress levels are low in the convecting interiors of the icy moons. Kubo *et al*. argue that grain size–sensitive creep of Ice I and Ice II plausibly dominates the evolution and dynamics of the interiors of the medium to large icy moons of the outer solar system. Ice II is considerably more viscous than Ice I. The transition from Ice I to Ice II, which occurs at depth, is accompanied by an increase in viscosity of four orders of magnitude. If grain size–sensitive creep does not operate, then the increase in viscosity would be six orders of magnitude. So if grain size–sensitive creep is not taken into account as a deformation mechanism, estimates for viscosities of the interiors of the icy moons are off by about two orders of magnitude. Such a difference would have profound implications for interpreting their evolution and dynamics. But this also has implications for the theoretical and experimental research now needed. Modeling of planetary dynamics cannot be based solely on mineral physics calculations of single-crystal behavior, nor on experiments on single crystals of ice. We need to understand the bulk physical and mechanical behavior of the rocks formed from ice and its high-pressure polymorphs in order to better understand the icy moons of the outer solar system.

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STRUCTURAL BIOLOGY

Architectural Options for a Fatty Acid Synthase

Multiple enzymes produce fatty acids in cells. These enzymes assemble into large complexes that are quite different in animals and fungi, but still carry out the same chemical synthesis.

Stuart Smith

The constituent enzymes of conserved biosynthetic processes do not necessarily assume the same organization across species. Take the enzymes required for the de novo biosynthesis of fatty acids, important cellular energy storage and structural molecules. In prokaryotes, the enzymes are freestanding individual proteins, whereas in eukaryotes, they are covalently linked into large, multifunctional polypeptides. Surprisingly, in eukaryotes, evolution of fatty acid synthases has proceeded along entirely different lines, resulting in two distinct architectural forms: a 2.6-megadalton barrel-shaped structure in fungi and a 0.54-megadalton X-shaped structure in animals (see the figure).

The animal fatty acid synthase plays an essential role in embryogenesis and energy homeostasis and is a target for the development of both anti-obesity and anti-cancer agents. Furthermore, it has served as a useful paradigm for understanding the structural and functional organization of multimodular enzymatic assembly lines that synthesize polyketides, important pharmacological agents. These megasynthases can contain multiple fatty acid synthase–like functional modules on a single polypeptide chain. Despite interest in fatty acid synthases, structural information has been limited to low-resolution (16 to 21 Å) electron micrographic reconstructions which, regarding animal forms, have been interpreted to support two quite different models. Crystals of both eukaryotic synthases were obtained several decades ago, but had not been exploited for structural analysis. This impasse finally has been overcome by Ban and colleagues. On page 1263 and 1258 in this issue, they report the successful application of x-ray crystallography to derive electron density maps for both a fungal (*1*) and a mammalian fatty acid synthase (*2*) to a resolution of ~5 Å. This breakthrough provides new insights into the architecture of these megasynthases and resolves the ongoing controversy over the animal enzyme structure.

Although the resolution is insufficient to identify amino acid side chains, or trace the complete backbone of individual subunits, the authors have fit three-dimensional structures of homologous individual bacterial proteins into the electron density maps of both synthases to reveal the location of most of the functional domains. The 5 Å structures agree well with the lower resolution electron micrographic structures (*3*, *4*). In both crystal structures, electron densities that could not be assigned to catalytic domains may represent interdomain regions that stabilize these large oligomeric complexes. The location of the acyl carrier protein (ACP) domain that carries reaction intermediates to the various catalytic centers also could not be unambiguously assigned in either structure, presumably because of its inherent mobility.

The fungal fatty acid synthase is a barrel-shaped dodecamer constructed from two nonidentical polypeptides ($\alpha_6\beta_6$). As predicted from earlier studies, the perimeter of the equatorial region of the barrel is derived from the α chains and the sides of the barrel from the β-chains. The barrel is divided by a central wheel-like structure into two reaction chambers. The equatorial region consists of three alternating β-ketoacyl synthase (KS) and β-ketoacyl reductase (KR) homodimers, with one active site of each dimer directed toward either the upper or lower chamber. Four monomeric domains that constitute the barrel's sides have active centers directed toward the interior. The authors speculate that the six ACP domains are tethered to the central wheel, with three directed toward each of the two chambers. Thus, each chamber contains three copies of all of the functional domains with interior-facing active sites. Small pores in the sides of the barrel allow diffusion of

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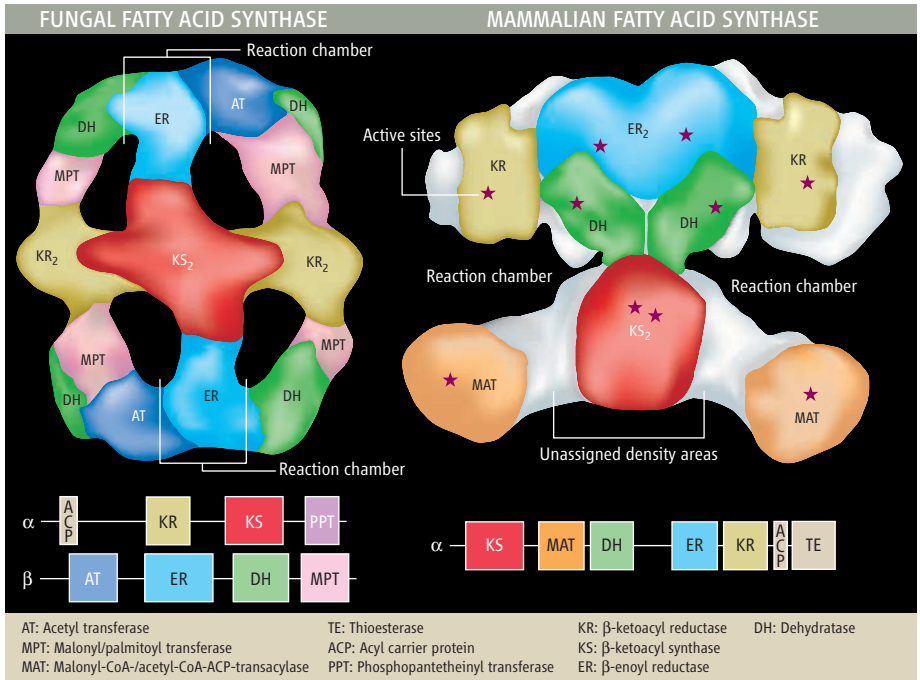
the substrates acetyl and malonyl–coenzyme A (CoA) into the reaction chambers and exiting of the palmitoyl-CoA product (*3*).

The original model for the animal fatty acid synthase, formulated in the 1980s, envisioned two subunits (α_2) orientated in a fully extended antiparallel arrangement in which a noncatalytic "central core" stabilized a dimeric structure. Despite evidence questioning its validity, this model still enjoys some support (*5*, *6*). Its proponents attempted to fit an x-ray crystal structure for the thioesterase (TE) domain and a modeled monomeric KS domain into a low-resolution electron micrographic structure but, as revealed by the new 5 Å resolution structure, both domains were incorrectly positioned (*7*). This outcome illustrates the perils of this approach. Although it would have been more reassuring if Ban and colleagues had provided some objective assessment of the "goodness of fit" of the homologous crystal structures into their electron density maps, the 5 Å resolution of their structure strongly suggests that they have got it right. Indeed, their structure agrees well with a model derived from mutant complementation (*8*), cross-linking (*9*), and electron micrographic (*4*) experiments. The new model depicts two coiled subunits oriented head to head, with paired KS domains stabilizing the dimer (*10*). The crystal structure confirms the dimeric nature of the KS domains but also reveals some surprises. The β-enoyl reductase (ER) domains are also dimeric and the dehydratase (DH) domains appear pseudodimeric, with each pseudosubunit coming from adjacent regions of the same polypeptide. Furthermore, the central core of the synthase appears to be interspersed along the "arms" of the structure.

The animal fatty acid synthase can be conveniently characterized as comprising a "body" (the ER and KS dimers and pseudodimeric DH pairs) with two "arms" (KR monomers) and "legs" [monomeric malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) domains]. The ACP and TE domains which, because of inherent mobility, could not be assigned unambiguously to the electron density map, most likely are located at the end of the two arms, by virtue of their location in the primary sequence, adjacent to the KR domains. Active sites of the two sets of catalytic domains are oriented facing each of the two lateral clefts in the structure, thus forming the two chambers. Interestingly, these reaction chambers do not appear in identical conformations; the distances between active sites associated with the arms and legs are different and blurred electron density, possibly attributable to the ACP and/or TE domains, is visible only on one side of the structure. It has been suggested that the synthesis of fatty acids at the two sites may function asynchronously (*4*), with one chamber engaged in carbon-chain elongation and the other in β-carbon processing. By superimposing the two sides of the synthase dimer on each other, Ban and colleagues have cleverly exploited this lateral asymmetry to reveal hinge regions that may facilitate the adoption of different conformations by this enzyme.



Distinct organization of two eukaryotic fatty acid synthases. (**Left**) Fungal fatty acid synthase assumes a barrel-like shape (260 Å high, 230 Å wide). (**Right**) Mammalian (porcine) fatty acid synthase is an asymmetric X-shape (210 Å high, 180 Å wide, 90 Å deep). Side views are shown. In the fungal enzyme, acetyl transferase loads the acetyl primer substrate whereas the malonyl/palmitoyl transferase loads malonyl moieties and releases the palmitoyl-CoA product. The animal synthase loads both substrates via the malonyl-CoA-/Acetyl CoA-ACP transacylase and unloads free palmitic acids via a thioesterase. The acyl carrier protein of the fungal synthase is posttranslationally modified by phophopantetheinyl transferases that are likely localized as timers at the barrel apices.

What new insights may be gleaned from the animal fatty acid synthase structure that might be applicable to other megasynthases? One surprising aspect of the animal structure is the substantial intersubunit contacts between the pairs of ER and DH domains along the pseudo-twofold axis of symmetry. Many modules associated with polyketide synthases completely lack these enzymatic domains that are required for β-carbon processing reactions. In addition, whereas the TE domains of the animal fatty acid synthase are monomeric, those associated with the terminal modules of polyketide synthases are dimeric. These considerations indicate that, despite similarities in the ordering of their component domains, the synthases involved in fatty acid and polyketide production may rely on different interactions to stabilize their complex architecture. It is hoped that the successful application of crystallographic analysis to the fatty acid synthase system will spark interest in developing high-resolution structures for the modular polyketide synthases and reveal how multiple modules are linked together to form an enzymatic assembly line.

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