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Bacteria on steroids

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When it comes to biochemical ingenuity, microbes can do just about anything. In PNAS, Wang et al. (1) report the isolation and physiology of a bacterium that can perform a highly specialized feat. Given mineral salts, it can live on testosterone alone. Testosterone will be familiar to many readers as an essential component of the human hormone bouquet and as an anabolic supplement used by body builders to rapidly grow muscle tissue on a high-protein diet. The bacterium that Wang et al. have isolated, *Phosphitispora* sp. strain TUW77, does not use testosterone as a food additive, it is the main course. The bacterium uses a minuscule portion of the testosterone molecule as its sole carbon and energy source. *Phosphitispora* sp. strain TUW77 is quite the opposite of a voracious bacterial weightlifter. On the contrary, it is the consummate gourmet, one of the pickiest eaters in town. Instead of enjoying a three-course menu on the 19 carbon atoms in testosterone, it carves out one single methyl group (CH_3) from the testosterone molecule and satisfies all of its carbon and energy needs from that one methyl group—center cut, *filet d'hormon*—converting testosterone into two other mammalian sex hormones that it excretes as metabolic end products in the process. Central to the specialized metabolism of *Phosphitispora* sp. TUW77 is not a newly invented biochemical machinery but an elegant application of the most ancient pathway of CO_2 fixation known, the Wood–Ljungdahl (or acetyl-CoA) pathway (2). This report is a classic example of biochemical evolution at work.

What environments even harbor enough testosterone to support bacterial growth? Testosterone and related steroid hormones occur in the effluent of municipal wastewater treatment plants at concentrations up to micrograms per liter, and previous work had shown that estrogens, including estradiol, were being synthesized in estuary sediment that was rich in steroid hormones (3). But synthesized by whom? Using a strategy of natural enrichment via the food chain, Wang et al. sampled the gut microbiome of the blue-spotted mudskipper, which inhabits steroid-rich estuary sediments of the northwest Pacific Ocean. Then, selecting for growth on testosterone alone, they isolated the bacterial hormone connoisseur *Phosphitispora* sp. TUW77, a strict anaerobe, based on its ability to use testosterone as the sole carbon and energy source. That provided an opportunity to grow the bacterium in laboratory cultures to characterize the enzymes and pathways involved.

A synopsis of the *Phosphitispora* sp. TUW77 testosterone-metabolizing pathway is shown in Fig. 1. The bacterium takes up testosterone from the environment and activates it by

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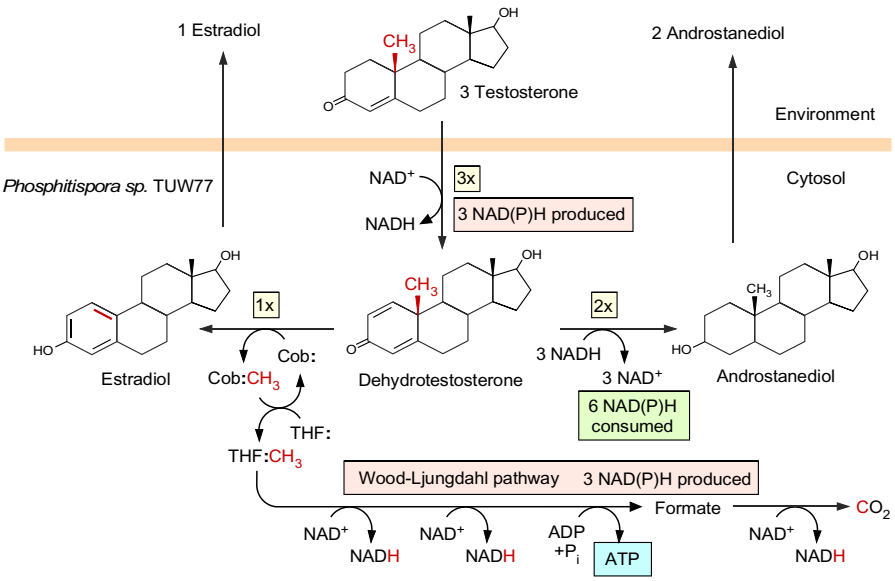

Fig. 1. Simplified summary of testosterone fermentation (1) in *Phosphitispora* sp. TUW77. Cob, cobalamine, or cobamide. Cobamides are a group of cobalt-containing compounds that include cobalamin and similar compounds that differ slightly with respect to the structure of the lower ligand (4). THF, tetrahydrofolate. ":," cofactor electron pairs that bind methyl groups. The beige line symbolizes the plasma membrane. See Wang et al. (1) for details.

introducing an additional double bond at the lower left end of the dehydrotestosterone (DT) molecule shown in the figure. It then uses a cobalamine-dependent enzyme to remove the methyl group from one-third of the DT pool. The electron pair subtending the methyl group remains on the steroid backbone, generating estradiol, an extremely potent sex hormone that moderates a variety of processes, including pregnancy, in humans. The methyl group is transferred to tetrahydrofolate (THF), where it is subsequently oxidized to CO_2 via the enzymes of the Wood–Ljungdahl pathway. This generates three molecules of reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH), which have to be reoxidized so that metabolism does not come to a halt. DT serves as the electron acceptor for NAD(P)H oxidation, regenerating $NAD(P)^+$. *Phosphitispora* converts the remaining two-thirds of the DT pool to androstanediol, another steroid, by reducing its three double bonds. Three testosterone molecules were activated to DT, generating three NAD(P)H, and one methyl group was oxidized to CO_2, generating three more NAD(P)H, such that six NAD(P)H have to be oxidized in order to maintain redox balance. The two remaining molecules of DT that did not undergo methyl transfer serve as the electron acceptor, yielding androstanediol and consuming three NAD(P)H each, or six NAD(P)H total, which generates redox balance.

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Phosphitospora sp. strain TUW77 does not go to all this enzymatic effort out of boredom. It uses the process to conserve energy. One reaction of methyl oxidation via the Wood–Ljungdahl (WL) pathway involves the generation of formate, $HCOO^-$, from formyltetrahydrofolate. The reaction is catalyzed by formyltetrahydrofolate synthetase (FTHFS). There is enough energy released in that reaction to permit the synthesis of adenosine triphosphate (ATP) using formylphosphate as an intermediate (5), which phosphorylates adenosine diphosphate (ADP) to ATP. For every three molecules of testosterone converted to estrogen and androgens (57 carbon atoms total), one single ATP is generated. That is an admittedly modest energy yield from moving 57 carbon atoms around, but the genus *Phosphitispora* is related to Clostridia, which are famous for growth on extremely low energy budgets (6). If there is enough testosterone around, one ATP per three testosterone is sufficient to support growth. Because the electron acceptor, DT, is generated internally by the bacterium during metabolism, the overall ATP-generating process is a fermentation.

And how does *Phosphitispora* sp. TUW77 satisfy its carbon needs for growth? For this, it also uses the Wood–Ljungdahl pathway but in the direction that synthesizes acetyl-CoA (2). This involves condensation of CO_2 with the methyl group that it gleaned from testosterone (1) by an exquisitely ancient enzyme called carbon monoxide dehydrogenase/acetyl-CoA synthase, or CODH/ACS for short (7, 8). This reaction requires the synthesis of reduced ferredoxin, a strong reductant, which is not as simple as it might seem starting with testosterone-generated NAD(P)H, a weak reductant, as the electron donor (1). Generating reduced ferredoxin with NAD(P)H requires electrons to flow energetically uphill. That would be thermodynamically unfavorable, were it not for a process called flavin-based electron bifurcation (9). It is not yet known exactly which electron-bifurcating enzymes *Phosphitispora* employs to generate reduced ferredoxin, because there are now many enzymes known that can catalyze this reaction (9, 10). In any event, the generation of reduced ferredoxin in *Phosphitispora* will likely involve flavin-based electron bifurcation, a process that is as ancient as metabolism itself (11, 12).

Beyond the use of ancient enzymes for modern applications, the evolutionary and ecological implications of the findings reported by Wang et al. (1) are significant. First, they show that a reaction previously thought to be specific to eukaryotes, the synthesis of an aromatic ring in a steroid (Fig. 1, *Left*), can be performed by a prokaryote. In addition, the canonical enzyme used by eukaryotes to generate that aromatic ring, aromatase, is O_2-dependent, whereas the cobalamine-dependent enzyme used by *Phosphitispora* is O_2-independent (1). This uncovers further biochemical antiquity because i) cobalamin and other cobamides (cobalt-containing compounds with a variety of lower ligands found in prokaryotes) are ancient cofactors that trace all the way back to the very first cells on Earth (4) and because ii) steroid synthesis can be traced deep into eukaryotic history. The four-ringed steroid carbon backbone, sterane, with various methylation patterns, can be preserved for over a billion years in sedimentary rocks (13), permitting crucial insights into the anaerobic evolutionary past of a young eukaryotic lineage (14). Could it be that prokaryotes interfered with steroid hormone functions of early eukaryotes, or that they still do so in modern environments? Given the surprising findings by Wang et al. (1), it needs to be on the map of possibilities.

Even the name *Phosphitispora* itself points to another ancient trait. The genus was named for the ability to use phosphite in energy metabolism. The originally described *Phosphitispora* species, *P. fastidiosa*, can use phosphite as a source of energy and electrons by converting phosphite, NAD^+, and AMP into NADH and ADP in a remarkable single-enzyme reaction (15). Phosphite utilization could be a primordial biochemical trait, as it is a candidate entry point of phosphorous into early metabolism (15), and phosphite is synthesized naturally in H_2-producing hydrothermal vents (16), a possible site of biochemical genesis. *Phosphitispora* sp. strain TUW77 does not utilize phosphite (1) but *P. fastidiosa* uses the acetyl-CoA pathway (15), which has the uniquely ancient distinction that the enzymes catalyzing the entire pathway from H_2 and CO_2 to pyruvate can be replaced by native nickel (Ni^0) (17, 18), a natural catalyst that is geochemically synthesized in H_2-producing hydrothermal vents (19).

All organisms have preserved some traces of chemical antiquity in metabolism (20), *Phosphitispora* sp. strain TUW77 has retained more than its fair share (1). Its carbon and energy metabolism link primordial carbon fixation pathways to environmental hormone synthesis, using enzymes and cofactors that were present in the last universal common ancestor. It is the first bacterium described that can make a living from a fermentation that converts one human steroid hormone into another, but it will probably not be the last.

1. P. H. Wang *et al.*, Bacterial estrogenesis without oxygen: Wood–Ljungdahl pathway likely contributed to the emergence of estrogens in the biosphere. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2422930122 (2025).
2. G. Fuchs, Alternative pathways of carbon dioxide fixation: Insights into the early evolution of life? *Annu. Rev. Microbiol.* **65**, 631–658 (2011).
3. C. J. Shih *et al.*, Biochemical mechanisms and microorganisms involved in anaerobic testosterone metabolism in estuarine sediments. *Front. Microbiol.* **8**, 1520 (2017).
4. L. D. Modjewski *et al.*, Evidence for corrin biosynthesis in the last universal common ancestor. *FEBS J.* **292**, 827–850 (2025).
5. L. R. Celeste *et al.*, Mechanism of N^{10}-formyltetrahydrofolate synthetase derived from complexes with intermediates and inhibitors. *Protein Sci.* **21**, 219–228 (2011).
6. K. Schuchmann, V. Müller, Autotrophy at the thermodynamic limit of life: A model for energy conservation in acetogenic bacteria. *Nat. Rev. Microbiol.* **12**, 809–821 (2014).
7. A. Biester, D. A. Grahame, C. L. Drennan, Capturing a methanogenic carbon monoxide dehydrogenase/acetyl-CoA synthase complex via cryogenic electron microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2410995121 (2024).
8. M. D. Yin *et al.*, Conformational dynamics of a multienzyme complex in anaerobic carbon fixation. *Science* **387**, 498–504 (2025).
9. W. Buckel, R. Thauer, Flavin-based electron bifurcation, a new mechanism of biological energy coupling. *Chem. Rev.* **118**, 3862–3886 (2018).
10. G. J. Schut *et al.*, An abundant and diverse new family of electron bifurcating enzymes with a non-canonical catalytic mechanism. *Front. Microbiol.* **13**, 946711 (2022).
11. E. S. Boyd, M. J. Amenabar, S. Poudel, A. S. Templeton, Bioenergetic constraints on the origin of autotrophic metabolism. *Philos. Trans. R. Soc. A* **378**, 20190151 (2020).
12. M. Brabender *et al.*, Ferredoxin reduction by hydrogen with iron functions as an evolutionary precursor of flavin-based electron bifurcation. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2318969121 (2024).
13. J. J. Brocks *et al.*, Lost world of complex life and the late rise of the eukaryotic crown. *Nature* **618**, 767–773 (2023).
14. D. B. Mills *et al.*, Eukaryogenesis and oxygen in Earth history. *Nat. Ecol. Evol.* **6**, 520–532 (2022).
15. Z. Mao *et al.*, AMP-dependent phosphite dehydrogenase, a phosphorylating enzyme in dissimilatory phosphite oxidation. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2309743120 (2023).
16. M. A. Pasek *et al.*, Serpentinization as a route to liberating phosphorus on habitable worlds. *Geochim. Cosmochim. Acta* **336**, 332–340 (2022).
17. M. Preiner *et al.*, A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism. *Nat. Ecol. Evol.* **4**, 534–542 (2020).
18. T. Beyazay *et al.*, Influence of composition of nickel-iron nanoparticles for abiotic CO_2 conversion to early prebiotic organics. *Angew. Chem. Int. Ed. Engl.* **62**, e202218189 (2023).
19. J. A. Chamberlain, C. R. McLeod, R. J. Traill, G. R. Lachance, Native metals in the Muskox intrusion. *Can. J. Earth Sci.* **2**, 188–215 (1965).
20. N. Mrnjavac *et al.*, Chemical antiquity in metabolism. *Acc. Chem. Res.* **57**, 2267–2278 (2024).