Furan fatty acids (FuFAs), characterized by a central furan moiety, are widely dispersed in nature, but their biosynthetic origins are not clear. A new study from Lemke et al. employs a full court press of genetics, genomics, biochemical, and advanced analytical techniques to dissect the biosynthetic pathway of mono- and dimethyl FuFAs and their intermediates in two related bacteria. These findings lay the foundation both for detailed study of these novel enzymes and for gaining further insights into FuFA functions.

Furan fatty acids (FuFAs) are heterocyclic fatty acids produced in algae, plants, and microorganisms and taken up by animals in their diets (1, 2). FuFAs are implicated as second messengers in cellular pathways that protect from oxidative damage (2), and their characteristic furan rings can also provide direct protection against oxidants by scavenging hydroxyl and peroxyl radicals (3). FuFAs have been shown to provide health benefits (4, 5) and also have potential industrial uses as feedstocks for the production of lubricants, fuel additives, and biofuels (2). Understanding the full scope of FuFA biology and producing enough FuFAs for health and industrial applications would both benefit from having a defined biosynthetic pathway(s), but a complete pathway remains unknown.

The furans in FuFAs are typically found in the middle of the fatty acid chain, with a carboxyalkyl chain containing 7, 9, 11, or 13 carbon atoms and a propyl or pentyl side chain flanking each side; the furan rings can also be decorated with 0, 1, or 2 methyl groups (4). Previous explorations in two algae species have suggested a likely route from two different starting materials (Fig. 1) (6). The initial step involves a lipoxygenase-catalyzed oxidation to form 13-hydroperoxy-9,11-alkandienoic acid followed by electron shifts, ring closure, and double bond rearrangement to produce the FuFA, which becomes methylated (4). A distinct pathway was proposed in marine bacteria (7). Based on the intermediates that were isolated, FuFA biosynthesis was proposed to start with the methylation of cis-vaccenic acid (also known as 18:1 11Z) to form 11-Me-18:1, followed by desaturation to generate 11-Me-18:2, and finally incorporation of oxygen to form the furan ring (Fig. 1) (7). A similar pathway was also proposed in the photosynthetic bacterium Rhodobacter sphaeroides (3) after RSP2144 (UfaM) was identified as a SAM-dependent methylase that converts vaccenic acid to E-11-methyltetradeca-12-enoic acid (11Me-12t-18:1), a potential intermediate for FuFA biosynthesis. The previous report (4) also pointed to the RSP1087-1091 operon involved in FuFA synthesis, but the other intermediates and enzymes in this pathway remained unclear.

In the current study, Lemke et al. report elegant work defining the entire mono- and dimethyl FuFA biosynthesis pathway in photosynthetic bacteria (8). The authors had previously identified a R. sphaeroides mutant lacking the ChrR antisigma factor with increased abundance of FuFA, which served as a key to their success in dissecting the function of the RSP1087-1091 operon. The authors individually deleted each gene in the operon in the ΔChrR mutant, ruling out the involvement of RSP1087, 1088, and 1089. Deletion of RSP1091 led to depletion of methyl 9-(3-methyl-5-pentylfuran-2-yl) nonanoate (9M5-FuFA) and accumulation of 11Me-12t-18:1, implying that RSP1091 plays an important role in producing an intermediate between 11Me-12t-18:1 and 9M5-FuFA. Deletion of RSP1090 in ΔChrR resulted in a new product that GC-MS and NMR analysis identified as the doubly unsaturated 11Me-10t,12t-18:2. The authors next generated a strain containing ChrR but lacking UfaM, RSP1091, and RSP1090 and found that the strain produced neither detectable FuFA nor its proposed intermediates. Overexpression of UfaM in this strain led to the accumulation of 11Me-12t-18:1, co-expression of UfaM and RSP1091 generated 11Me-10t,12t-18:2, and overexpression of all three genes led to the production of 9M5-FuFA. These results suggested that UfaM, RSP1091, and RSP1090 might operate sequentially to make 9M5-FuFA (Fig. 1).

In vitro assays of recombinant protein incubated with their potential substrates confirmed the proposed reactions. RSP1091 (renamed UfaD) functions as a fatty acyl desaturase with an unexpected homology to phytoene desaturase, involved in the evolutionarily distinct carotene biosynthetic pathway, rather than other characterized fatty acid desaturases. UfaD’s product was only observed when the cells were grown aerobically, suggesting that the enzyme may use molecular oxygen in its catalytic reaction. This was confirmed by oxygen labeling experiments showing that RSP1090 (renamed UfaO) functions as a fatty acid-modifying enzyme that incorporates molecular oxygen into 11Me-10t,12t-18:2 to produce 9M5-FuFA.

Genes related to UfaM, UfaD, and UfaO have been reported in other bacteria (9), begging the question whether or not they carry out the same biosynthetic functions. Overexpression of three Rhodopseudomonas palustris homologs in the R. sphaeroides ΔUfaMΔUfaDΔUfaO triple mutant restored 9M5-FuFA production. Deleting the genes in the native strain abolished 9M5-FuFA accumulation, which could be rescued by introducing an appropriate plasmid. These data convincingly confirm the existence of a similar FuFA biosynthetic pathway in other bacteria. Finally, in addition to 9M5-FuFA, the authors noted the
presence in *R. palustrus* of the corresponding dimethyl compound, 9D5-FuFA. They tested the two genes (RPA3082 and RPA0924) annotated as SAM-dependent fatty acid–modifying enzymes, and thus likely methylases, to see if either played a role in 9D5-FuFA synthesis. Whereas RPA3082 deletion had no effect on fatty acid profiles, inactivation of RPA0924 was associated with the loss of 9D5-FuFA, consistent with a role in 9D5-FuFA synthesis, and its expression in *R. Sphaeroides* ΔChrR restored 9D5-FuFA accumulation. *In vitro* incubation of RPA0924 with SAM and 9M5-FuFA also resulted in 9D5-FuFA accumulation, confirming it as a 9M5-FuFA methylase (renamed FuFM).

This tour de force of biochemical and genetic approaches establishes three novel enzymes in the FuFA biosynthetic pathway and stimulates new questions. For example, what is the function of the adjacent RSP1087-1089? Are there any physiological benefits to further methylating 9M5-FuFA to 9D5-FuFA? The existence of distinct synthetic pathways in plants, algae, and photosynthetic bacteria suggests the existence of a strong evolutionary pressure that resulted in convergent strategies to produce FuFA as radical scavengers for protection against the deleterious effect of UV radiation. Will following the homologues of UfaM, UfaD, and Ufa help us to identify important enzymes in FuFA biosynthesis in plants, algae, and other organisms? More specifically, could the RPA0924 homolog in algae work as a FuFM, given similarities between the pathways (Fig. 1)? Finally, what does the uniqueness of the pathway teach us about the evolution of FuFA biosynthesis? Fatty acyl monooxygenases are known to be involved in fatty acid metabolism (10), and UfaO is a potential new class of monooxygenases, which catalyzes the ring formation to stabilize the chain to prevent cleavage. The present work (8) lays the foundation for further studies answering these and related questions by creating unique strains and methodology to study FuFA biosynthesis and biology and for engineering their accumulation in plants and/or other production hosts.

**Editors’ Pick Highlight:** *Furan fatty acid biosynthesis*

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**Funding and additional information—**This work was supported by Department of Energy Center for Advanced Energy and Bio-products Innovation Award DE-SC0018420 (to X.-H. Y. and J. S.) and Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, United States Department of Energy Grant DOE KC0304000 (to J. S.).

**Conflict of interest—**The authors declare that they have no conflicts of interests with the contents of this article.

**Abbreviations—**The abbreviations used are: FuFA, furan fatty acid; 11Me-12r-18:1, E-11-methyloctadeca-12-enoic acid; 9M5-FuFA, methyl 9-(3-methyl-5-pentylfuran-2-yl) nonanoate.

**References**


**J. Biol. Chem. (2020) 295(29) 9802–9803 | 9803**