

A type III PKS makes the DIFference

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Differentiation-inducing factor 1 is a modified polyketide natural product involved in the differentiation of *Dictyostelium discoideum* cells. A new study shows that a type III polyketide synthase existing in an unusual association with type I fatty acid synthase domains is responsible for biosynthesis of this signaling compound.

*Dictyostelium discoideum*¹ is a free-living amoeba during its vegetative growth phase, but upon starvation it switches to a multicellular developmental phase that is induced by cyclic AMP (<http://www.dictybase.org>). The multicellular aggregate forms a slug that migrates toward heat and light. It then develops into a fruiting body made up of different cell types that form a mass of spore cells supported atop a stalk. Small signaling molecules called differentiation-inducing factors (DIFs) orchestrate the differentiation of cell types, such as the prestalk and prestalk cells, in the multicellular slug². DIF-1 (Fig. 1a) is a well-characterized polyketide-based molecule that induces the differentiation of pstO, one type of prestalk cell³. Taking advantage of the recently available genome sequence of *D. discoideum*, Austin *et al.* have identified the enzyme (named “Steely2”) that is responsible for the synthesis of the DIF scaffold⁴.

Polyketides are chemically and structurally diverse secondary metabolites that are synthesized by polyketide synthases (PKSs) present in both prokaryotes and eukaryotes⁵. Recently, attention has been focused on both the ability of PKSs to synthesize complex chemical scaffolds and the possibility of engineering them to produce new antimicrobial compounds⁶. PKSs generate diverse molecules by taking advantage of reactivities in a way similar to that of fatty acid synthases (FASs), which carry out an iterative cycle of two-carbon chain extension through a ketoacyl synthase domain. However, compared to FASs, PKSs have an expanded capacity because they are able to off-load products after partial processing without going through a complete set of reactions, which can include steps such as reductions and dehydrations. Type III PKSs are small homodimeric enzymes that perform multiple enzymatic activities at a single site⁷. Chalcone synthase (CHS) from the plant

Medicago sativa, which performs the first step in flavonoid biosynthesis, is one biochemically and structurally well-characterized type III system. Type III PKSs that belong to the CHS family were initially thought to be specific to plants, in which they produce the chalcone precursor for a variety of molecules involved in diverse roles such as microbial defense, UV protection and pigment formation. However, with the availability of sequenced genomes of many organisms, it is becoming clear that type III PKSs are present in several bacteria and other organisms as well, and some of them produce functionally important secondary metabolites. For example, RppA, the first type III PKS to be identified and characterized from a bacterial source (*Streptomyces griseus*), is involved in melanin production. A more recent report of the functional role of a type III PKS identified outside the plant kingdom is in the soil bacterium *Azotobacter vinelandii*, in which the enzyme has a role in formation of the cyst, a protective lipid coat⁸.

DIF-1 is based on a phloroglucinol skeleton and has been presumed to arise by a pathway involving PKS synthesis of phlorocaprophenone (PCP; Fig. 1a). However, until now few molecular details have been elucidated for the biosynthetic pathway that produces DIF-1, other than for the enzyme responsible for the final methylation step. Sequencing of the *D. discoideum* genome revealed the pres-

ence of nearly 40 genes homologous to PKSs. Using bioinformatic tools, Austin *et al.* identified two open reading frames, which they named “Steely1” and “Steely2,” in which a type III PKS was associated with typical type I FAS domains. Surprisingly, in Steely2, the type III PKS module at its C terminus replaces the thioesterase domain normally expected at that position in most modular enzymes (Fig. 1b). The authors hypothesized that the FAS domains could provide a hexanoyl coenzyme A (CoA) starter molecule to the type III PKS, which could then carry out three cycles of acetyl group extension and cyclization to produce the phloroglucinol scaffold necessary for DIF synthesis. To test this, they performed *in vitro* assays in which hexanoyl CoA-primed reactions with Steely2 produced the DIF scaffold, but those with Steely1 did not. To further show that one of these proteins is actually responsible for DIF scaffold synthesis, they created mutant strains in which the Steely proteins were individually disrupted. Whereas the *Steely1*⁻ mutant was not defective in DIF-1 synthesis, the *Steely2*⁻ mutants did not produce DIF-1 and resulted in a developmental phenotype expected for cells lacking DIF-1. Supplementing *Steely2*⁻ mutants with DIF-1 restored normal development, which supports the assignment of Steely2 as being responsible for the synthesis of DIF-1. The crystal structure of the type III PKS domain of

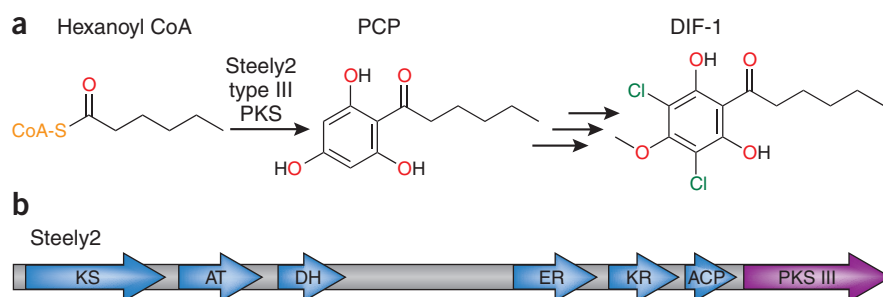


Figure 1 DIF-1 synthesis in *D. discoideum*. (a) The type III PKS of Steely2 synthesizes the PCP scaffold, which is further modified by chlorination and methylation reactions to form DIF-1. (b) The domain organization of Steely2, in which typical FAS domains (KS, ketosynthase; AT, acyltransferase; DH, dehydratase; ER, enoylreductase; KR, ketoreductase and ACP, acyl carrier protein) are associated with a type III PKS module.

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Steely1 confirmed that it has the characteristic fold and the catalytic triad as observed in the related CHS family of enzymes.

Although the work identifies the enzyme for DIF-1 precursor synthesis, it seems as though it is the tip of the iceberg. DIF-1 is responsible for the differentiation of only one out of many *D. discoideum* cell types, and more DIFs are being identified from this organism⁹. It is likely that these chemically similar molecules are produced by similar pathways, yet it remains to be seen exactly how they are produced and regulated for the differentiation of multiple cell types. Therefore, this study by Austin *et al.* provides a starting point for probing the biochemical and functional role of the putative PKS genes, including that of *Steely1*, and others responsible for DIF production in *D. discoideum*.

The metabolite diversity generated by subtle changes in some of the type III PKSs

that have been characterized *in vitro*, for example from *Mycobacterium tuberculosis*¹⁰, highlights the limitations of homology modeling and even of experimentally determined structures (in some cases) in predicting the diversity generated by PKS enzymes. Therefore, it is imperative to probe these enzymes using a combination of *in vivo* and *in vitro* techniques, such as was used by Austin *et al.*, to obtain a holistic view of their function. It is also worth noting that domain swapping in modular PKSs has been used as a strategy to generate new polyketides⁶. The newly identified role of type III PKSs in a modular system in which they replace the thioesterase domain to allow release of growing polyketide chains adds another dimension to the metabolite diversity that can be generated by domain-swapping experiments. Such a methodology would also benefit from the emerging structural information on

some of the modular FAS systems. Thus, the natural occurrence of these domains in varied contexts further highlights the potential of using the domain-swapping strategy for generating chemical diversity.

1. Chisholm, R.L. *et al. Nucleic Acids Res.* **34**, D423–D427 (2006).
2. Morris, H.R., Taylor, G.W., Masento, M.S., Jermyn, K.A. & Kay, R.R. *Nature* **328**, 811–814 (1987).
3. Thompson, C.R. & Kay, R.R. *Mol. Cell* **6**, 1509–1514 (2000).
4. Austin, M.B. *et al. Nat. Chem. Biol.* **2**, 494–502 (2006).
5. Staunton, J. & Weissman, K.J. *Nat. Prod. Rep.* **18**, 380–416 (2001).
6. Weissman, K.J. & Leadlay, P.F. *Nat. Rev. Microbiol.* **3**, 925–936 (2005).
7. Austin, M.B. & Noel, J.P. *Nat. Prod. Rep.* **20**, 79–110 (2003).
8. Funa, N., Ozawa, H., Hirata, A. & Horinouchi, S. *Proc. Natl. Acad. Sci. USA* **103**, 6356–6361 (2006).
9. Saito, T. *et al. Biochim. Biophys. Acta* **1760**, 754–761 (2006).
10. Sankaranarayanan, R. *et al. Nat. Struct. Mol. Biol.* **11**, 894–900 (2004).

Copper and nitric oxide meet in the plasma

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Nitrite is an inorganic anion essential in cell signaling and vascular biology. A new study shows that the multicopper oxidase ceruloplasmin is critical for maintaining plasma nitrite, revealing a new link between copper and nitric oxide homeostasis.

Once considered as merely a toxic food additive, inorganic nitrite (NO_2^-) is now a molecule of considerable interest as a messenger of cell signaling in health and disease¹. Nitric oxide (NO) is an endogenously produced gas that functions as an essential signaling molecule². Although most NO is synthesized from arginine and oxygen by NO synthases, nitrite functions as a stable reservoir of NO after reduction by hemoglobin and other proteins¹. However, far from serving simply as an inert reservoir, NO_2^- also functions directly as an essential messenger in vascular biology³; thus defining the mechanisms of NO_2^- synthesis and metabolism is crucial. Though auto-oxidation of NO to NO_2^- occurs readily in aqueous solution, the kinetics of this reaction preclude a physiological role for this process in NO_2^- synthesis. Transition metals within metalloproteins are a

rich source of facile electron transfer reactions, and in this issue of *Nature Chemical Biology* Shiva *et al.*⁴ show that ceruloplasmin functions as an NO oxidase essential for maintaining plasma NO_2^- .

Ceruloplasmin is a multicopper oxidase characterized by three types of spectroscopically distinct copper ions. Six copper ions are incorporated into ceruloplasmin during synthesis in hepatocytes as a secreted plasma protein and in glia and macrophages as a glycosylphosphatidylinositol (GPI)-linked plasma-membrane protein⁵. Aceruloplasminemia is an inherited neurodegenerative disease characterized by tissue iron accumulation secondary to loss-of-function mutations in the ceruloplasmin gene. Recognition of this disease revealed an essential role for ceruloplasmin in the oxidation of ferrous iron that is necessary for iron efflux from cells⁶. Interestingly, restoration of systemic iron homeostasis in aceruloplasminemia requires only 10% of the ceruloplasmin concentration, suggesting that the substantial increase in serum ceruloplasmin observed in inflammation and infection may be necessary for oxidation of plasma substrates other than ferrous iron.

Shiva *et al.* hypothesized that NO is a physiological substrate of ceruloplasmin that results in the generation of plasma NO_2^- . Observing that plasma can catalyze NO_2^- formation at physiologic hemoglobin concentrations, the authors showed that a redox-active plasma protein of high molecular weight functions as an NO oxidase. Removal of ceruloplasmin from plasma using specific antibodies decreased plasma nitrite content by 50%, whereas addition of ceruloplasmin to plasma increased NO_2^- production in the presence of an NO donor. The plasma nitrite concentrations and NO oxidase activity of mice and people with aceruloplasminemia were significantly lower than those of controls. Experiments with metal chelators and erythrocytes confirmed that it is ceruloplasmin that accounts for this plasma NO oxidase activity. Given the lower plasma nitrite concentration seen in aceruloplasminemia and the known role of NO_2^- in oxygen-dependent vascular responsiveness¹, the authors next examined the physiologic relevance of these observations using a mouse model of liver injury that occurs after impaired liver blood flow. Notably, as compared to

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