Figures and figure supplements

A terpene synthase-cytochrome P450 cluster in *Dictyostelium discoideum* produces a novel trisnorsesquiterpene

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Figure 1. Structure elucidation of DdTPS8 product and biosynthetic mechanism. (A) Structure of discoidol. (B) Contiguous spin systems indicated by bold lines observed in discoidol by 1H,1H-COSY NMR, single headed arrows indicate diagnostic HMBC correlations. (C) Important NOESY correlations that are indicated by double headed arrows observed in discoidol. (D) Biosynthetic mechanism from farnesyl diphosphate (FDP) to discoidol catalyzed by DdTPS8. See also Figure 1—figure supplements 1–4.

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Figure 1—figure supplement 1. Determination of the absolute configuration of discoidol.
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Figure 1—figure supplement 2. Investigation of the reprotonation step in the cyclization mechanism of discoidol.
DOI: https://doi.org/10.7554/eLife.44352.004
Figure 1—figure supplement 3. Investigation of the 1,2-hydride migration by incubation of (3–13C,2–2H)FDP with DdTPS8. DOI: https://doi.org/10.7554/eLife.44352.005
Figure 1—figure supplement 4. Incubation experiments with (12–13C)FDP and (13–13C)FDP and DdTPS8.
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Figure 2. DdTPS8 insertional mutant and its volatile profile. (A) Schematic presentation of DdTPS8 gene with an insert of 1.6 kb. (B) Volatiles were collected from the headspace of the cultures and analyzed using GC-MS. Total ion chromatograms are shown. 1, unknown compound; 2, unidentified compound; 3, unidentified sesquiterpene hydrocarbon; 4, unidentified compound; 5, β-maaliene; 6, aristolene; 7, calarene; 8–10, unidentified sesquiterpene hydrocarbons; 11, nerolidol. See also Figure 2—figure supplement 1.

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Figure 2—figure supplement 1. Verification of DdTPS8 insertion mutant. DOI: https://doi.org/10.7554/eLife.44352.008
Figure 3. Cytochrome P450 (CYP) genes associated with DdT8. (A) Expression pattern of three CYP genes that showed highest level of coexpression coefficient with DdT8. The cartoons show the six stages of multicellular development of D. discoideum: individual cells (0 hr), streaming (8 hr), loose aggregate (10 hr), slug (16 hr), Mexican hat (20 hr) and fruiting bodies (24 hr). (B) DdT8 and CYP521A1 are neighbor genes. The number above the black line indicates the length of the intergenic region in base pairs.

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Figure 4. Volatile profiles of E. coli B21-DE3-Star expressing different combinations of CYP521A1, CYP508C1, the P450 reductase gene RedB, and the terpene synthase gene DdTPS8. Volatiles were collected from the headspace of the induced bacterial cultures using PDMS tubes and analyzed using GC-TDU-MS. The extracted ion chromatograms for m/z 105 (A) and the mass spectra of the DdTPS8 product (B) and CYP521A1 product (C) are shown. See also Figure 4—figure supplement 1.

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Figure 4—figure supplement 1. Activity of DdTPS8 and CYP521A1 in cell-free enzyme assays in the presence of (E,E)-FDP.

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Figure 5. Mass spectra (A–C) and structures (D) of DdTPS8 products and CYP521A1 products derived from unlabeled farnesyl diphosphate (FDP) (A), 13C15-FDP (B), and 1,1-2H2,11-13C-FDP (C + D). The genes were coexpressed in E. coli BL21-DE3-Star together with the P450 reductase gene RedB. Crude protein extracts were incubated with unlabeled or labeled (E,E)-FDP and volatile enzyme products were collected from the headspace of the assays using PDMS tubes. Product analysis was performed with GC-TDU-MS. See also Figure 5—figure supplement 1.

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Figure 5—figure supplement 1. Formation of discodie by CYP521A1.
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Figure 6. Developmental phenotype of the DdTPS8 knockout mutant. Wild type AX4 cells (WT) and mutant DdTPS8 cells (mutant) were grown separately in HL5 to the log phase, washed in buffer, and plated clonally on dark nitrocellulose filters. The filters were cut in half and placed next to each other in one dish. The cells were incubated in the dark at 22°C and photographed from above with a dissecting microscope at the indicated times. This experiment was independently performed three times with same results and the data shown represent one of the replicates. DOI: https://doi.org/10.7554/eLife.44352.014
Figure 7. TPS-CYP gene clusters in D. discoideum (A) and D. purpureum (B). Green blocks depict TPS genes, red blocks indicate CYP genes. The blue block indicates a non-TPS/CYP gene. The numbers above the black lines indicate length in base pairs.

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