BIOSYNTHESIS

Metal matters

A study of an insect prenyltransferase demonstrates that the product specificity of this bifunctional enzyme can be regulated by the presence of different divalent metal cofactors, resulting, for example, in the production of the precursors for either insect defense compounds or developmental hormones.

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Terpenoids are the largest group of natural products, containing more than 55,000 identified compounds that have many essential functions in all domains of life. Examples include the well-known sterols, juvenile sesquiterpene hormones and various monoterpene-, diterpene- and triterpene-derived natural products for defenses in animals and plants. Despite their immense diversity, terpenoids are all ultimately produced from simple C_5 linear allylic diphosphate precursors. The mechanisms for induction and regulation of terpenoid biosynthetic pathways are diverse, and many remain obscure. Transcription factors are known to function as key regulators of metabolic pathways[1], and the many cases of coexpression of genes from metabolic gene clusters[2,3] suggest that chromatin-level regulation of natural product biosynthesis may exist[4]. A recent study by Frick *et al.*[5] of a multifunctional diphosphate synthase demonstrates a new regulation mechanism that controls product specificity on the basis of the local concentrations of particular metal ions.

Short-chain isoprenyl diphosphate synthase (scIDS) enzymes catalyze the reactions that yield C_{10}, C_{15} and C_{20} prenyl diphosphates, which are the precursors of monoterpenes, sesquiterpenes and diterpenes, respectively[6]. Although most scIDSs produce single products, several multifunctional scIDSs that are able to catalyze the formation of either C_{10} or C_{15} products have been reported from insects and plants[7]. scIDSs use a trinuclear metal cluster cofactor for activation of a 'head-to-tail' alkylation reaction that couples C_5 and/or C_{10} allylic cosubstrates to yield longer-chain products[8]. Of interest here are the allylic cosubstrates isopentenyl pyrophosphate (IPP, C_5), dimethylallyl pyrophosphate (DMAPP, C_5) and geranyl pyrophosphate (GPP, C_{10}) and the products GPP and farnesyl pyrophosphate (FPP, C_{15}). A previous study of a bifunctional scIDS from an aphid suggests that product chain length is determined by the size of the hydrophobic pocket in the scIDS active 'center'[9]. Studies of a partially purified FPP synthase and of cell homogenates from a lepidopteran have indicated that alterations in Mg^{2+} and Mn^{2+} concentrations affected the accumulation ratios of scIDS products of different lengths[10].

Motivated by these reports, Frick *et al.*[5] probed the specific influence of various divalent cation cofactors on the bifunctionality of a beetle scIDS (*Pc*IDS1, from *Phaedon cochleariae*). The core of the study consisted of a series of *in vitro* *Pc*IDS1 assays testing a diversity of both allylic substrates (IPP, DMAPP and GPP) and separate divalent cations (Co^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}). In the assays that combined IPP and DMAPP as substrates, maximum *Pc*IDS1 activity was observed with Co^{2+} as the metal cofactor. A notable additional result from these assays was that the ratios of product accumulation (GPP versus FPP) varied substantially depending on which of the metal cofactors was present (**Fig. 1**). *Pc*IDS1 produced about 96% GPP and only 4% FPP in the presence of Co^{2+} or Mn^{2+}, whereas it produced 18% GPP and 82% FPP in the presence of Mg^{2+}. Follow-up assays that varied the relative concentration of these metals indicated that the Mg^{2+}-catalyzed activity of *Pc*IDS1 is abolished as soon as Co^{2+} reaches its optimal concentration.

Rigorous kinetic studies further bolstered their assertion that *Pc*IDS1 has an energetic preference for Co^{2+} with DMAPP as an allylic cosubstrate for C_{10} GPP production but showed that C_{15} FPP production was favored when Mg^{2+} was the cofactor. Theoretical modeling of hypothetical reaction energies indicated that *Pc*IDS1 has a conspicuously higher affinity for Co^{2+} than for Mg^{2+}. Cation quantification studies of *P. cochleariae* larval tissues reinforced the physiological plausibility that these organisms may indeed control the product specificity of scIDSs through changes in

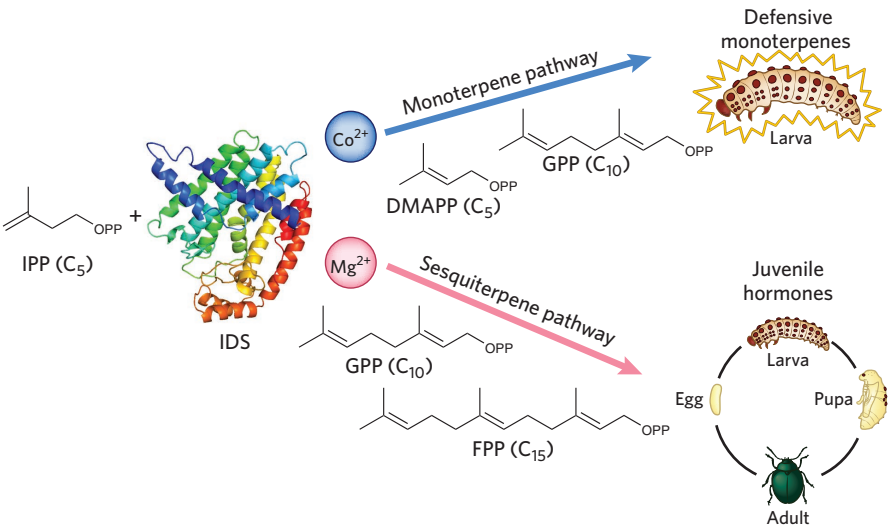

Figure 1 | Regulation of terpenoid pathways by metal cofactors. A mustard leaf beetle (*P. cochleariae*) enzyme *Pc*IDS1 (here represented by an avian farnesyl pyrophosphate synthetase, Protein Data Bank code 1UBV) alters its product specificity on the basis of the presence of Co^{2+} or Mg^{2+}. With Co^{2+} as a cofactor, *Pc*IDS1 preferentially combines cosubstrates IPP and DMAPP to produce GPP, the precursor for monoterpene metabolism (blue arrow). Monoterpenes such as chrysomelidial are known to be important in insect chemical defense. With Mg^{2+} as a cofactor, *Pc*IDS1 preferentially combines cosubstrates IPP and GPP to produce FPP, the precursor for sesquiterpene metabolism (pink arrow), producing sesquiterpene compounds as insect juvenile hormones that regulate many aspects of insect development.

the local concentrations of these metal ions. Finally, Frick *et al.*[5] used size-exclusion chromatography to show that the *Pc*IDS1 apo enzyme (lacking a metal cofactor), *Pc*IDS with Co^{2+} and *Pc*IDS1 with Mg^{2+} all eluted at different volumes, indicating that the hydrodynamic volume, and thus the quaternary structure of the protein, is altered by the various divalent cofactors. Resolving the three-dimensional structures of *Pc*IDS1 with Co^{2+} or Mg^{2+} will be required to characterize the precise chemical mechanism underlying the observed metal cofactor–dependent regulation of product specificity.

The discovery of this metal ion concentration–dependent enzyme product specificity reveals a new type of metabolic 'regulation'. In contrast to alternative splicing mechanisms, which generate multiple gene products from a single genomic locus, this metal ion-dependent regulatory mechanism allows a single enzyme to selectively control the metabolites it produces, thus potentially altering the flow of carbon into separate metabolic pathways. This type of 'adjustable' enzyme may afford insects an efficient mechanism for the generation of the chemical diversity that is critical for adaptation to ever-changing ecological contexts. Systematic investigation of the effects of diverse metal cofactors on various metalloproteins may reveal more examples of this regulatory mechanism.

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References

1. Butelli, E. *et al. Nat. Biotechnol.* **26**, 1301–1308 (2008).
2. Qi, X. *et al. Proc. Natl. Acad. Sci. USA* **101**, 8233–8238 (2004).
3. Field, B. & Osbourn, A. *Science* **320**, 543–547 (2008).
4. Hurst, L.D., Pál, C. & Lercher, M.J. *Nat. Rev. Genet.* **5**, 299–310 (2004).
5. Frick, S. *et al. Proc. Natl. Acad. Sci. USA* **110**, 4194–4199 (2013).
6. Gershenzon, J. & Dudareva, N. *Nat. Chem. Biol.* **3**, 408–414 (2007).
7. Vandermoten, S., Haubruge, E. & Cusson, M. *Cell Mol. Life Sci.* **66**, 3685–3695 (2009).
8. Aaron, J.A. & Christianson, D.W. *Pure Appl. Chem.* **82**, 1585–1597 (2010).
9. Vandermoten, S. *et al. Insect Biochem. Mol. Biol.* **39**, 707–716 (2009).
10. Sen, S.E. *et al. Insect Biochem. Mol. Biol.* **37**, 29–40 (2007).

Competing financial interests

The authors declare no competing financial interests.