Breaking the Mold: A Distinct Class of Terpenoid Cyclases Redefines Abscisic Acid Biosynthesis

The crystal structures of BcABA3 and its homologs reveal a distinct class of terpenoid cyclases that lack canonical motifs. This enzyme architecture is characterized by a unique glutamate-chelated Mg^{2+} cluster and a distal Zn^{2+} anchor, which enable fungal abscisic acid biosynthesis and provide a versatile platform for the production of novel bacterial terpenoids.

Terpenoid cyclases (TCs) are the molecular architects behind the most diverse family of natural products, orchestrating the complex cyclization of linear isoprenoid precursors into intricate ring structures. For decades, the biochemical understanding of this enzyme family has been grounded in a strict structural requirement: the presence of aspartate-rich motifs, such as DDXXD or NSE/DTE.[1] These motifs are considered indispensable for coordinating the divalent metal ions (typically Mg^{2+}) that trigger the ionization of the substrate's pyrophosphate group. However, nature often defies established rules. The discovery of BcABA3, a key enzyme in the phytopathogenic fungus *Botrytis cinerea*, challenged this paradigm. Unlike plants, which synthesize the stress hormone abscisic acid (ABA) indirectly from carotenoids, these fungi utilize a direct pathway involving the cyclization of farnesyl pyrophosphate (FPP) by BcABA3.[2] Despite catalyzing a classic sesquiterpene cyclization, BcABA3 lacks the signature aspartate-rich motifs.[3] To elucidate the molecular basis of this evolutionary anomaly, the research team led by Rey-Ting Guo (Hangzhou Normal University, China) solved the high-resolution crystal structures of BcABA3 and its homologs using diffraction data collected at **TPS 05A** and **TPS 07A** of the NSRRC.

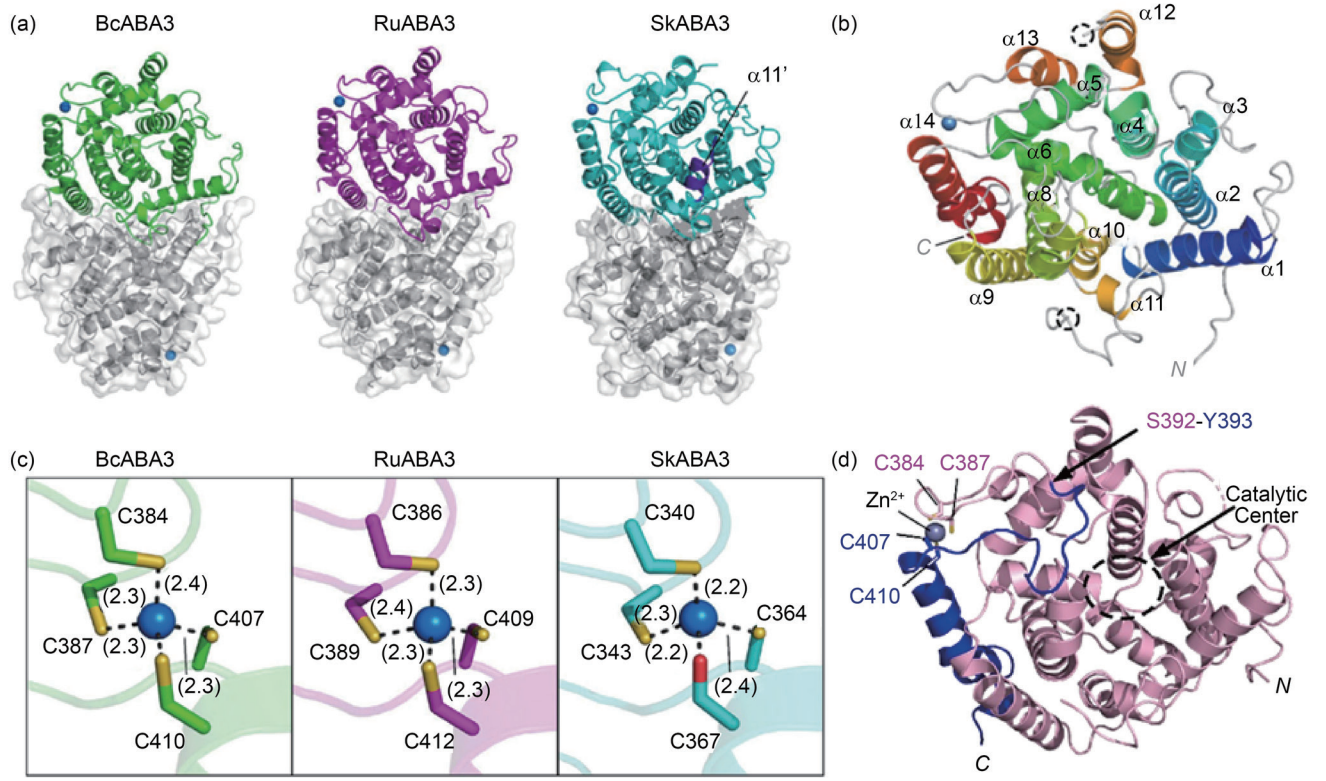

Fig. 1: Structural architecture and essential distal zinc anchor of ABA3-like TCs. (a) Comparative overall structures of the fungal BcABA3 and RuABA3 and the bacterial SkABA3. Despite their diverse origins, all three display a highly conserved homodimeric configuration. (b) Topology of a single BcABA3 polypeptide chain. The enzyme exhibits an all-α-helix fold (labeled α1–α14), featuring a unique C-terminal extension (α11–α14), which is characteristic of this cyclase class. (c) Close-up view of the metal coordination site on the loop between helices α13 and α14. The zinc ion (Zn^{2+}, blue sphere) is coordinated in a tetrahedral geometry by conserved cysteine residues, forming a structural motif distant from the active center. (d) Structural role of the Zn^{2+} site. The loop containing the Zn-binding motif extends to cover the top of the catalytic center (indicated by the dashed circle). The blue region corresponds to the C-terminal segment absent in BcABA3-S, a naturally occurring short variant. The inactivity of this truncated variant demonstrates that the Zn-stabilized cap is essential for maintaining active site integrity. [Reproduced from Ref. 4]

The path to determining these structures required overcoming significant challenges in protein stability. Initial attempts to crystallize full-length BcABA3 were hampered by the protein's labile nature. By employing a strategy of N-terminal truncation and utilizing the more stable homolog RuABA3 (from *Rutstroemia sp.*), the team successfully determined the structures of BcABA3, RuABA3, and a bacterial homolog, SkABA3 (from *Shimazuella kribbensis*) (**Fig. 1(a)**). Structural analysis reveals that this distinct class of TCs adopts an all-α-helix fold. While the N-terminal topology (helices α1–α10) resembles that of canonical TCs, the C-terminal region is uniquely extended with four additional helices (α11–α14) (**Fig. 1(b)**). A striking feature was identified within the extensive loop connecting helices α13 and α14: a specific metal-binding site coordinated in a tetrahedral geometry by cysteine residues (**Fig. 1(c)**). Atomic spectrometric analysis confirmed this to be a Zinc (Zn^{2+}) ion. Although located remotely from the active center, mutagenesis data indicate that this Zn^{2+} site functions as a crucial structural anchor. It stabilizes the loop extending over the active site, maintaining the architectural integrity required for catalysis, a finding consistent with the inactivity of natural variants lacking this C-terminal region (**Fig. 1(d)**).[3]

The most significant departure from canonical enzyme mechanisms is found in the architecture of the active site. By determining the complex structures of RuABA3 with a substrate analog (FSPP) and SkABA3 with the reaction product (PPi) (**Fig. 2(a)**), the researchers identified a novel metal-binding strategy. In the complete absence of aspartate-rich motifs, these enzymes utilize a strictly conserved glutamate residue (E124 in BcABA3) to coordinate the Mg^{2+} cluster required for substrate ionization (**Fig. 2(b)**). Additionally, the molecular recognition of the pyrophosphate moiety is mediated by a distinctive arrangement of residues: four basic residues (R202, K249, H250, and R312) and two tyrosine residues (Y242 and Y313) establish a precise hydrogen-bond network (**Fig. 2(b)**). This network features a conserved arginine-tyrosine (RY) pair. While RY pairs are common in Type I TCs, the pair in BcABA3 serves a dual function by interacting

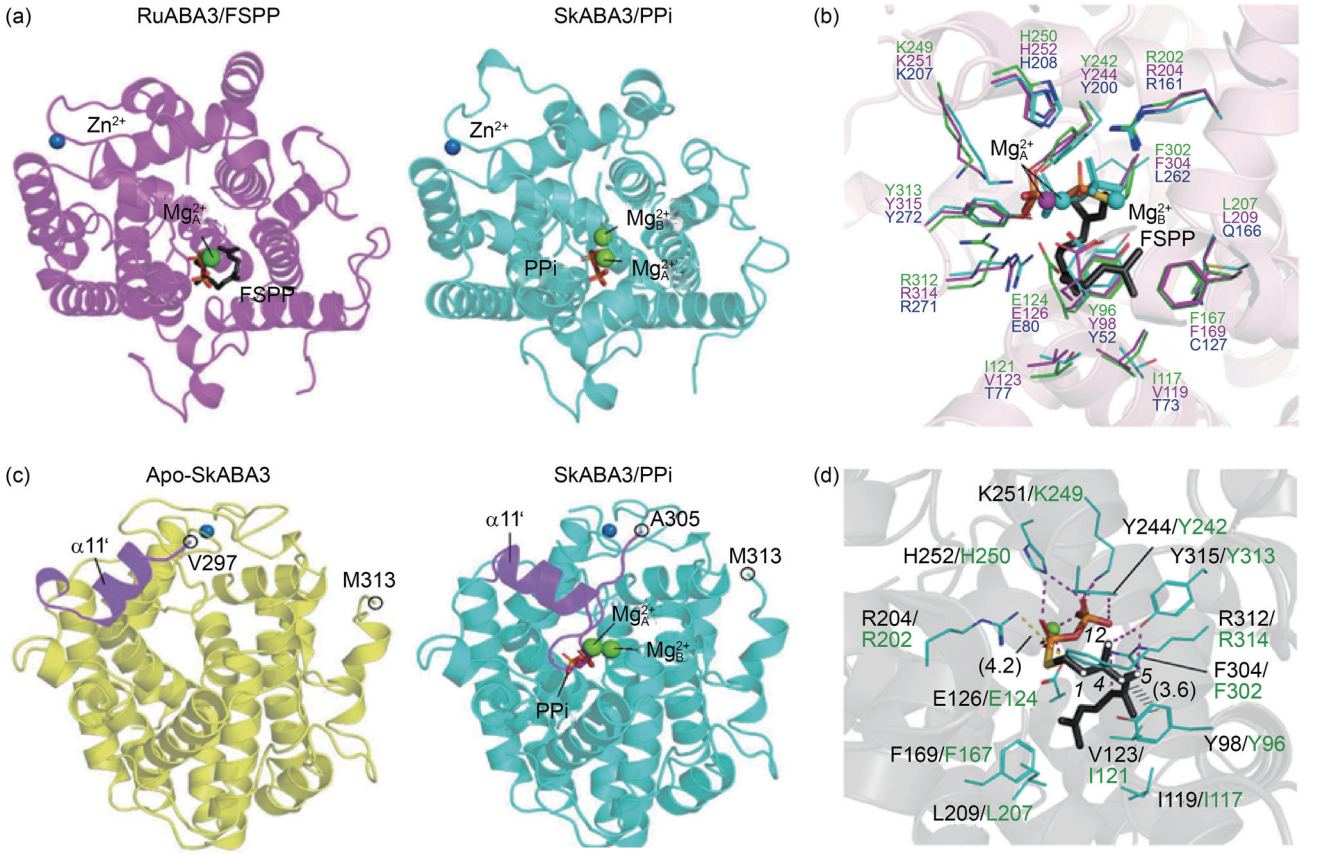

Fig. 2: Unique active site architecture and catalytic mechanism of ABA3-like cyclases. (a) Structures of RuABA3 bound to the substrate analog FSPP (magenta) and SkABA3 bound to the product PPi (cyan) show the ligand positioned deep within a central hydrophobic pocket. (b) Comparison of the substrate-binding pockets of apo-BcABA3 (green), RuABA3-FSPP (magenta), and SkABA3-PPi (cyan) demonstrates that, in the absence of a canonical aspartate-rich motif, a strictly conserved glutamate residue coordinates the Mg^{2+} cluster (spheres) required for catalysis. The pyrophosphate group is stabilized by a unique arrangement of basic residues and tyrosines, differing from classical TCs. (c) Substrate-driven conformational changes in SkABA3: upon ligand binding, helix α11' rotates approximately 90° (purple), positioning the adjacent loop region to cover the active site, which likely protects reactive carbocation intermediates. (d) The proposed substrate interaction network in the RuABA3-FSPP complex: the aromatic ring of residue Y96 (and Y98 in SkABA3) provides critical packing interactions that stabilize the hydrocarbon tail of the substrate. Mutagenesis studies indicate that this specific shaping of the hydrophobic pocket is essential for directing the cyclization cascade. [Reproduced from Ref. 4]

with both the pyrophosphate group and the hydrocarbon portion of the substrate.

A detailed structural comparison further revealed the dynamic features of the catalytic cycle. In the SkABA3 structure, helix α11' rotates by approximately 90° upon ligand binding, accompanied by the movement of a loop that closes over the active site (**Fig. 2(c)**). This induced-fit mechanism likely protects the highly reactive carbocation intermediates from premature quenching by the solvent. The reaction proceeds through a distinct pathway involving neutral intermediates, such as (E)-β-farnesene and allofarnesene.[3] The study demonstrated that the shape of the hydrophobic pocket directs this cascade; residues such as Y96 provide critical packing interactions to stabilize intermediates (**Fig. 2(d)**). Mutation of these pocket-lining residues altered the reaction pathway, causing the enzyme to stall and release intermediates rather than the final product.

The characterization of the bacterial homolog SkABA3 further broadens the biological significance of this work. Although SkABA3 shares the same "ABA3-like" fold and active site features, it produces a chemically distinct and uncharacterized sesquiterpenoid isomer. This demonstrates that the newly defined enzyme class is not confined to fungal ABA biosynthesis but instead represents a versatile evolutionary platform for synthesizing diverse natural products. Since terpenoids are a major source of pharmacological agents—including essential antibiotics and chemotherapeutics—this finding has considerable potential for pharmaceutical applications. Using the structural blueprint of ABA3, researchers can now search microbial genomes for similar cryptic enzymes, potentially leading to the discovery of new therapeutic lead compounds. These structural insights collectively establish a foundation for discovering and engineering novel bioactive compounds within the extensive microbial terpenoid repertoire. (Reported by Chun-Hsiang Huang, NSRRC)

This report features the work of Rey-Ting Guo and his collaborators published in Nat. Commun. ***16****, 207 (2025).*

TPS 05A Protein Microcrystallography
TPS 07A Micro-focus Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

References

1. D. W. Christianson, Chem. Rev. **117**, 11570 (2017).
2. V. Siewers, L. Kokkelink, J. Smedsgaard, P. Tudzynski, Appl. Environ. Microbiol. **72**, 4619 (2006).
3. J. Takino, T. Kozaki, Y. Sato, C. Liu, T. Ozaki, A. Minami, H. Oikawa, J. Am. Chem. Soc. **140**, 12392 (2018).
4. S. Li, J.-W. Huang, J. Min, H. Li, M. Ning, S. Zhou, Y. Yang, C.-C. Chen, R.-T. Guo, Nat. Commun. **16**, 207 (2025).