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Spotlight

The BAHD and the bold: the mitochondria's role in alkaloid artistry

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In a recent study, Zeng *et al.* uncovered 3β-tigloyloxytropane synthase (TS) in *Atropa belladonna*, characterizing its mitochondrial localization and substrate specificity. The discovery of this enzyme opens up new bioengineering possibilities for tropane alkaloids (TAs), enhancing the potential for sustainable agriculture and expanding our knowledge of TA biosynthesis.

Humans have used plant specialized metabolites as medicines for millennia. Among these, TAs stand out with their unique 8-azabicyclo[3.2.1]octane core, featuring a cycloheptane ring and a nitrogen bridge, known as the tropane moiety. Over 300 TAs have been identified across various plant families, such as Solanaceae, Convolvulaceae, Rhizophoraceae, and Erythroxylaceae [1]. Recent research has shed light on the biosynthesis pathways of hyoscyamine and scopolamine in *A. belladonna* [2], also known as deadly nightshade, and the cocaine biosynthetic pathway in the coca plant [3]. Calystegines, a subset of TAs derived from 3β-tropanol (pseudotropine), are prevalent in both solanaceous as well as brassicaceous plants. Vegetables within the Solanaceae family include tomatoes, potatoes, eggplants, and hot peppers, while the Brassicaceae family includes cruciferous vegetables (i.e., broccoli and cabbage) as well as *arabidopsis (Arabidopsis thaliana)* and rapeseed [4]. TAs are of interest because they show potential as treatments for metabolic disorders in humans. Specifically, 3β-benzoyloxytropane (tropacocaine) could serve as an ophthalmic and spinal anesthetic, while 3β-tigloyloxytropane (tigloidine or tropogline) has applications in treating neurodegenerative diseases [5]. However, our understanding of the biosynthesis of 3β-tropanol-derived TAs remains limited. In a groundbreaking study, Zeng *et al.* isolated the enzyme TS from *A. belladonna*. This enzyme, part of the BAHD acyltransferase (BAHD-AT) family [6], was surprisingly found in the mitochondria. This discovery not only provides new insights into the cellular synthesis of TAs but also paves the way for the synthetic biology production of 3β-tropanol-derived TAs [7].

In their study, Zeng *et al*. unraveled the genetics behind the biosynthesis of 3β-tigloyloxytropane. Their initial step involved a comparative analysis of the metabolite's presence across various tissues of *A. belladonna* [7]. By correlating the levels of 3β-tigloyloxytropane with gene expression patterns, they pinpointed the responsible structural genes. The researchers observed that the metabolite predominantly accumulated in the plant's primary and secondary roots. Among the 46 BAHD-ATs identified, one enzyme, aptly named TS, paralleled this specific pattern of expression. A closer examination into the enzyme's lineage through phylogenetic analysis placed TS in the company of functionally characterized BAHD-ATs known for their affinity to short-chain acyl-CoA thioesters. This cluster also included EcCS and EcBAHD8, enzymes instrumental in the formation of cocaine, which utilize methylecgonine with a 3β-2-carbomethoxy tropane ring skeleton as the acyl acceptor [8]. Advancing their research, Zeng *et al*. synthesized recombinant TS and conducted experiments that showcased its ability to synthesize 3β-tigloyloxytropane when provided with 3β-tropanol and tigloyl CoA, through a series of esterification reactions (Figure 1). Further experimentation with a variety of acyl donors revealed that while acetyl CoA and benzoyl CoA could act as substitutes, TS exhibited a marked preference for tigloyl-CoA, underscoring its specificity and potential for targeted applications in metabolic engineering [7]. This study not only illuminates the pathway of 3β-tigloyloxytropane synthesis but also highlights the enzyme's promising role in the burgeoning field of synthetic biology.

Utilizing advanced techniques like molecular docking and site-directed mutagenesis, Zeng *et al*. elucidated the catalytic mechanism of TS [7]. The model posited His162 as the pivotal base catalyst within the catalytic pocket of TS. Confirming this, mutating His162 led to a total loss of enzymatic function. Other mutations, aligning with the model's predictions for the binding pocket, similarly reduced the activity of TS. Kinetic studies revealed the preference of TS for short-chain acyl thioesters over benzoyl-CoA, suggesting that steric hindrance is an important factor for substrate specificity. Mutations informed by the docking model alleviated this hindrance, enhancing benzoyl-CoA activity and demonstrating the model's utility in refining enzyme efficiency.

This research paves the way for bioengineering advancements, highlighted by the synthesis of 3β-tigloyloxytropane in tobacco and *Escherichia coli*. In tobacco, coexpression of the seven genes involved in 3β-tigloyloxytropane biosynthesis yielded low production levels. However, supplementing with 3β-tropanol and tiglic acid significantly boosted production. In *E. coli*, initial attempts failed, but incorporating an isobutyryl CoA synthase from *Pseudomonas chlororaphis* dramatically increased 3β-tigloyloxytropane levels, surpassing those in tobacco [7].

Typically, BAHD-ATs exhibit cytosolic activity. However, this study presents the unprecedented localization of a BAHD-AT within the mitochondria. This discovery

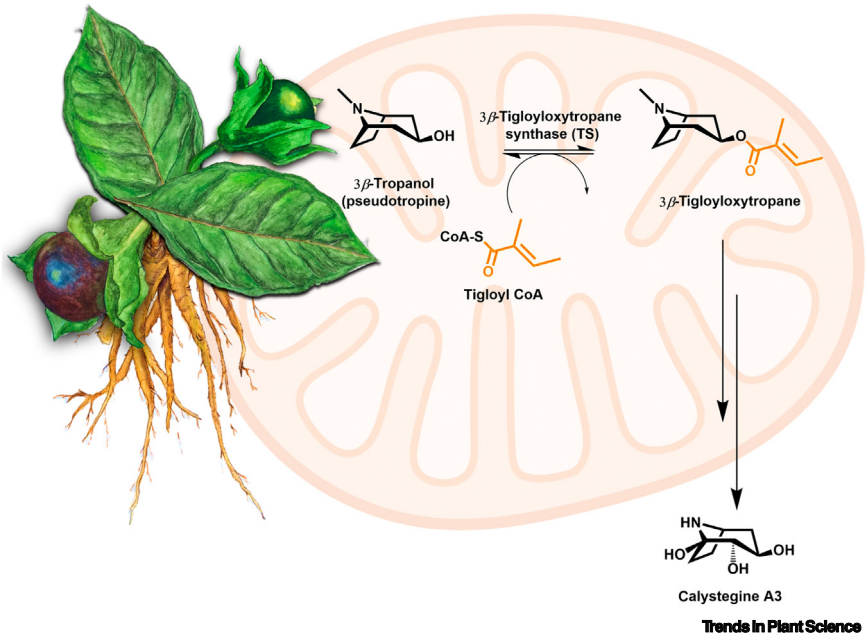

Figure 1. Activity of 3β-tigloyloxytropane synthase (TS) is present in the mitochondria of the solanaceous plant *Atropa belladonna*.

suggests a potential research trajectory exploring the targeting of BAHD-ATs to nontraditional organelles, which could enhance substrate accessibility and, consequently, ester production for pharmaceutical and industrial applications. Future research may delve into the evolution of the mitochondrial targeting signal peptide sequence and investigate the presence of analogous sequences within and beyond the Solanaceae.

The strategic manipulation of calystegines in crops holds profound implications for both agriculture and our comprehension of the ecological functions of these alkaloids. A nuanced understanding of calystegine ecology could illuminate their role in bolstering agricultural resilience and advancing sustainable farming practices. Eliminating these alkaloids from key agricultural species could enhance the bioavailability and absorption of nutrients, benefiting consumer health [9]. Moreover, because calystegines and other alkaloids typically confer a bitter taste to plants, their removal might result in more palatable vegetables. Additionally, considering the potential of calystegines to act as natural pest deterrents, their careful management could also contribute to reducing reliance on synthetic pesticides, aligning with eco-friendly agricultural goals. Thus, the targeted breeding of calystegine-limited crops could signify a pivotal step towards more sustainable and health-conscious food production.

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Declaration of interests

The authors declare no conflicts of interests.

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References

1. Kim, N. *et al.* (2016) Tropane and granatane alkaloid biosynthesis: a systematic analysis. *Molecules* 21, 1510
2. Huang, J.P. *et al.* (2021) Tropane alkaloid biosynthesis: a centennial review. *Nat. Prod. Rep.* 38, 1634–1658
3. Chavez, B.G. *et al.* (2022) Elucidation of tropane alkaloid biosynthesis in *Erythroxylum coca* using a microbial pathway discovery platform. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2215372119
4. Biastoff, S. and Dräger, B. (2007) Calystegines. In *The Alkaloids: Chemistry and Biology* (Cordell, G.A., ed.), pp. 49–102, Academic Press
5. Sanghvi, I. *et al.* (1968) Pharmacology of a potential anti-Parkinson agent: tigloidine. *Eur. J. Pharmacol.* 4, 246–253
6. Moghe, G. *et al.* (2023) BAHD company: the ever-expanding roles of the BAHD acyltransferase gene family in plants. *Annu. Rev. Plant Biol.* 74, 165–194
7. Zeng, J. *et al.* (2024) Discovering a mitochondrion-localized BAHD acyltransferase involved in calystegine biosynthesis and engineering the production of 3β-tigloyloxytropane. *Nat. Commun.* 15, 3623
8. Schmidt, G.W. *et al.* (2015) The last step in cocaine biosynthesis is catalyzed by a BAHD acyltransferase. *Plant Physiol.* 167, 89–101
9. Haraguchi, M. *et al.* (2003) Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). *J. Agric. Food Chem.* 51, 4995–5000