# 学 位 論 文 の 要 約

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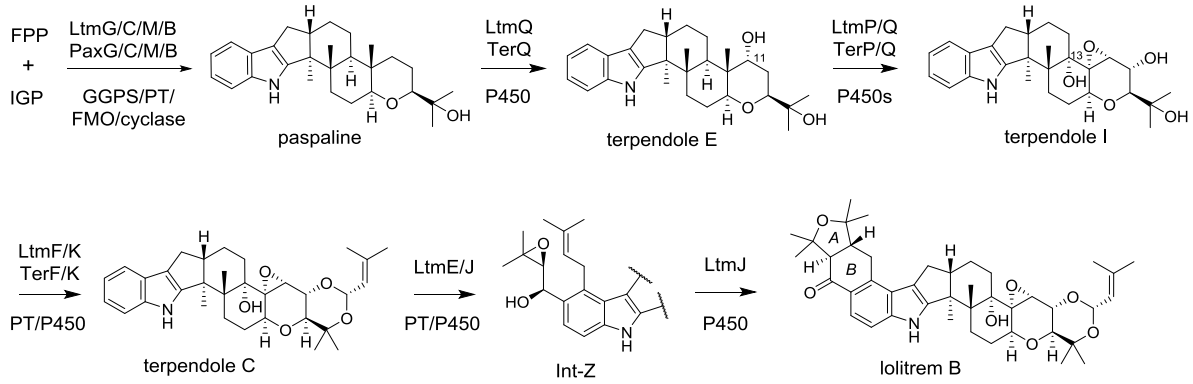
## 学 位 論 文 題 名

Studies on Unusual Oxidative Transformations in Fungal Natural Product Biosynthesis
（糸状菌由来天然物の生合成に関与する特異な酸化的変換に関する研究）

Oxidative transformations play critical roles in structural diversification of natural products, conferring numerous biological activities derived from the chemical structures. With the extensive biosynthetic studies reported in the past decades, simple oxidations, such as hydroxylation and epoxidation of substrates, and their reaction mechanism have been well elucidated. However, biological system often employs highly elaborated oxidations, which frequently accompany skeletal rearrangements and cyclizations. Currently, these unusual oxidations have not been understood well and even the biosynthetic gene(s) remain unidentified in many cases. To achieve the enzymatic total synthesis of complex natural products, dissection of their biosynthetic pathway, particularly the unusual oxidations, is an urgent issue. In this thesis, I focused on the two subjects concerning the unusual oxidative processes in the fungal natural product biosynthesis that largely contribute to the structural diversification.

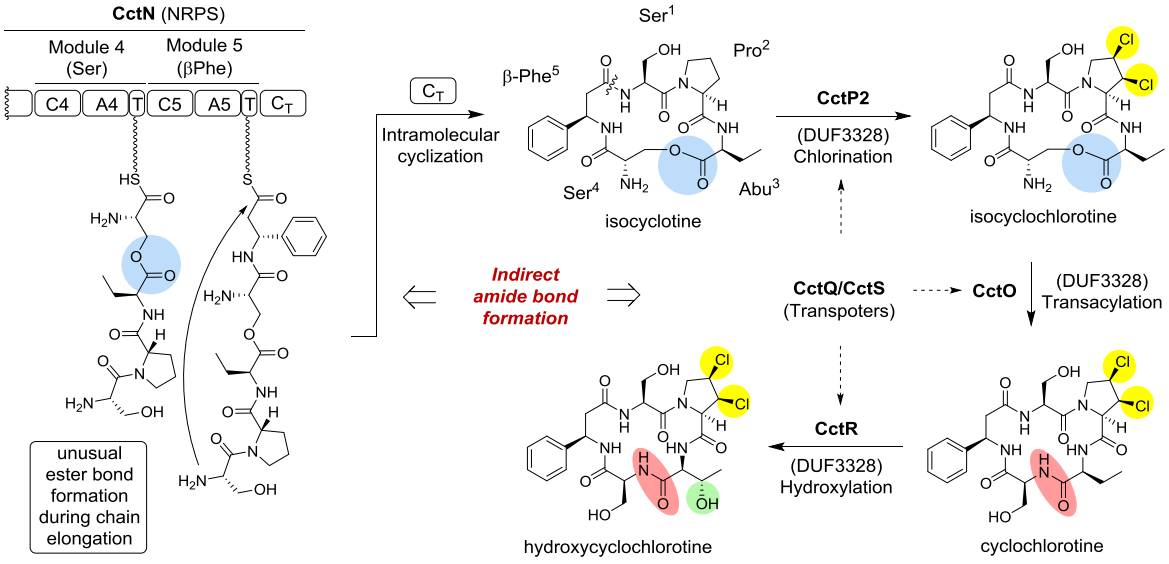
Chapter 1 described typical catalytic mechanisms and reaction examples of common oxidases and related enzymes. The highly reliable and efficient CRISPR-Cas9/*Aspergillus oryzae* heterologous expression system that employed in this thesis is also introduced. This hot spot (HS)-knock-in method allowed me to effectively reconstitute the biosynthetic pathways of lolitrems and cyclochlorotines in Chapter 2 and Chapter 3.

In Chapter 2, biosynthetic study of indole diterpene lolitrem B was carried out (Scheme 1). Ten genes in total were introduced into *A. oryzae* and achieved total biosynthesis of lolitrem B. The HS-knock-in method enabled facile expression of the biosynthetic genes in various combinations, yielding the biosynthetic intermediates in high yield. Given that the prenyltransferase LtmE and the cytochrome P450 LtmJ are solely responsible for the construction of the unique 5/6 bicyclic (A/B rings) structure, the functional analysis of these two enzymes was then conducted by the bioconversion experiments. Using the transformant AO-*ltmEJ* and known IDTs/ISTs as substrates, LtmEJ were found to accept the IDTs/ISTs containing a C-13/C-9 hydroxy group. During this experiment, I also identified compounds having an epoxyalcohol on prenyl moiety as LtmJ-catalyzed cyclization intermediates. Based on the structures of these intermediates, the mechanism of LtmJ-catalyzed oxidative cyclization was proposed, which is triggered by the H-abstraction and rapid epoxide opening. The proposed mechanism was further supported by the density functional theory calculation and the synthetic approach with the model compound. The unveiled late-stage modification route of lolitrems shows that oxidative transformations were employed for structural diversification of paspaline-derived IDTs.




Scheme 1. Biosynthetic pathway of lolitrem B.

In Chapter 3, I focused on the biosynthesis of cyclochlorotines, whose biosynthetic gene cluster lacks conventional oxidases/modification enzymes, although halogenation and hydroxylation are expected to be involved. Gene-knock out experiments revealed the involvement of three Domain-Unknown-Function (DUF3328) proteins, CctP2/CctO/CctR in the biosynthetic pathway (Scheme 2). After the nonribosomal peptide synthetase (NRPS) CctN constructing isocyclotine, which contains unusual internal ester linkage between Abu[3] and Ser[4], CctP2 catalyzes dichlorination of Pro[3] residue. Subsequently, the CctO-mediated transacylation gives cyclochlorotine, followed by CctR-catalyzed hydroxylation which furnishes the final product, hydroxycyclochlorotine. The proposed biosynthetic route of cyclochlorotines was further confirmed by the heterologous expression in *A. oryzae*. In addition, two transporters, CctQ and CctS were found to be essential for functional reconstitution of DUF3328 proteins. Subcellular localization examination of CctQ and CctS with fluorescent tags suggested that these two proteins locate in unspecific organelle, indicating the same subcellular localization of CctP2/CctO/CctR. Based on these findings, an intracellular trafficking system was proposed for biosynthesis of cyclochlorotines. Through the studies on CctP2/CctO/CctR, together with other studies concerning DUF3328 proteins, a new family of fungal oxidase was proposed.




Scheme 2. Biosynthetic pathway of cyclochlorotine and its analogues.