Advances in Microbiology 微生物前沿, 2020, 9(4), 141-155
Published Online December 2020 in Hans. http://www.hanspub.org/journal/amb
https://doi.org/10.12677/amb.2020.94020

Hans汉斯

微生物卤化酶及其应用研究进展

郑哲麟，胡文达，何亚文*

上海交通大学生命科学技术学院，微生物代谢国家重点实验室，教育部代谢与发育科学国际合作联合实验室，上海
Email: *yawenhe@sjtu.edu.cn

收稿日期：2020年11月15日；录用日期：2020年12月3日；发布日期：2020年12月10日

摘　要

由于卤素原子具有强大的电负性，它的存在可以改变化合物的物理化学性质和生物活性。尽管我们可以通过化学反应合成卤化物，但能量消耗大，易对环境造成污染。许多天然化合物含有卤素原子，自然界演化出多种卤化酶来负责这些化合物的卤化。本文综述了目前发现的各种卤化酶及其特征以及催化机理，简介了如何通过人工改造拓宽卤化酶的催化底物和提高其热稳定性两方面的研究进展，为进一步促进卤化酶在工业催化方面的应用提供理论依据。

关键词

卤化物生物合成，卤过氧化物酶，α-酮戊二酸依赖型卤化酶，黄素依赖型卤化酶，S-腺苷甲硫氨酸依赖性氟化酶

Research Progress in Microbial Halogenases and Their Industrial Applications

Zhelin Zheng, Wenda Hu, Yawen He*

State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai
Email: *yawenhe@sjtu.edu.cn

Received: Nov. 15th, 2020; accepted: Dec. 3rd, 2020; published: Dec. 10th, 2020

Abstract

As the halogens have a large electronegativity, the introduction of a halogen atom to small mole-

*通讯作者。

文章引用: 郑哲麟, 胡文达, 何亚文. 微生物卤化酶及其应用研究进展[J]. 微生物前沿, 2020, 9(4): 141-155.
DOI: 10.12677/amb.2020.94020

cules can have a profound effect on their bioactivity, physical and chemical properties. Halides can be synthesized through chemical reactions, which require high energy consumption and result in environmental pollution. Many natural compounds contain halogen atoms, and a range of halogenases have been identified to be responsible for the halogenation. In this review, the microbial halogenases identified and their characteristics and catalytic mechanisms were first introduced. The current strategies to broaden the catalytic substrates of halogenases and to improve the thermal stability were then summarized. The information provides clues for the development of highly efficient halogenases in industrial catalysis.

Keywords

Biosynthesis of Halogenated Compounds, Haloperoxidases, *α*-Ketoglutarate-Dependent Halogenases, Flavin-Dependent Halogenases, S-Adenosylmethionine-Dependent Fluorinases

1. 引言

卤素原子广泛存在于各类天然产物以及化工产品中。由于卤素原子具有强大的电负性，它的存在可以提高化合物的生物活性，改变化合物的物理化学性质，因此，卤族元素在药物、农药和新型材料中得以广泛的应用。部分临床药物氯霉素、万古霉素和瑞贝卡霉素含有卤素原子[1] [2] [3] [4]；杀虫剂蚍虫啉和噻虫嗪、除草剂乙草胺和莠去津也含有卤素原子[5] [6]；许多具有优良性能的有机聚合物都含有卤素原子[7] [8]。因此，卤化物正受到科学界和工业界的广泛关注。

上个世纪，卤化有机化合物主要是通过化学方法合成，这种卤化方式导致了额外的能量消耗和极大的环境污染。随着天然卤化物生物合成途径研究的深入，其生物合成途径中的各种卤化酶给科学家们带来了新的思路。目前已经发现的卤化酶按照其作用机制和辅酶(辅基)主要分为四种类型：卤过氧化物酶(haloperoxidases, HPO)、*α*-酮戊二酸依赖型卤化酶(*α*-ketoglutarate-dependent halogenases, KG-Hal)、黄素依赖型卤化酶(flavin-dependent halogenases, F-Hal)和 S-腺苷甲硫氨酸依赖性氟化酶(S-adenosylmethionine-dependent fluorinases) (图 1) [9]。大部分卤化酶是以氯、溴或碘作为卤素供体，以氯最为常见，只有 S-腺苷甲硫氨酸依赖性卤化酶是氟化酶，只以氟元素作为卤素供体。

本领域已有多篇综述介绍了卤化酶的特征[9] [10] [11]，在此基础上，本文结合最新研究成果，系统简介卤化酶的分类、作用机制和应用研究进展，以飨读者。

2. HPO

HPO 是最早被发现和鉴定的一类天然卤化酶，它利用过氧化氢使卤素阴离子氧化产生次卤酸作为卤化剂，与底物发生卤化反应。HPO 可以利用氯、溴和碘元素进行卤化反应，不能与氟元素发生反应，因此，HPO 可以分为氯过氧化物酶(chloroperoxidase, CPO)、溴过氧化物酶(bromoperoxidase, BPO)和碘过氧化物酶(iodoperoxidase, IPO)。其中，CPO 可以利用氯、溴和碘进行卤化反应，而 BPO 只能利用溴和碘，不能利用氯化物。按照参与反应的辅基，卤过氧化物酶主要分成血红素依赖型卤过氧化物酶(heme-iron-dependent haloperoxidases, Heme-HPO)和钒依赖型卤过氧化物酶(vanadium-dependent haloperoxidases, V-HPO)。

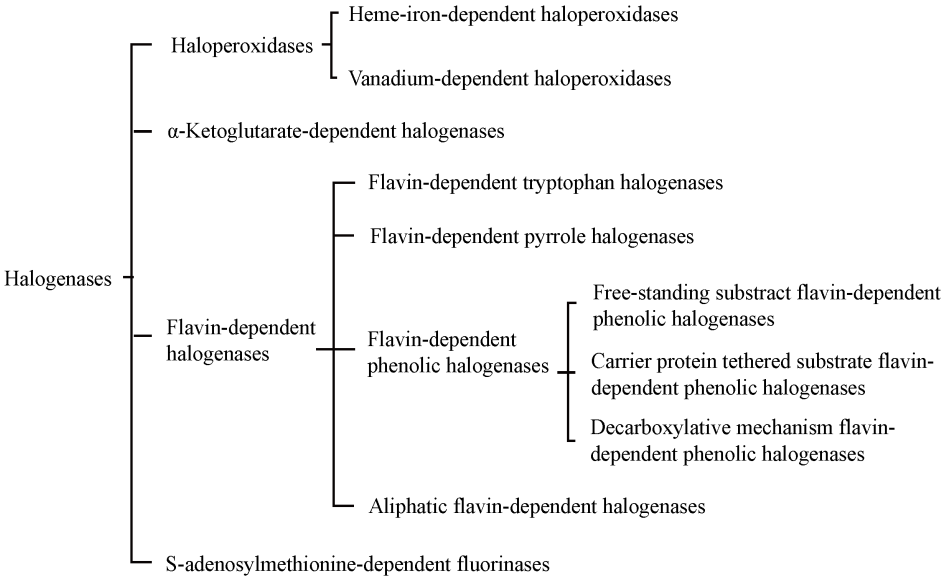

Figure 1. Overview and classification of microbial halogenase [9]
图 1. 微生物卤化酶概况与分类[9]

2.1. Heme-HPO

第一个 Heme-HPO 来自于海洋真菌 *Caldariomyces fumago*，参与卡尔里霉素(caldariomycin)生物合成。它是一种糖基化修饰酶，由 8 个 *α*-螺旋组成独特的三级结构，含有血红素辅基，氯化 1，3-环戊二烯形成卡尔里霉素[12]。此外，该酶还具有过氧化物酶、过氧化氢酶和细胞色素 P450 活性[12]。Heme-HPO 的催化循环起始于血红素铁与水分子结合，随后被过氧化氢取代生成 Fe(III)复合物。在催化位点的谷氨酸(Glu183)的作用下，导致 O-O 键断裂，Fe(III)复合物失去羟基形成 Fe(IV)-OXO 复合物。卤素离子和复合物结合，以次卤酸的形式释放，使底物卤化[13] [14]。Heme-HPO 缺乏底物选择性(substrate selectivity)和区域选择性(regioselectivity)，可能产生自由扩散的次卤酸，与许多易受亲电攻击的底物发生反应(图 2) [15] [16]。

2.2. V-HPO

V-HPO 主要存在于海洋生物海绵、红藻等[17]，但在地衣、真菌和细菌中也有发现[18] [19]。整个 V-HPO 呈双锥形，与酸性磷酸酶的结构高度相似。在催化循环的最初阶段，钒离子含有四个氧配位位点，剩余的一个配位位点被组氨酸残基占据。在过氧化氢的存在下，过氧化氢与钒离子的最远端氧配体结合，失去一分子羟基，导致环氧化物中间体的形成。卤素离子进攻环氧基团，导致次卤酸的形成。游离的次卤酸攻击底物完成卤化反应(图 3) [20] [21]。

大部分 V-HPO 无法特异性地选择底物，仅少部分 V-HPO 具有底物特异选择性。首次报道的具有底物选择性的 V-HPO 是珊瑚藻(*Corallina officinalis*)中的溴过氧化物酶 V-BPO，负责倍半萜类物质的非对映性溴化和环化反应，催化倍半萜类物质 e (E)-(+)-nerolidol 生成 *α*-snyderol，*β*-snyderol 和(+)-3*β*-bromo-8-epicaparrapi oxide [22]。此外，链霉菌(*Streptomyces*) sp.CNQ-525 中鉴定并体外表达的钒依赖型卤过氧化物酶 NapH1 具有高度的底物选择性，参与 napyradiomycin 的生物合成，负责萜类化合物 SF2415B1 氯化生成 SF2415B3 [23]。链霉菌 sp. CNH-121 中的钒依赖型氯过氧化物酶 Mcl24 特异催化 merochlorin 前体氯化生成 merochlorin-A 和 merochlorin-B。

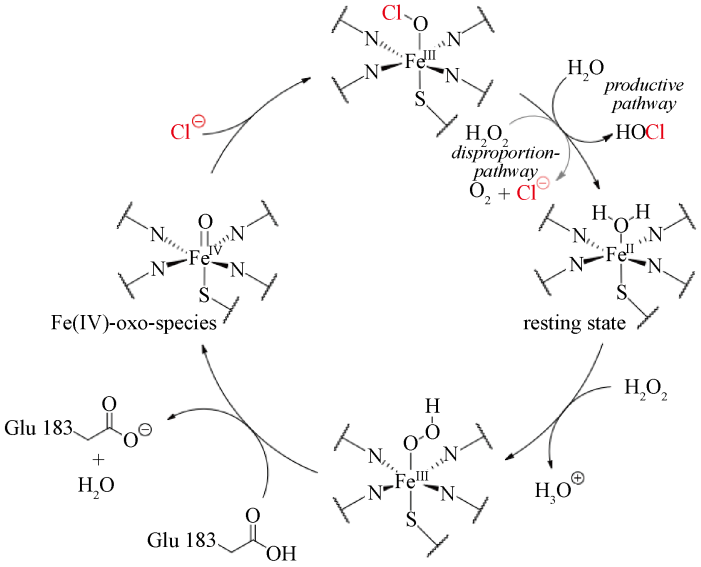

Figure 2. Catalytic cycle of Heme-HPO [10]. In the resting state, water is bound to the heme-iron, which is subsequently replaced by hydrogen peroxide. After protonation of this complex by a catalytic glutamate (Glu183), water is eliminated creating the actual active species, the Fe(IV)-oxo complex. A halide, in this case chloride, binds to the Fe(IV)-oxo species and is released as hypochloric acid, regenerating the heme-site by hydrolysis with water

图 2. Heme-HPO 的催化循环[10]。在起始状态时，水与血红素铁结合。随后水被过氧化氢取代，生成 Fe(III)-OOH 复合物。通过催化谷氨酸(Glu183)质子化该复合物后，形成 Fe(IV)-oxo 复合物。之后，氯离子与 Fe(IV)-oxo 复合物结合，释放游离的次氯酸，并通过水的水解再生血红素铁

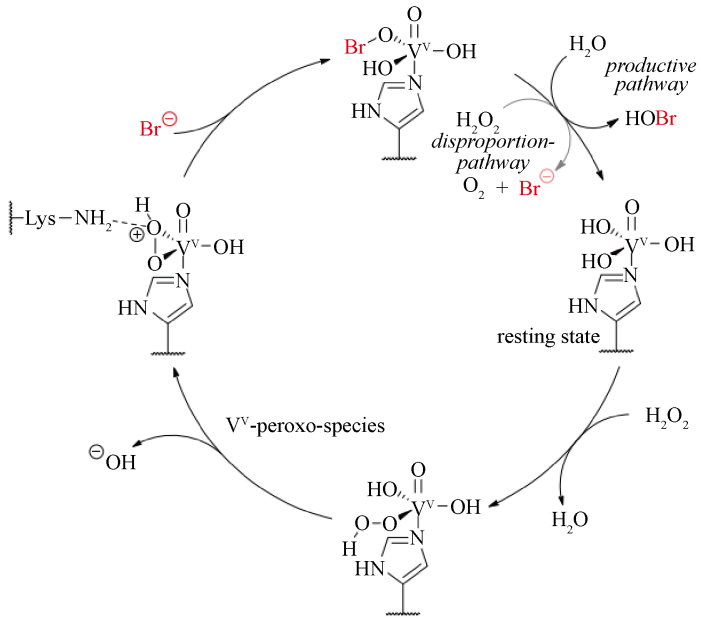

Figure 3. Catalytic cycle of V-HPO [10]. In its resting state, vanadium contains four oxygen ligands, while the free coordination site is occupied by a catalytic histidine residue. In presence of hydrogen peroxide, a hydroxyl group is substituted by peroxide. Upon elimination of a hydroxide ion, a cycloperoxo-species is generated, which is stabilized by a catalytic lysine residue. This cyclic intermediate is opened by addition of a halide, in this case bromide, which can then be hydrolyzed by water, leading to the release of hypobromic acid. During catalysis, the vanadium does not alter its oxidation state (V)

图 3. V-HPO 的催化循环[10]。在起始状态，钒含有四个氧配体，而自由配位位点被催化组氨酸残基占据。在过氧化氢作用下，一个羟基被过氧化物取代。在去除一份子氢氧化物离子后，产生环过氧化物中间体。接下来，卤素原子进攻环过氧化物中间体，之后被水分子水解，释放出次卤酸。在催化过程中，钒不会改变其氧化状态

虽然 HPO 能催化许多化合物产生卤化反应，但要么像 Heme-HPO 一样不具有底物特异性，要么像部分 V-HPO 一样具有高度的底物特异性和狭窄的底物谱，因此，HPO 难以作为良好的生物催化剂在工业生产中得到广泛的应用。

3. KG-Hal

KG-Hal 是依赖于 α-酮戊二酸和非血红素铁的一类卤化酶，将卤素原子装配到未活化的 SP3-型杂化碳原子中心，具有一定的底物选择性和区域选择性[24]。首次发现的 KG-Hal 是丁香假单胞菌(*Pseudomonas syringae*)中的 SyrB2，参与非核糖体肽 Syringomycin E 的生物合成。SyrB2 无法卤化游离的 L-亮氨酸，L-亮氨酸必须与肽基载体蛋白(peptidyl carrier proteins, PCP)结合才能完成卤化反应。体外酶活测定发现只有环境中存在 Fe(II)、O_2 和 Cl^- 时，SyrB2 才有氯化活性[25]。SyrB2 的核心由八个反平行的 β-折叠‘jelly-roll’模体组成，核心铁蛋白存在两个组氨酸配体。在催化起始状态时，α-酮戊二酸、氯离子和水分子与催化中心的 Fe(II)结合，底物进入活性位点将水分子除去并诱导氧气结合。此后，α-KG 脱羧导致高能的 Fe(IV)-oxo 中间体形成，并从底物中夺去一个氢原子。氯原子进攻底物的自由基，使底物氯化并再生 Fe(II)中心(图 4) [26]。

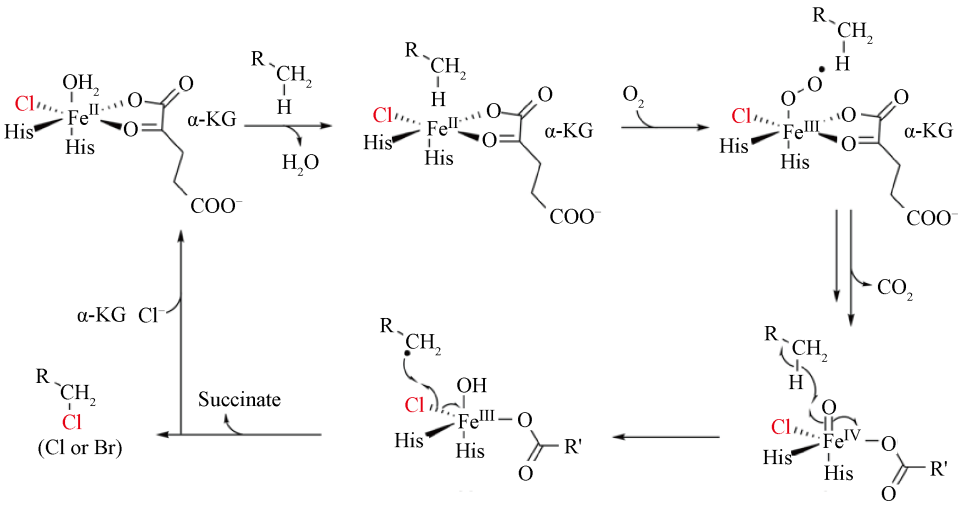

Figure 4. Catalytic cycle of KG-Hal [9]. In its resting state, α-KG, chloride ions and water molecules combine with Fe(II) in the catalytic center, and subsequently the substrate enters the active site to remove a molecule of water and combine with oxygen. After that, α-KG removes one molecule of carboxyl group to form Fe(IV)-oxo intermediate, and takes away one hydrogen atom of the substrate. Finally, the halogen atoms attack the substrate, chlorinating the substrate and regenerating the Fe(II) center

图 4. KG-Hal 的催化循环[9]。在起始状态时，α-KG、氯离子和水分子与催化中心的 Fe(II)结合，然后底物进入活性位点，去除一分子水并与氧气结合。此后，α-KG 脱去一分子羧基，形成 Fe(IV)-oxo 中间体，并夺去底物的一个氢原子。最后卤素原子进攻底物，使底物氯化并再生 Fe(II)中心

除了催化与载体蛋白结合的底物，KG-Hal 也能卤化独立底物。WelO5 是第一个被鉴定可以卤化游离底物的酶，也是第一个能将卤素原子安装到非活化碳原子上的卤化酶，它参与 Welwitindolinone 生物合成，负责 12-epi-Fischerindole U 和 12-epi-Hapalindole C 中脂肪链上碳原子的氯化[27] [28]。BesD 是第一个发现的卤化游离氨基酸的 KG-Hal，与 SyrB2 和 WelO5 的基因同源性较低，负责氯化游离的赖氨酸产生 4-Cl-赖氨酸[29]。此外，在马杜拉放线菌(*Actinomadura*) sp. ATCC 39365 的 adeadecin 生物合成途径中发现的第一个 α-酮戊二酸依赖型核苷类卤化酶 AdeV，能分别卤化游离的核苷类物质 2'-dAMP、2',3'-ddAMP 或 2'-dIMP，分别生成 2'-Cl-2'-dAMP、2'-Cl-2',3'-ddAMP 或 2'-Cl-2'-Dimp [30]。

KG-Hal 主要以氨基酸作为底物，难以分离纯化，Kcat 值低，这些特点限制了它们在工业生物催化中的应用。

4. F-Hal

F-Hal 属于黄素依赖型单加氧酶超家族，需要利用黄素还原酶(Flavin-Reductase, Fre)产生的还原型黄素二核苷酸(FADH2)来活化分子氧，生成 FAD-OOH 参与卤化反应[31] [32]。首例黄素依赖型色氨酸 7-卤化酶 PrnA 的晶体学分析显示，其底物和黄素结合位点在空间上存在位阻，被一个长约 10 Å 的通道隔开，底物无法与辅因子的直接进行相互作用[33] [34]。在催化起始阶段，FADH2 活化分子氧，生成 FAD-OOH 复合物。然后，卤化物对 FAD-OOH 亲核攻击，导致次氯酸的生成。最终卤化物阴离子与底物的芳香环反应，从而产生 Wheland 中间体，然后由保守的谷氨酸残基(E346)去质子化，得到卤化产物(图 5) [35]。PrnA 中高度保守基序(WxWxIP)与负责结合 FADH2 的基序(GxGxxG)是鉴定 F-Hal 基因的特征基序。

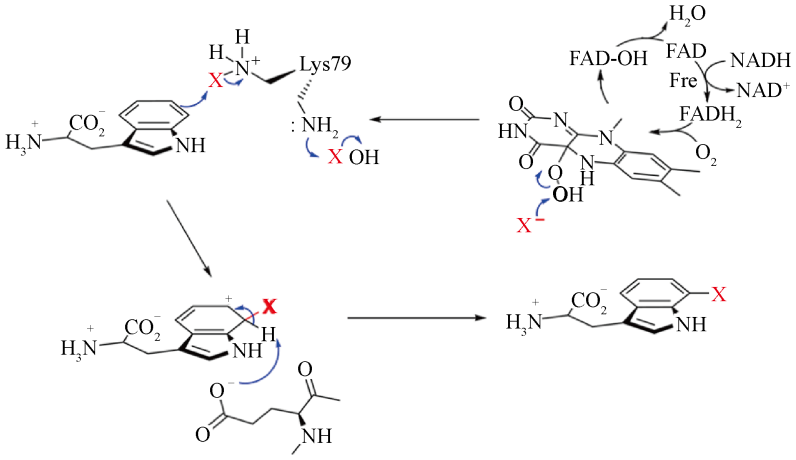

Figure 5. Catalytic cycle of F-Hal [9]. In the resting stage, FADH2 activates molecular oxygen to form FAD-OOH complex. Subsequently, the halide nucleophilically attacks FAD-OOH, resulting in the formation of hypochlorous acid. Finally the halide anion reacts with the aromatic ring of the substrate to produce the Wheland intermediate, which is then deprotonated by the conserved glutamic acid residue (E346) to obtain the halogenated product

图 5. F-Hal 的催化循环[9]。在催化起始阶段，FADH2 活化分子氧，生成 FAD-OOH 复合物。然后，卤化物对 FAD-OOH 亲核攻击，导致次氯酸的生成。最终卤化物阴离子与底物的芳香环反应，从而产生 Wheland 中间体，然后由保守的谷氨酸残基(E346)去质子化，得到卤化产物

4.1. 黄素依赖型色氨酸卤化酶(Flavin-Dependent Tryptophan Halogenases, FDT-Hal)

FDT-Hal 首先在荧光假单胞菌(*Pseudomonas fluorescens*)吡咯菌素(pyrrolnitrin, Prn)生物合成途径中被鉴定[36] [37]。广谱抗真菌化合物吡咯菌素生物合成需要两个色氨酸卤化酶：PrnA 氯化色氨酸的 7 号位，属于色氨酸 7-卤化酶；PrnC 氯化吡咯中间体。随后，从产气列契瓦尼尔氏菌(*Lechevalieria aerocolonigenes*)瑞贝卡霉素生物合成途径中发现并鉴定出另一种色氨酸-7-卤化酶 RebH，它与 PrnA 有 55%的序列同源性，负责色氨酸的 7 号位氯化[38]。

库茨涅尔氏菌(*Kutzneria*) sp.744 中二氯化非核糖体肽(kutzneride)生物合成途径需要两个色氨酸卤化酶(KtzQ 和 KtzR)和一个 Fe(II)/α-KG 依赖性卤化酶(KtzD) [39] [40]。其中 KtzQ 催化 7-氯色氨酸的形成，KtzR 随后卤化 6 号位，生成 6,7-二氯色氨酸。最近还鉴定了许多游离的色氨酸 6-卤化酶，海洋白浅灰链

霉菌(*Streptomyces albogriseolus*)中的 ThaL 负责合成 6-氯化色氨酸，参与吲哚生物碱 thienodolin 噻吩环生物合成[41]；毒三素链霉菌(*Streptomyces toxytricini*)的 SttH 和紫黑链霉菌(*Streptomyces violaceusniger*)SPC6 中的 Th-Hal 也有色氨酸 6-卤化酶活性[42]。

皱胃链霉菌(*Streptomyces rugosporus*)中的 PyrH 是第一个被研究的色氨酸 5-卤化酶，负责吡咯菌霉素生物合成，PyrH 氯化色氨酸的 5 号位碳[43]。色氨酸-5-卤化酶 ClaH 参与了钩状链霉菌(*Streptomyces uncialis*)中双吲哚生物碱克拉多尼酰胺(cladoniamide)的生物合成，负责色氨酸的氯化[44]。在羊毛硫抗生素 NAI-107 生物合成途径中，色氨酸 5-卤化酶 MibH 负责肽链中色氨酸残基的卤化，而不是卤化游离色氨酸[45]。

4.2. 黄素依赖型吡咯卤化酶(Flavin-Dependent Pyrrole Halogenases, FDPR-Hal)

FDPR-Hal 是能卤化吡咯类底物的卤化酶，已发现的 FDPR-Hal 主要包括两类：一类酶能卤化游离的吡咯，如上述所提到的 PrnC；另一类卤化吡咯-2-羧基硫酯底物。一个典型的例子是来自假单胞菌藤黄绿菌素(pyoluteorin)生物合成途径中的 PltA [46]。PltA 的 C 端含有一个阻止酶与底物结合的裂隙(cleft)，是一个独特的螺旋区域(helical region)；底物的结合引起酶构象变化，允许底物进入裂隙。与色氨酸卤化酶相比，PrnC 和 PltA 似乎都具有很强的区域选择性，大多数体外酶活产物是单氯化和二氯化产物的混合物[47]。与 PltA 类似的吡咯卤化酶还包括游动放线菌(Actinoplanes) sp. ATCC3302 中参与五氯联苯(pentachloropseduilin)生物合成的 HalB 和链霉菌 sp. CNQ-418 中参与 chlorizidine A 生物合成的 Clz5 [48] [49]。此外，藤黄紫假交替单胞菌(*Pseudoalteromonas luteoviolacea*)中还存在一个有趣的吡咯卤化酶 Bmp2。Bmp2 与同基因簇的硫酯酶 Bmp1 和脯氨酸腺苷酸转移酶 Bmp4 相关。Bmp2 负责溴化吡咯-2-羧基-S-Bmp1 复合物，生成一溴、二溴和三溴产物[50]。不同于迄今为止所研究的 F-Hal，Bmp2 催化底物溴化和碘化，但无法完成氯化反应[50]。与相应的芳香族氯化物或溴化物相比，芳香族碘化物通常更具反应性，更易于后续的化学反应。因此，Bmp2 能催化碘化反应这一特征引起了科学家的兴趣。

4.3. 黄素依赖型酚类卤化酶(Flavin-Dependent Phenolic Halogenases, FDP-Hal)

4.3.1. 作用于游离底物的黄素依赖型酚类卤化酶 (Free-Standing Substract Flavin-Dependent Phenolic Halogenases)

厚垣普可尼亚菌(*Pochonia chlamydosporia*)中的卤化酶 Rdc2 和 *Chaetomium chiversii* 中的卤化酶 RadH 都作用于游离的酚类底物，参与间苯二酸内酯类代谢产物的生物合成[51] [52] [53]。在体外反应体系中 Rdc2 能卤化多种大环类天然产物，如玉米赤霉烯酮(zearaleone)、dihydrosorcylide、弯孢霉菌素(curvularin)和姜黄素(curcumin)等[54]；如果芳香环上存在多个羟基时，Rdc2 催化的氯化或溴化反应均能形成二卤代产物。

作用于独立底物的黄素依赖型酚类卤化酶还包括 Gsfl、AclH、CazI、AcOTAhal 和 GedL，它们分别参与了一系列真菌天然产物，包括灰黄霉素(griseofulvin) (Gsfl)、aspirochlorine (AclH)、chaetoviridin (CazI)、赭曲霉毒素(ochratoxin) (AcOTAhal)和 dihydrogeodin (GedL) [55]-[60]的生物合成。它们中的大部分与 Rdc2 和 RadH 一样，卤化芳香环酚羟基的邻位碳原子，但 AcOTAhal 卤化底物芳香环酚羟基的对位碳原子。盘基网柄菌(*Dictyostelium discoideum*)中的 ChlA 参与分化诱导因子 1 (DIF-1)生物合成，负责卤化 THPH，产生氯化 THPH 或二氯化 THPH [61]，而安丝菌素(ansamitocin)生物合成途径中的 Asm12 能卤化游离的酚类底物[62] [63]。链霉菌 sp. CNQ-525 napyradiomycin 生物合成途径中 NapH2 能卤化萘醌前体的 C2 位置，促进后续化合物的异戊二烯化[64]。最近，在藤黄绿菌素的生物合成中还发现了一种酚类卤化酶 PltM，负责间苯三酚的一氯化和二氯化。此外，通过体外酶活分析，PltM 对一些酚醛和苯胺衍生物也具有卤化

活性[65]。

4.3.2. 作用于载体蛋白-底物中间体的黄素依赖型酚类卤化酶 (Carrier Protein Tethered Substrate Flavin-Dependent Phenolic Halogenases)

玫瑰产色链霉菌(*Streptomyces roseochromogenes*)中参与氯新生霉素(chlorobiocin)生物合成的 Clo-Hal、地中海拟分枝酸菌(*Amycolatopsis mediterranei*)中参与巴尔赫霉素(Balhimycin)生物合成的 BhaA、淡紫灰链霉菌(*Streptomyces lavendulae*)中负责氯化合成 Complestatin 的 ComH 和浮游放线菌(*Actinoplanes teichomyceticus*)中参与替考拉宁(teicoplanin)生物合成的 Tcp2 [66] [67] [68] [69]都是作用于载体蛋白-底物中间体黄素依赖型酚类卤化酶。这些酶都是负责卤化 PCP-酪氨酸或 PCP-*β*-羟基酪氨酸中间体，生成相应的产物。氯化物卤化酶 CndH 的晶体结构已被解析，其底物是 PCP-酪氨酸中间体，具有较大的非极性表面口袋，可以容纳载体蛋白[70]。

C-1027 是球孢链霉菌(*Streptomyces globisporus*)合成的一个色素蛋白类抗肿瘤类产物，SgcC3 是其生物合成途径中的一个卤化酶[71]。异源表达与体外酶活实验表明：只有当底物与载体蛋白 SgcC2 相连时，SgcC3 才具有卤化活性，表明这个酶催化的真正底物是(S)-或(R)-*β*-酪氨酸-S-SgcC2 [72]。这一类的酚类卤化酶还包括 Ram20 和 End30，它们分别在脂肽类抗生素雷莫拉宁(ramoplanin)和恩拉霉素(enduracidin)生物合成过程负责卤化羟苯基甘氨酸残基[73] [74]。

4.3.3. 遵循脱羧卤化机制的黄素依赖型酚类卤化酶 (Decarboxylative Mechanism Flavin-Dependent Phenolic Halogenases)

藤黄紫假交替单胞菌中的甲苯酚脱羧溴化酶 Bmp5 是第一个被鉴定的遵循脱羧卤化机制的黄素依赖型酚类卤化酶[75]。Bmp5 与其他黄素依赖性卤化酶氨基酸序列同源性不大，与已知的单组分黄素依赖性单加氧酶具有一定的序列同源性。Bmp5 不需要额外的黄素还原酶，但需要溴化物和 NADPH 同时存在，表明 Bmp5 本身具有黄素还原和溴化双重酶活。在溴化物、NADPH 和 FAD 存在下，Bmp5 与 4-羟基苯甲酸反应形成 3-溴-4-羟基苯甲酸，通过脱羧进行第二次溴化，得到二溴苯酚。Bmp5 没有氯化活性，但形成碘酚类物质，表明该蛋白质中存在高度进化的溴化物或碘化物结合位点，而其他黄素依赖性卤代酶均不存在[75]。

4.4. 脂肪族黄素依赖型卤化酶(Aliphatic Flavin-Dependent Halogenases, AFD-Hal)

AFD-Hal 能够卤化脂肪链的 C-H 键。委内瑞拉链霉菌(*Streptomyces venezuelae*)的氯霉素卤化酶 CmlS 晶体结构的解析显示出在其它 F-Hal 中未发现的结构特征：包括存在与活性位点相连的 T 形通道；FAD 通过碳 8*α* 与卤化酶共价结合，而不是 5 号位碳；存在许多与激活剂或底物载体蛋白结合的非极性表面口袋；活性位点残基甘氨酸 E44 负责催化次氯酸的生成，酪氨酸 Y350 负责稳定烯醇化中间体，该烯醇中间体充当亲核试剂以生成氯化产物[76]。

最近，Chankhamjon 等[77]在米曲霉(*Aspergillus oryzae*) RIB40 中鉴定出的一种双功能甲基转移酶卤化酶 AoiQ，负责腐皮壳菌素(diaporthin)的甲基化和氯化双重反应，生成双氯化腐皮壳菌素衍生物。卤化的位置表明了 AoiQ 与之前的 F-Hal 存在不同的催化机制[77]，基因组分析表明 AoiQ 同源酶也存在于其他真菌中。

4.5. 改造黄素依赖型卤化酶，开发新型生物催化剂

4.5.1. 扩宽黄素依赖型卤化酶的底物选择性

通过定点诱变来改变或提高黄素依赖性色氨酸卤化酶的区域选择性。PrnA 是色氨酸-7-溴卤化酶，将活性位点残基的苯丙氨酸(F103)突变为丙氨酸，导致 7-溴色氨酸和 5-溴化色氨酸以 2:1 的比率形成[78]。

Shepherd 等[79]用同样的方法突变卤化酶 SttH，得到一个三突变体改变了该酶的区域选择性，卤化位点从3-吲哚丙酸酯的 6 号位碳变为 5 号位碳。

Andorfer 等[80]通过采用随机诱变和定向进化方法，对色氨酸-7-卤化酶 RebH 进行了六轮筛选，发现了两个 RebH 突变体，能卤化吲哚类底物的 6 号位碳和 5 号位碳。Payne 等[81]对野生型卤化酶 RebH 进行四次易错 PCR (error PCR)后，野生型 RebH 被突变为一个五突变体，该突变体能够区域选择性卤化吲哚类底物的 4 号位碳。

4.5.2. 提高黄素依赖型卤化酶的活性和稳定性

通常黄素依赖性卤化酶 Kcat 值较低，稳定性较差以及易被底物产物所抑制，所以很难有极高的活性。两种策略可以改善 F1-Hal 的生物催化性能，一种策略是寻找嗜热生物的卤化酶，因为热稳定酶具有寿命较长、对有机溶剂有较高的耐受性和不易水解等优点。Chen 等[82]从紫黑链霉菌 SPC6 中鉴定出嗜热色氨酸-6-卤化酶(Th-Hal)，其区域选择性与类似的色氨酸-6-卤化酶 SttH 相同，但熔融温度(Tm)比 SttH 高近 10℃，且 Kcat 值也高于许多其他色氨酸卤化酶。Takahashi 等[83]将 Th-Hal 与嗜热的芽孢杆菌的黄素还原酶结合在一起，该反应体系能在 45℃条件下与多种非天然底物发生卤化反应。Th-Hal 的晶体结构发现：Th-Hal 表面存在大量极性残基，导致表面产生大量电荷，阻止蛋白质聚集，增加与水的氢键数量，这赋予了 Th-Hal 的热稳定性[84]。

另一种策略是利用易错 PCR 进行定向进化，利用温度作为筛选指标，从中筛选出具有热稳定性的突变体。Poor 等[85] [86]对卤化酶 RebH 进行了三轮易错 PCR，发现两个突变体的 Topt 值和 Tm 值比野生型高七到八倍，且两者均具有比野生型更长更稳定的反应时间，但 Kcat 值降低。其中一个突变体的三维结构特征与 Th-Hal 类似，突变体表面的谷氨酰胺突变为精氨酸，导致表面电荷增加。其他部分电荷密度降低，以减少与附近残基的排斥，N-端丝氨酸突变为脯氨酸，提高了酶的稳定性。

4.5.3. 利用黄素依赖型卤化酶合成非天然产物

将 F-Hal 掺入天然产物的生物合成途径中，可以增加产物的多样性。Sanchez 等[87]将瑞贝卡霉素和星形孢菌素生物合成基因基因重组，即将编码不同区域选择性色氨酸卤化酶的基因导入至天然产物生物合成基因簇中，从而形成氯化吲哚并咔唑非天然产物的不同区域异构体。Roy 等[88]将色氨酸 7-卤化酶引入生产 pacidamycin 的链霉菌菌株中，形成了氯化的 pacidamycin。

5. S-腺苷甲硫氨酸依赖型氟化酶(S-Adenosylmethionine-Dependent Fluorinases)

首个氟化酶 FlA 来自于卡特利链霉菌(*Streptomyces cattleya*)的氟乙酸盐和 4-氟苏氨酸生物合成途径，介导 S-腺苷甲硫氨酸(SAM)向 5'-氟-5'-脱氧腺苷(5'-FDA)的转化[89] [90]。在催化循环的初始阶段，溶剂化的氟化物离子以低亲和力结合到氟化酶的一个活性口袋中，交换水分子，通过氢键与蛋白质的极性基团连接；由于 SAM 与氟化酶的亲和力比氟化物高 1000 倍左右，更容易结合到氟化酶活性位点，驱使氟化物离子脱溶剂化，作为亲核试剂进攻 SAM 核糖的 5'-碳原子，导致了 C-S 键的断裂和 C-F 键的生成，最终生成了 5'-氟-5'-脱氧腺苷中间产物(图 6) [91]。

通过序列比对发现 FlA 同源酶在链霉菌 sp. MA37、巴西诺卡菌(*Norcardia brasiliensis*)和游动放线菌 sp. N902-109 中均存在，它们都能氟化 SAM 生成 5'-氟-5'-脱氧腺苷(5'-FDA)。不同的是，这三种酶的 Kcat 值均高于氟化酶 FlA，且具有更高的 Kcat/Km 值[92]。此外，Huang 等还从星海链霉菌(*Streptomyces Xinghaiensis*) NRRL B-17474 中发现了与 FlA 相似的氟化酶 FlA4，负责 SAM 的氟化[93]。

5.1. S-腺苷甲硫氨酸依赖型氟化酶在 PET 中的应用

PET (Positron Emission Computed Tomography)是一种定量无创医学成像技术，需要将生命代谢中必

须物质，如：葡萄糖、蛋白质、核酸、脂肪酸，标记上放射性同位素(如 ^{18}F，^{11}C 等)，注入人体后作为示踪剂。合成 18F-PET 示踪剂的传统方法需要多个步骤和/或苛刻的反应条件，因为氟离子作为亲核试剂在水溶液中的反应能力有限，需在严格的无水条件下高温加热。氟化酶介导的卤化反应已经成为 PET 探针合成的主要方式，无需干燥或与专门的配体配位，直接使用氟化物的水溶液，以提高其在有机溶剂中的反应活性，可以以较高的放射化学产率(Radiochemical yield, RCY)迅速生成所需的氟化物[94]。

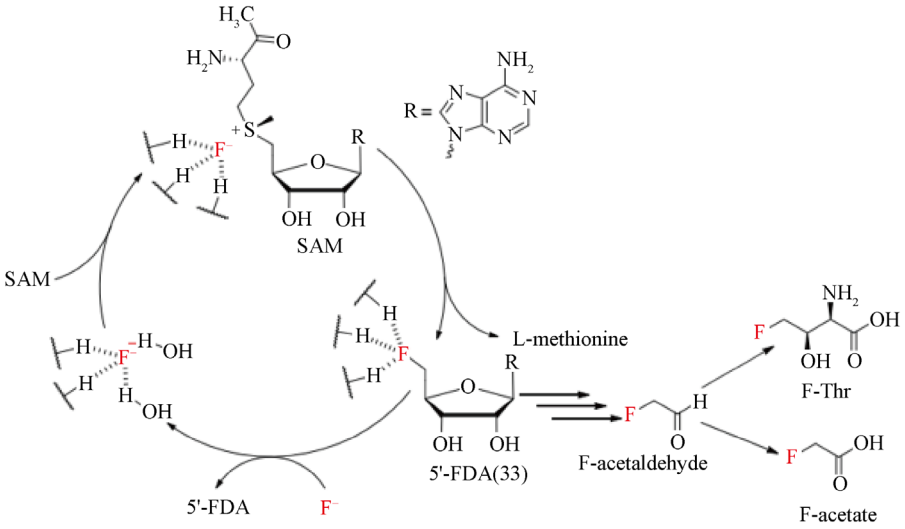

Figure 6. Catalytic cycle of S-adenosylmethionine-dependent fluorinases [10]. In the resting stage, the solvated fluoride ion binds to an active pocket of fluorinase, exchanges water molecules, and binds to the polar group of the protein through hydrogen bonding; subsequently S-adenosylmethionine binds to the active site of fluorinase and drives the desolvation of fluoride ions. The desolvated fluoride ions act as nucleophiles where it attacks the 5'-carbon atom of S-adenosylmethionine ribose to generate 5'-fluorine-5'-deoxyadenosine (5'-FDA) and L-methionine (L-Met)

图 6. S-腺苷甲硫氨酸依赖型氟化酶的催化循环[10]。在起始阶段，溶剂化的氟化物离子结合到氟化酶的一个活性口袋中，交换水分子，通过氢键与蛋白质的极性基团连接；接下来 S-腺苷甲硫氨酸结合到氟化酶活性位点，驱使氟化物离子脱溶剂化，脱溶剂化的氟化物离子作为亲核试剂，进攻 S-腺苷甲硫氨酸核糖的 5'-碳原子，生成了 5'-氟-5'-脱氧腺苷(5'-FDA)和 L-甲硫氨酸(L-Met)

5.2. S-腺苷甲硫氨酸依赖型氟化酶在天然产物合成中的作用

在药物化学、农业化学以及材料化学领域，氟元素占据着极其重要的地位，如何将氟元素通过安全高效的催化方式引入有机物成为许多科学家努力的方向和目标。化学合成 C-F 键的方法复杂，难以实现底物选择性氟化，氟化酶的发现给了科学家们新的研究灵感和方向，通过发酵简便制备含氟化合物十分简便且高效。Eustaquio 等[95]首先在 *Salinispora tropica* 中使用编码氟化酶 FlA 的基因取代编码 SalL 的基因，从而工程化制备抗癌药物 salinosporamide A。此外，还可以通过改变底物的方式生物合成含氟聚酮化合物。Hong 等[96]利用氟乙酰-CoA 作为 II 型聚酮化合物合酶 PKS 的底物，生物合成含氟的放线菌素。Walker 等[97]利用氟丙二酰基-CoA 作为起始反应物，将氟元素引入许多聚酮类天然化合物(如红霉素、雷帕霉素)中，拓宽天然氟化产物生物合成的范围。最近，Thuronyi 等[98]又在工程化的大肠杆菌中使用氟丙二酸作为原料生产氟化二酮化合物 2-氟-3-羟基丁酸酯，这种氟化的二酮化合物可以用作单体，生产氟化聚羟基链烷酸酯(PHA)生物塑料。

6. 总结与展望

卤素原子作为一个功能基团，可以改变物质的物理化学性质。自然界中存在着许多含有卤素原子的

天然化合物，这些化合物是由天然卤化酶催化形成的。目前已经发现和鉴定了四大类卤化酶，这四类卤化酶具有不同的催化机制，海量基因组数据分析将有助于发现并鉴定新型的卤化酶。

传统的化学卤化反应容易产生有毒的副产物，能源消耗过大。使用卤化酶作为生物催化剂能提高化合物的生物活性，减少污染，减少有毒副产物的产生以及合成区域选择性的卤化底物。四类卤化酶中，α-KG 依赖型卤化酶、黄素依赖型卤化酶以及氟化酶具有区域和立体选择性，具有一定的工业生物催化应用潜力，但要在工业上大规模使用，还有很长的路要走。采用遗传、生化和结构生物学等手段深入系统研究这些卤化酶的作用机理，在此基础上开展系统的改造，改变其底物范围，改进区域选择性和稳定性，开发出更适合工业应用的卤化酶生物催化剂是未来研究重点。

参考文献

[1] Xu, Z., *et al*. (2014) Halogen Bond: Its Role beyond Drug Target Binding Affinity for Drug Discovery and Development. *Journal of Chemical Information and Modeling*, **54**, 69-78. https://doi.org/10.1021/ci400539q

[2] Lu, Y., *et al*. (2012) Halogen Bonding for Rational Drug Design and New Drug Discovery. *Expert Opinion on Drug Discovery*, **7**, 375-383. https://doi.org/10.1517/17460441.2012.678829

[3] Zhou, Y., *et al*. (2016) Next Generation of Fluorine-Containing Pharmaceuticals, Compounds Currently in Phase II-III Clinical Trials of Major Pharmaceutical Companies: New Structural Trends and Therapeutic Areas. *Chemical Reviews*, **116**, 422-518. https://doi.org/10.1021/acs.chemrev.5b00392

[4] Wang, J., *et al*. (2014) Fluorine in Pharmaceutical Industry: Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001-2011). *Chemcial Reviews*, **114**, 2432-2506. https://doi.org/10.1021/cr4002879

[5] Jeschke, P. (2010) The Unique Role of Halogen Substituents in the Design of Modern Agrochemicals. *Pest Management Science*, **66**, 10-27. https://doi.org/10.1002/ps.1829

[6] Jeschke, P. (2004) The Unique Role of Fluorine in the Design of Active Ingredients for Modern Crop Protection. *Chembiochem*, **5**, 571-589. https://doi.org/10.1002/cbic.200300833

[7] Berger, G., *et al*. (2015) Halogen Bonding in Polymer Science: From Crystal Engineering to Functional Supramolecular Polymers and Materials. *Polymer Chemistry*, **6**, 3559-3580. https://doi.org/10.1039/C5PY00354G

[8] Tang, M.L. and Bao, Z.N. (2011) Halogenated Materials as Organic Semiconductors. *Chemistry of Materials*, **23**, 446-455. https://doi.org/10.1021/cm102182x

[9] Latham, J., *et al*. (2018) Development of Halogenase Enzymes for Use in Synthesis. *Chemical Reviews*, **118**, 232-269. https://doi.org/10.1021/acs.chemrev.7b00032

[10] Fejzagic, A.V., *et al*. (2019) Halogenating Enzymes for Active Agent Synthesis: First Steps Are Done and Many Have to Follow. *Molecules*, **24**, 4008. https://doi.org/10.3390/molecules24214008

[11] 曾佳, 占纪勋. 黄素依赖型卤化酶的研究进展及应用[J]. 生命科学仪器, 2019, 17(Z1): 34-45.

[12] Sundaramoorthy, M., *et al*. (1995) The Crystal Structure of Chloroperoxidase: A Heme Peroxidase-Cytochrome P450 Functional Hybrid. *Structure*, **3**, 1367-1377. https://doi.org/10.1016/S0969-2126(01)00274-X

[13] Butler, A. and Sandy, M. (2009) Mechanistic Considerations of Halogenating Enzymes. *Nature*, **460**, 848-854. https://doi.org/10.1038/nature08303

[14] Neumann, C.S., *et al*. (2008) Halogenation Strategies in Natural Product Biosynthesis. *Chemical Biology*, **15**, 99-109. https://doi.org/10.1016/j.chembiol.2008.01.006

[15] Vazquez-duhalt, R., *et al*. (2001) Biocatalytic Chlorination of Aromatic Hydrocarbons by Chloroperoxidase of *Caldariomyces fumago*. *Phytochemistry*, **58**, 929-933. https://doi.org/10.1016/S0031-9422(01)00326-0

[16] Geigert, J., *et al*. (1983) Novel Haloperoxidase Substrates. Alkynes and Cyclopropanes. *Journal of Biological Chemistry*, **258**, 2273-2277.

[17] Butler, A. and Walker, J.V. (1993) Marine Haloperoxidases. *Chemical Reviews*, **93**, 1937-1944. https://doi.org/10.1021/cr00021a014

[18] Iton, N., *et al*. (1986) Characterization of Non-Heme Type Bromoperoxidase in *Corallina pilulifera*. *Journal of Biological Chemistry*, **261**, 5194-5200.

[19] Kaysser, L., *et al*. (2012) Merochlorins A-D, Cyclic Meroterpenoid Antibiotics Biosynthesized in Divergent Pathways with Vanadium-Dependent Chloroperoxidases. *Journal of the American Chemical Society*, **134**, 11988-11991. https://doi.org/10.1021/ja305665f

[20] Wischang, D., Brucher, O. and Hartung, J. (2011) Bromoperoxidases and Functional Enzyme Mimics as Catalysts for Oxidative Bromination—A Sustainable Synthetic Approach. *Coordination Chemistry Reviews*, **255**, 2204-2217. https://doi.org/10.1016/j.ccr.2011.04.003

[21] Wischang, D., *et al*. (2011) Vanadate(v)-Dependent Bromoperoxidase Immobilized on Magnetic Beads as Reusable Catalyst for Oxidative Bromination. *Green Chemistry*, **13**, 102-108. https://doi.org/10.1039/C0GC00499E

[22] Carter-Fanklin, J.N., *et al*. (2004) Vanadium Bromoperoxidase-Catalyzed Biosynthesis of Halogenated Marine Natural Products. *Journal of the American Chemical Society*, **26**, 15060-15066. https://doi.org/10.1021/ja047925p

[23] Bernhardt, P., *et al*. (2011) A Stereoselective Vanadium-Dependent Chloroperoxidase in Bacterial Antibiotic Biosynthesis. *Journal of the American Chemical Society*, **133**, 4268-4270. https://doi.org/10.1021/ja201088k

[24] Voss, M., *et al*. (2020) Exploring the Biocatalytic Potential of Fe/α-Ketoglutarate-Dependent Halogenases. *Chemistry*, **26**, 7336-7345. https://doi.org/10.1002/chem.201905752

[25] Vaillancourt, F.H., *et al*. (2005) SyrB2 in Syringomycin E Biosynthesis Is a Non-Herne Fe-II Alpha-Ketoglutarate- and O_2-Dependent Halogenase. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 10111-10116. https://doi.org/10.1073/pnas.0504412102

[26] Blaslak, L.C., *et al*. (2006) Crystal Structure of the Non-Haem Iron Halogenase SyrB2 in Syringomycin Biosynthesis. *Nature*, **440**, 368-371. https://doi.org/10.1038/nature04544

[27] Hillwig, M.L. and Liu, X.Y. (2014) A New Family of Iron-Dependent Halogenases Acts on Freestanding Substrates. *Nature Chemical Biology*, **10**, 921-923. https://doi.org/10.1038/nchembio.1625

[28] Hillwig, M.L., *et al*. (2014) Biosynthesis of Ambiguine Indole Alkaloids in Cyanobacterium *Fischerella ambigua*. *ACS Chemical Biology*, **9**, 372-377. https://doi.org/10.1021/cb400681n

[29] Neugebauer, M.E., *et al*. (2019) A Family of Radical Halogenases for the Engineering of Amino-Acid-Based Products. *Nature Chemical Biology*, **15**, 1009-1016. https://doi.org/10.1038/s41589-019-0355-x

[30] Zhao, C., *et al*. (2020) An Fe^{2+}- and α-Ketoglutarate-Dependent Halogenase Acts on Nucleotide Substrates. *Angewandte Chemie International Edition*, **59**, 9478-9484. https://doi.org/10.1002/anie.201914994

[31] Huijbers, M.M., *et al*. (2014) Flavin Dependent Monooxygenases. *Archives of Biochemistry and Biophysics*, **544**, 2-17. https://doi.org/10.1016/j.abb.2013.12.005

[32] Visitsatthawong, S., *et al*. (2015) Mechanism of Oxygen Activation in a Flavin-Dependent Monooxygenase: A Nearly Barrierless Formation of C4a-Hydroperoxyflavin via Proton-Coupled Electron Transfer. *Journal of the American Chemical Society*, **137**, 9363-9374. https://doi.org/10.1021/jacs.5b04328

[33] Zhu, X.F., *et al*. (2009) Structural Insights into Regioselectivity in the Enzymatic Chlorination of Tryptophan. *Journal of Molecular Biology*, **391**, 74-85. https://doi.org/10.1016/j.jmb.2009.06.008

[34] Dong, C.J., *et al*. (2004) Crystallization and X-Ray Diffraction of a Halogenating Enzyme, Tryptophan 7-Halogenase, from *Pseudomonas fluorescens*. *Acta Crystallographica Section D—Structural Biology*, **60**, 1438-1440. https://doi.org/10.1107/S0907444904012521

[35] Dong, C.J., *et al*. (2005) Tryptophan 7-Halogenase (PrnA) Structure Suggests a Mechanism for Regioselective Chlorination. *Science*, **309**, 2216-2219. https://doi.org/10.1126/science.1116510

[36] Kirner, S., *et al*. (1998) Functions Encoded by Pyrrolnitrin Biosynthetic Genes from *Pseudomonas fluorescens*. *Journal of Bacteriology*, **180**, 1939-1943. https://doi.org/10.1128/JB.180.7.1939-1943.1998

[37] Kirner, S., *et al*. (1996) The Non-Haem Chloroperoxidase from *Pseudomonas fluorescens* and Its Rrelationship to Pyrrolnitrin Biosynthesis. *Microbiology*, **142**, 2129-2135. https://doi.org/10.1099/13500872-142-8-2129

[38] Sanchez, C., *et al*. (2002) The Biosynthetic Gene Cluster for the Antitumor Rebeccamycin: Characterization and Generation of Indolocarbazole Derivatives. *Chemical Biology*, **9**, 519-531. https://doi.org/10.1016/S1074-5521(02)00126-6

[39] Heemstra, J.R. and Walsh, C.T. (2008) Tandem Action of the O2- and FADH2-Dependent Halogenases KtzQ and KtzR Produce 6,7-Dichlorotryptophan for Kutzneride Assembly. *Journal of the American Chemical Society*, **130**, 14024-14025. https://doi.org/10.1021/ja806467a

[40] Fujimori, D.G., *et al*. (2007) Cloning and Characterization of the Biosynthetic Gene Cluster for Kutznerides. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16498-16503. https://doi.org/10.1073/pnas.0708242104

[41] Milbredt, D., *et al*. (2014) A Tryptophan 6-Halogenase and an Amidotransferase Are Involved in Thienodolin Biosynthesis. *Chembiochem*, **15**, 1011-1020. https://doi.org/10.1002/cbic.201400016

[42] Menon, B.R.K., *et al*. (2016) Structure and Biocatalytic Scope of Thermophilic Flavin-Dependent Halogenase and Flavin Reductase Enzymes. *Organic & Biomolecular Chemistry*, **14**, 9354-9361. https://doi.org/10.1039/C6OB01861K

[43] Zehner, S., *et al*. (2005) A Regioselective Tryptophan 5-Hhalogenase Is Involved in Pyrroindomycin Biosynthesis in

Streptomyces rugosporus LL-42D005. *Chemical Biology*, **12**, 445-452. https://doi.org/10.1016/j.chembiol.2005.02.005

[44] Du, Y.L., *et al*. (2015) Expansion of Bisindole Biosynthetic Pathways by Combinatorial Construction. *ACS Synthetic Biology*, **4**, 682-688. https://doi.org/10.1021/sb5003218

[45] Ortega, M.A., *et al*. (2017) Two Flavoenzymes Catalyze the Post-Translational Generation of 5-Chlorotryptophan and 2-Aminovinyl-Cysteine during NAI-107 Biosynthesis. *ACS Chemical Biology*, **12**, 548-557. https://doi.org/10.1021/acschembio.6b01031

[46] Hammer, P.E., Hill, D.S., Lam, S.T., Van Pée, K.H. and Ligon, J.M. (1997) Four Genes from *Pseudomonas fluorescens* That Encode the Biosynthesis of Pyrrolnitrin. *Applied and Environmental Microbiology*, **63**, 2147-2154. https://doi.org/10.1128/AEM.63.6.2147-2154.1997

[47] Dorrestein, P.C., *et al*. (2005) Dichlorination of a Pyrrolyl-S-Carrier Protein by FADH2-Dependent Halogenase PltA during Pyoluteorin Biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 13843-13848. https://doi.org/10.1073/pnas.0506964102

[48] Wynands, I., *et al*. (2004) A Novel Halogenase Gene from the Pentachloropseudilin Producer Actinoplanes sp. ATCC 33002 and Detection of *in Vitro* Halogenase Activity. *FEMS Microbiology Letters*, **237**, 363-367. https://doi.org/10.1111/j.1574-6968.2004.tb09718.x

[49] Mantovani, S.M. and Moore, B.S. (2013) Flavin-Linked Oxidase Catalyzes Pyrrolizine Formation of Dichloropyrrole-Containing Polyketide Extender Unit in Chlorizidine A. *Journal of the American Chemical Society*, **135**, 18032-18035. https://doi.org/10.1021/ja409520v

[50] El Gamal, A., *et al*. (2016) Biosynthesis of Coral Settlement Cue Tetrabromopyrrole in Marine Bacteria by a Uniquely Adapted Brominase-Thioesterase Enzyme Pair. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 3797-3802. https://doi.org/10.1073/pnas.1519695113

[51] Zhou, H., *et al*. (2010) Insights into Radicicol Biosynthesis via Heterologous Synthesis of Intermediates and Analogs. *Journal of Biological Chemistry*, **285**, 41412-41421. https://doi.org/10.1074/jbc.M110.183574

[52] Zeng, J., *et al*. (2011) Specific Inhibition of the Halogenase for Radicicol Biosynthesis by Bromide at the Transcriptional Level in *Pochonia chlamydosporia*. *Biotechnology Letters*, **33**, 333-338. https://doi.org/10.1007/s10529-010-0427-1

[53] Wang, S.H., *et al*. (2008) Functional Characterization of the Biosynthesis of Radicicol, an Hsp90 Inhibitor Resorcylic Acid Lactone from *Chaetomium chiversii*. *Chemical Biology*, **15**, 1328-1338. https://doi.org/10.1016/j.chembiol.2008.10.006

[54] Xu, F.C., *et al*. (2016) Selective Biochlorination of Hydroxyquinolines by a Flavin-Dependent Halogenase. *Tetrahedron Letters*, **57**, 5262-5265. https://doi.org/10.1016/j.tetlet.2016.10.044

[55] Chool, Y.H., Cacho, R. and Tang, Y. (2010) Identification of the Viridicatumtoxin and Griseofulvin Gene Clusters from *Penicillium aethiopicum*. *Chemical Biology*, **17**, 483-494. https://doi.org/10.1016/j.chembiol.2010.03.015

[56] Chankhamjon, P., *et al*. (2014) Biosynthesis of the Halogenated Mycotoxin Aspirochlorine in Koji Mold Involves a Cryptic Amino Acid Conversion. *Angewandte Chemie—International Edition*, **53**, 13409-13413. https://doi.org/10.1002/anie.201407624

[57] Winter, J.M., *et al*. (2012) Identification and Characterization of the Chaetoviridin and Chaetomugilin Gene Cluster in Chaetomium Globosum Reveal Dual Functions of an Iterative Highly-Reducing Polyketide Synthase. *Journal of the American Chemical Society*, **134**, 17900-17903. https://doi.org/10.1021/ja3090498

[58] Susca, A., *et al*. (2016) Variation in Fumonisin and Ochratoxin Production Associated with Differences in Biosynthetic Gene Content in *Aspergillus niger* and a Welwitschiae Isolates from Multiple Crop and Geographic Origins. *Frontiers in Microbiology*, **7**, 1412. https://doi.org/10.3389/fmicb.2016.01412

[59] Nielsen, M.T., *et al*. (2013) Heterologous Reconstitution of the Intact Geodin Gene Cluster in *Aspergillus nidulans* through a Simple and Versatile PCR Based Approach. *PLoS ONE*, **8**, e72871. https://doi.org/10.1371/annotation/c05c3fd1-dd00-4840-891d-693c614aaaf9

[60] Yin, Y., *et al*. (2016) Polyketides in *Aspergillus terreus*: Biosynthesis Pathway Discovery and Application. *Applied Microbiology and Biotechnology*, **100**, 7787-7798. https://doi.org/10.1007/s00253-016-7733-z

[61] Neumann, C.S., *et al*. (2010) A Flavin-Dependent Halogenase Ccatalyzes the Chlorination Step in the Biosynthesis of Dictyostelium Differentiation-Inducing Factor 1. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 5798-5803. https://doi.org/10.1073/pnas.1001681107

[62] Yu, T.W., *et al*. (2010) The Biosynthetic Gene Cluster of the Maytansinoid Antitumor Agent Ansamitocin from *Actinosynnema pretiosum*. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 5798-5803.

[63] Spiteller, P., *et al*. (2003）The Post-Polyketide Synthase Modification Steps in the Biosynthesis of the Antitumor Agent

Ansamitocin by *Actinosynnema pretiosum*. *Journal of the American Chemical Society*, **125**, 14236-14237. https://doi.org/10.1021/ja038166y

[64] Winter, J.M., *et al*. (2007) Molecular Basis for Chloronium-Mediated Meroterpene Cyclization-Cloning, Sequencing, and Heterologous Expression of the Napyradiomycin Biosynthetic Gene Cluster. *Journal of Biological Chemistry*, **282**, 16362-16368. https://doi.org/10.1074/jbc.M611046200

[65] Mori, S., *et al*. (2019) Unusual Substrate and Halide Versatility of Phenolic Halogenase PltM. *Nature Communications*, **10**, 1255. https://doi.org/10.1038/s41467-019-09215-9

[66] Eustaquio, A.S., *et al*. (2003) Clorobiocin Biosynthesis in Streptomyces: Identification of the Halogenase and Generation of Structural Analogs. *Chemical Biology*, **10**, 279-288. https://doi.org/10.1016/S1074-5521(03)00051-6

[67] Pelzer, S., *et al*. (1999) Identification and Analysis of the Balhimycin Biosynthetic Gene Cluster and Its Use for Manipulating Glycopeptide Biosynthesis in *Amycolatopsis mediterranei* DSM5908. *Antimicrobial Agents and Chemotherapy*, **43**, 1565-1573. https://doi.org/10.1128/AAC.43.7.1565

[68] Chiu, H.T., *et al*. (2001) Molecular Cloning and Sequence Analysis of the Complestatin Biosynthetic Gene Cluster. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 8548-8553. https://doi.org/10.1073/pnas.151246498

[69] Park, O.K., *et al*. (2016) Generation of New Complestatin Analogues by Heterologous Expression of the Complestatin Biosynthetic Gene Cluster from *Streptomyces chartreusis* AN1542. *Chembiochem*, **17**, 1725-1731. https://doi.org/10.1002/cbic.201600241

[70] Buedenbender, S., Rachid, S., Müller, R. and Schulz, G.E. (2009) Structure and Action of the Myxobacterial Chondrochloren Halogenase CndH: A New Variant of FAD-Dependent Halogenases. *Journal of Molecular Biology*, **385**, 520-530. https://doi.org/10.1016/j.jmb.2008.10.057

[71] Lin, S.J., *et al*. (2008) Characterization of the Two-Component, FAD-Dependent Monooxygenase SgcC That Requires Carrier Protein-Tethered Substrates for the Biosynthesis of the Enediyne Antitumor Antibiotic C-1027. *Journal of the American Chemical Society*, **130**, 6616-6623. https://doi.org/10.1021/ja710601d

[72] Van, L.S.G., *et al*. (2009) Characterization of Sgce6, the Flavin Reductase Component Supporting FAD-Dependent Halogenation and Hydroxylation in the Biosynthesis of the Enediyne Antitumor Antibiotic C-1027. *FEMS Microbiology Letters*, **300**, 237-241. https://doi.org/10.1111/j.1574-6968.2009.01802.x

[73] Chen, J.S., *et al*. (2016) Investigation of Halogenation during the Biosynthesis of Ramoplanin in Actinoplanes sp ATCC33076. *Applied Microbiology and Biotechnology*, **100**, 289-298. https://doi.org/10.1007/s00253-015-7014-2

[74] Yin, X.H. and Zabriskie, T.M. (2006) The Enduracidin Biosynthetic Gene Cluster from *Streptomyces fungicidicus*. *Microbiology-SGM*, **152**, 2969-2983. https://doi.org/10.1099/mic.0.29043-0

[75] Agarwal, V., *et al*. (2014) Biosynthesis of Polybrominated Aromatic Organic Compounds by Marine Bacteria. *Nature Chemical Biology*, **10**, 640-647. https://doi.org/10.1038/nchembio.1564

[76] Podzellnska, K., *et al*. (2010) Chloramphenicol Biosynthesis: The Structure of CmlS, a Flavin-Dependent Halogenase Showing a Covalent Flavin-Aspartate Bond. *Journal of Molecular Biology*, **397**, 316-331. https://doi.org/10.1016/j.jmb.2010.01.020

[77] Chankham, J.P., *et al*. (2016) Regioselective Dichlorination of a Non-Activated Aliphatic Carbon Atom and Phenolic Bismethylation by a Multifunctional Fungal Flavoenzyme. *Angewandte Chemie International Edition*, **55**, 11955-11959. https://doi.org/10.1002/anie.201604516

[78] Lang, A., *et al*. (2011) Changing the Regioselectivity of the Tryptophan 7-Halogenase PrnA by Site-Directed Mutagenesis. *Angewandte Chemie International Edition*, **50**, 2951-2953. https://doi.org/10.1002/anie.201007896

[79] Shepherd, S.A., *et al*. (2016) A Structure-Guided Switch in the Regioselectivity of a Tryptophan Halogenase. *Chembiochem*, **17**, 821-824. https://doi.org/10.1002/cbic.201600051

[80] Andorfer, M.C., *et al*. (2016) Directed Evolution of RebH for Catalyst-Controlled Halogenation of Indole C-H Bonds. *Chemical Science*, **7**, 3720-3729. https://doi.org/10.1039/C5SC04680G

[81] Payne, J.T., *et al*. (2015) Directed Evolution of RebH for Site-Selective Halogenation of Large Biologically Active Molecules. *Angewandte Chemie International Edition*, **54**, 4226-4230. https://doi.org/10.1002/anie.201411901

[82] Chen, X., *et al*. (2013) Genome Sequence of Streptomyces Violaceusniger Strain SPC6, a Halotolerant Streptomycete That Exhibits Rapid Growth and Development. *Genome Announcements*, **1**, e00494-13. https://doi.org/10.1128/genomeA.00494-13

[83] Takahashi, S., *et al*. (2009) Characterization of a Flavin Reductase from a Thermophilic Dibenzothiophene-Desulfurizing Bacterium, *Bacillus subtilis* WU-S2B. *Journal Bioscience Bioengneering*, **107**, 38-41. https://doi.org/10.1016/j.jbiosc.2008.09.008

[84] Strickler, S.S., *et al*. (2006) Protein Stability and Surface Electrostatics: A Charged Relationship. *Biochemistry*, **45**, 2761-

2766. https://doi.org/10.1021/bi0600143

[85] Lawrence, M.S., *et al*. (2007) Supercharging Proteins can Impart Unusual Resilience. *Journal of the American Chemical Society*, **129**, 10110-10112. https://doi.org/10.1021/ja071641y

[86] Poor, C.B., *et al*. (2014) Improving the Stability and Catalyst Lifetime of the Halogenase RebH by Directed Evolution. *Chembiochem*, **15**, 1286-1289. https://doi.org/10.1002/cbic.201300780

[87] Sanchez, C., *et al*. (2005) Combinatorial Biosynthesis of Antitumor Indolocarbazole Compounds. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 461-466. https://doi.org/10.1073/pnas.0407809102

[88] Gruschow, S., *et al*. (2009) New Pacidamycin Antibiotics through Precursor-Directed Biosynthesis. *Chembiochem*, **10**, 355-360. https://doi.org/10.1002/cbic.200800575

[89] O'hagan, D., *et al*. (2002) Biochemistry: Biosynthesis of an Organofluorine Molecule. *Nature*, **416**, 279. https://doi.org/10.1038/416279a

[90] Schaffrath, C., *et al*. (2003) Isolation and Characterisation of 5'-Fluorode-Oxyadenosine Synthase, a Fluorination Enzyme from *Streptomyces cattleya*. *FEBS Letters*, **547**, 111-114. https://doi.org/10.1016/S0014-5793(03)00688-4

[91] Dong, C., *et al*. (2004) Crystal Structure and Mechanism of a Bacterial Fluorinating Enzyme. *Nature*, **427**, 561-565. https://doi.org/10.1038/nature02280

[92] Deng, H., *et al*. (2014) Identification of Fluorinases from Streptomyces sp MA37, *Norcardia brasiliensis*, and Actinoplanes sp N902-109 by Genome Mining. *Chembiochem*, **15**, 364-368. https://doi.org/10.1002/cbic.201300732

[93] Huang, S., *et al*. (2014) Fluoroacetate Biosynthesis from the Marine-Derived Bacterium *Streptomyces xinghaiensis* NRRL B-24674. *Organic & Biomolecular Chemistry*, **12**, 4828-4831. https://doi.org/10.1039/C4OB00970C

[94] O'hagan, D., *et al*. (2015) Enzymatic Fluorination and Biotechnological Developments of the Fluorinase. *Chemical Reviews*, **115**, 634-649. https://doi.org/10.1021/cr500209t

[95] Eustaquio, A.S., *et al*. (2010) Engineering Fluorometabolite Production: Fluorinase Expression in *Salinispora tropica* Yields Fluorosalinosporamide. *Journal of Nature Product*, **73**, 378-382. https://doi.org/10.1021/np900719u

[96] Hong, H., Spiteller, D. and Spencer, J.B. (2008) Incorporation of Fluoroacetate into an Aromatic Polyketide and Its Influence on the Mode of Cyclization. *Angewandte Chemie International Edition*, **47**, 6028-6032. https://doi.org/10.1002/anie.200801100

[97] Walker, M.C., *et al*. (2013) Expanding the Fluorine Chemistry of Living Systems Using Engineered Polyketide Synthase Pathways. *Science*, **341**, 1089-1094. https://doi.org/10.1126/science.1242345

[98] Thuronyi, B.W., *et al*. (2017) Engineered Fluorine Metabolism and Fluoropolymer Production in Living Cells. *Angewandte Chemie International Edition*, **56**, 13637-13640. https://doi.org/10.1002/anie.201706696