Trends in Genetics

CellPress

Spotlight

Convergent evolution for antibiotic biosynthesis in bacteria and animals

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Convergent evolution has been described for several metabolic pathways across the kingdoms of life. However, there is hitherto no evidence for such an interkingdom process for antimicrobials. A new report suggests that marine animals have evolved the ability to biosynthesize antimicrobial polyketides, in parallel with bacteria.

For decades, the marine world has provided human society with extremely diverse natural products. Several hundred new marine molecules produced by bacteria, fungi, algae, and invertebrates are identified from the world's oceans each year and have potential interest for food, industry, cosmetics, and health [1]. A large proportion of these biocompounds arise from so-called 'secondary metabolism', also known as specialized metabolism. They are mostly involved in intra- and interspecific communications and some are used as chemical weapons to survive in the seabed. Four decades of intensive chemical research, combined with the progressive explosion in the availability of genome resources, has deeply strengthened our knowledge of the architecture of secondary biosynthetic pathways in marine bacteria, fungi, and algae [2]. However, little is known about the production of secondary metabolites in the main marine invertebrate clades, including sponges, cnidarians, bryozoans, echinoderms, ascidians, and mollusks. Furthermore, we are not sure whether these complex molecules are synthesized by marine animals themselves, the microbes colonizing them, or a combination of both [3]. While most pathways elucidated in invertebrates in the past decade originated from their microbiomes, recent examples from corals, sea urchins, and sea cucumbers indicate that the hosts can also synthesize complex metabolites themselves [4–7]. Now, a new and remarkable report by Yue and colleagues from the research groups of Margaret McFall-Ngai and Baozhong Liu has provided surprising insights into the endogenous biosynthesis of polyketide antibiotics in a mollusk [8].

The mud-dwelling clam, *Meretrix petechialis*, is one of the most commercially maricultured shellfish in China (Figure 1A) and is an emerging model for studying reproduction and immune responses against pathogen invasion in mollusks. In this context, a transcriptomic analysis allowed Yue and colleagues to identify a nucleotide sequence, named *MpES*, that was predicted to encode a protein displaying a combination of domains characteristic of a class I polyketide synthase (PKS), a type of enzyme rarely found in animals but well described in bacteria and fungi for producing complex polyketides. The authors then went on to show that MpES shares some amino acid sequence similarity with the bacterial deoxyerythronolide B synthase (DEBS, Figure 1B), which is known for specifically driving the biosynthesis of a precursor of the broad-spectrum antibiotic erythromycin. However, the protein domain architectures of MpES and DEBS are markedly different and the sequence identity of MpES to DEBS is not substantially higher than the identity of MpES to other bacterial PKSs. Interestingly, *MpES* transcripts were found to preferentially accumulate in the shellfish mantle, a tissue acting as both a mechanical and biochemical barrier by interfacing directly with the environment.

These observations led Yue and colleagues to investigate the putative presence of erythromycin-like molecules in the mantle cells of *M. petechialis*. By combining complementary chromatography, immunocytochemistry, and molecular cytogenetic techniques, they demonstrated that a metabolite accumulates in structures along the epithelia of the mantle margin (Figure 1C). Additional transmission electronic microscopy analysis shed light on the storage of this molecule in specific mucous cells of the mantle and on the secretion of the metabolites outside the epithelium. While the authors claim that this antibiotic represents erythromycin itself, based on its mass-to-charge ratio and recognition by erythromycin-specific antibodies, additional research will be required to provide analytical evidence for this in the form of tandem mass spectrometry or nuclear magnetic resonance spectroscopy. Nevertheless, the molecule might well represent a cyclic polyketide in a way reminiscent of erythromycin or its precursors.

To determine a putative role for the MpES-predicted PKS, the authors then took advantage of a previously developed RNAi strategy in *M. petechialis*. RNAi targeting *MpES* significantly reduced both the level of *MpES* mRNA and the accumulation of corresponding metabolites in the shellfish mantle, providing firm evidence of a functional role of MpES in the synthesis of the putative polyketide metabolite in the mud-dwelling clam (Figure 1B). They also showed that antibacterial activity was substantially reduced after removing the putative polyketide metabolite using antibody-mediated affinity chromatography.

Lastly, to decipher polyketide biosynthesis in *M. petechialis* and to potentially broaden it to other animals, a combination of genetic breeding, expression analysis, and genome mining provided a compilation of data suggesting that: (i) the *MpES* DNA sequence is present in the *M. petechialis* genome, (ii) the *MpES*-encoding gene is expressed in the early developmental stages of the shellfish, (iii) two alleles of *MpES* segregate during reproduction, and

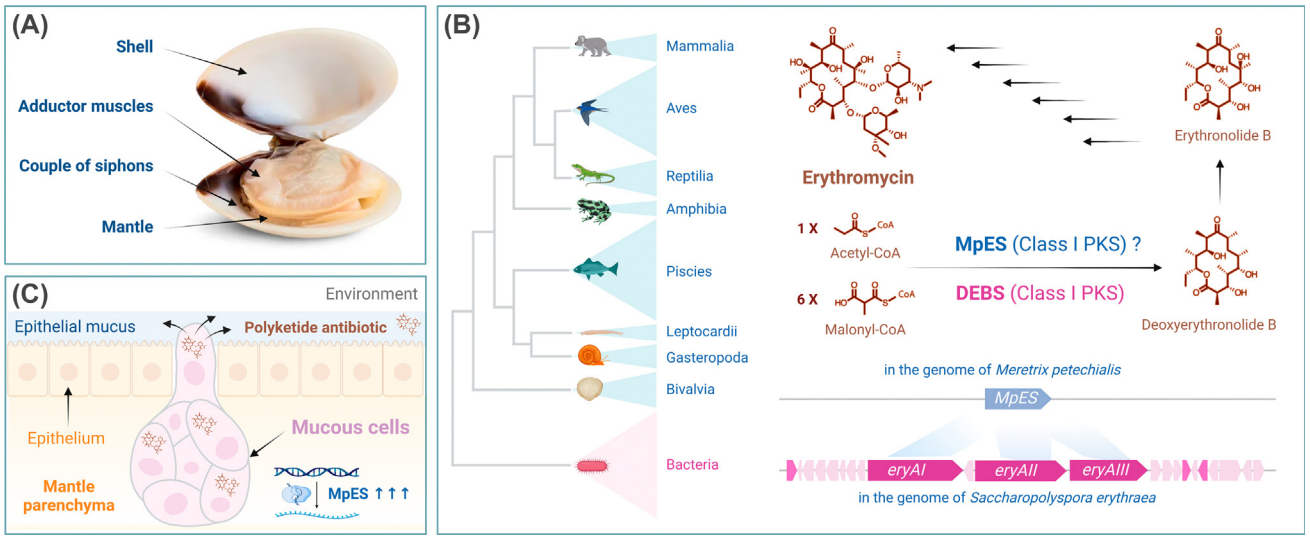

Figure 1. Endogenous biosynthesis of a polyketide antibiotic in the mud-dwelling clam *Meretrix petechialis*. (A) Anatomy of *M. petechialis*. (B) Yue and colleagues identified a nucleotide sequence, named *mpES* (in blue), predicted to encode a protein with a domain architecture similar to a type I polyketide synthase (PKS), in the bivalve *M. petechialis*. Further investigations revealed that MpES has distant amino acid sequence similarity to the bacterial deoxyerythronolide B synthase (DEBS, encoded by *eryAI-III*), which is known for specifically driving the biosynthesis of a precursor of erythromycin. In addition, explorations of eukaryotic genomic resources revealed that *MpES* gene homologs are sporadically found in roughly 30 additional species belonging to various animal clades within the tree of life (in sky blue). (C) Investigators also showed that *MpES* transcripts are preferentially detected in the shellfish mantle and that a possibly erythromycin-like metabolite is accumulated in specific mucous cells along the epithelium of the mantle margin and secreted outside the epithelium.

(iv) additional genes encoding proteins similar to enzymes involved in polyketide tailoring in bacteria can also be identified in the *M. petechialis* genome (although no evidence was presented for their involvement in a pathway with MpES). Further genomic explorations revealed that *MpES* gene homologs are sporadically found in roughly 30 additional eukaryotic species within the tree of life (e.g., reptilia, amphibia, fishes, and birds) (Figure 1B). In addition, phylogenetic inference provided compelling evidence that MpES is of animal origin.

In sum, Yue and colleagues shed light on the likely endogenous biosynthesis of a polyketide metabolite in the mud-dwelling clam *M. petechialis*, potentially making this mollusk the first animal known to produce a complex polyketide antibiotic. From an ecological point of view, this report teaches us how some marine invertebrates, devoid of any adaptive immune system, have co-opted a plethora of mechanical, cellular, and biochemical strategies for surviving in highly challenging environments. Above all, this finding provides a striking example of a convergent evolution of antimicrobial polyketides across distinct kingdoms and propels exciting research that will elucidate the full structure of the metabolite and its complete biosynthetic pathway. Major advances in multi-omics technologies may assist in identifying all the enzymes in such animal metabolic pathways, even in the absence of gene clustering [9,10]. Once these enzymes are characterized, it will be particularly important to decipher their tissue/cell/subcellular compartmentalization, to determine whether there is a higher level of complexity in this metabolic pathway, as is observed for most plant secondary metabolites. Finally, it is important to mention that such research could also find future applications in health biotechnology. The newly identified animal enzymes for polyketide biosynthesis could reinforce the toolkit for metabolic engineering, which had hitherto been filled by bacterial, fungal, and plant enzymes.

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Declaration of interests

No interests are declared.

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