Towards the genetically engineered synthesis of natural products

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During the 1950s a growing understanding of the structural relationships between natural products provided an interesting yet remote view of Nature's master plan for the elaboration of some 20000 small molecules. The obvious relationships between the biosynthetic pathways of polyketide, shikimate, mevalonate and amino acid origins led pioneers in the field to suggest plausible biogenetic schemes that received rigorous experimental testing in the ensuing decades. Testing began with *in vivo* feeding experiments, followed by cell-free systems and enzyme purification. Today, the library of structures being explored numbers over 100 000 and, the approaches used to elucidate biosynthetic pathways are very different. Enzyme purification is not the only option; the products of genes involved in a pathway can now be expressed recombinantly. The elucidation of the biosynthetic pathway for vitamin B_{12} provides an excellent example of the effect of molecular biology on this field; this work also shows that complex biosynthetic pathways can be reconstituted in bacterial lysates.

The difficulty in studying biosynthetic pathways *in vivo* became clear in our first experiments on the biosynthesis of vinblastine. The yield of target was miniscule (~0.1%), since the labeled precursors were diverted into pathways of primary metabolism [1]. Our luck improved when we began to study vitamin B_{12} synthesis in 1968. Armed with a home-made Fourier transform NMR spectrometer and liters of cells, we obtained 2–5% incorporations of the ^{13}C-enriched substrates 5-amino levulinic acid (ALA), porphobilinogen (PBG) and uro'gen III (Fig. 1). Later, with L. Siegel and G. Müller, we found the intermediates precorrins-2 and -3. But identifying the next intermediates was difficult. Over the next decade, a great deal was learned about enzymes 2, 3 and 4 (Fig. 1) but the steps between precorrin-3 and corrin remained cryptic.

In 1987, John Roth provided the key to further progress by showing that *Salmonella typhimurium* makes vitamin B_{12} anaerobically, and characterizing the corrin operon — the *cbi* genes. The corresponding genes of *Pseudomonas denitrificans*, the *cob* genes, then became accessible, so that by mid-1990 the complete repertoire of corrin-synthesizing enzymes for both the anaerobic and aerobic pathways was in hand; at the same time, collaborative work in Paris and Cambridge had established the pathway from precorrin-6x to the cobalt-free corrin, hydrogenobyrinic acid (see Fig. 1). Now came the fascinating problem of matching each gene product to its function. To deduce the function of the enzymes connecting precorrin-2 and -6x, precorrin-2 was biosynthetically labeled with ^{13}C, then incubated with each of the gene products in turn in the presence of $^{13}CH_3$ *S*-adenosylmethione (SAM). If new signals appeared in the NMR spectrum, a reaction had occurred. In this way enzyme 5 was found to methylate precorrin-2, producing precorrin-3. When precorrin-3 was incubated with oxygen and the CobG protein of *P. denitrificans* (enzyme 6, Fig. 1), the NMR spectrum changed to that of the novel hydroxylactone precorrin-3x. Although precorrin-3x has all of the functionality necessary for ring contraction, CobJ (enzyme 7) is required first to catalyze C-methylation at C-17. Enzyme 7 also shrinks the macrocycle (Fig. 1) leading to precorrin-4 [2], which is methylated by enzyme 8 ([3]; see Fig 1) and after one more step yields precorrin-6x. It took 20 years and an enormous international effort to find the first seven intermediates, yet thanks to recombinant DNA technology the remaining six structures have been discovered in the last three years!

With all of the enzymes required for aerobic synthesis of corrins overexpressed, we attempted *in vitro* reconstitution of the pathway. Mixing enzymes 1–5 with ALA and SAM produced precorrin-3 in >80% yield [3]. Adding enzymes 6–9 produced precorrin-6x; we are now optimizing the process to use all 12 enzymes to prepare corrin from ALA. The use of multi-enzyme synthesis to reach other complex targets, such as vinblastine and taxol, is under intensive study. There is already success in transferring the genes for alkaloid synthesis from the plant *Catharanthus roseus* to *Escherichia coli* [4]. It should thus be possible to use lysates of engineered *E. coli* to make a range of complex natural products not found in bacteria, avoiding the problems of plant collection and the associated ecological difficulties, as well as bypassing the metabolism of the living cell. We are entering a new era at the interface of chemistry and biology where organic chemists can actively control the pathway to natural products, rather than remaining spectators of Nature's synthetic strategies [4].

References

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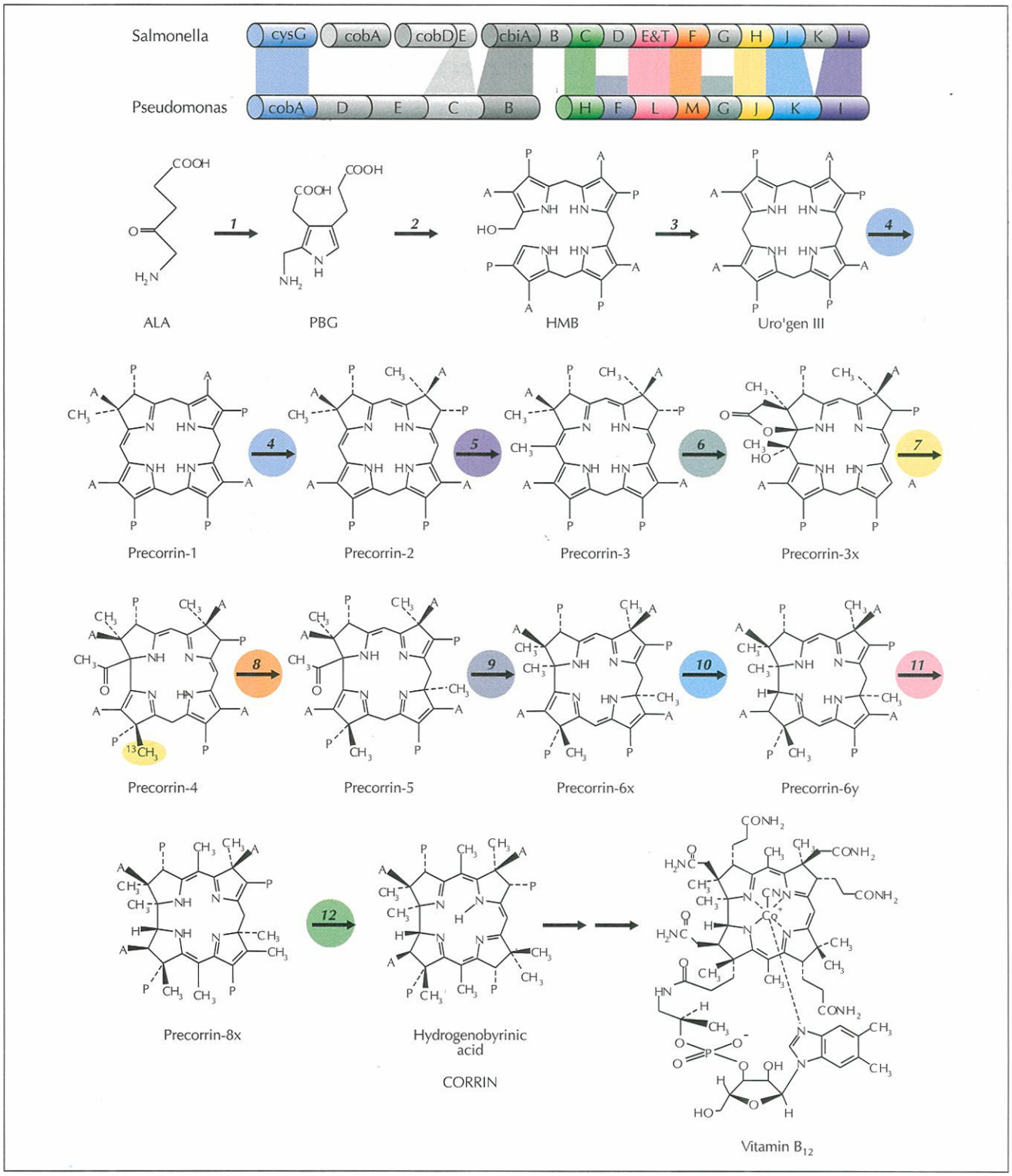

Fig. 1. The biosynthetic pathway of vitamin B_{12} synthesis. The *Salmonella* and *Pseudomonas* operons required for cobinamide and vitamin B_{12} biosynthesis are shown at the top of the figure; homologous genes are color-matched. Where the function of the gene is known, it is color-coded to the appropriate enzyme in the vitamin B_{12} biosynthetic pathway. The enzymes are: **1.** 5-amino levulinic acid (ALA) dehydratase (the product of the gene *hemB*) **2.** porphobilinogen (PBG) deaminase (*hemC*) **3.** Uro'gen III synthetase [cosynthase] (*hemD*) **4.** Uro'gen III methylase [M-1] (*cysG/cobA*) **5.** M-2 (*cobI, cbiL*) **6.** Precorrin-3x synthase (*cobG*) **7.** Ring contractase/ 17 methyl transferase [M-3] (*cobJ, cbiH*) **8.** M-4 (*cobM, cbiF*) **9.** M-5 (*cobF*) **10.** Reductase (*cobK, cbiJ*) **11.** Precorrin-8x synthase (M-6/decarboxylase) (*cobL, cbiE+T*) **12.** [1,5]-sigmatropic shiftase [hydrogenobyrinic acid synthase] (*cobH, cbiC*).A, CH_2COOH, P, CH_2CH_2COOH. Insertion of the new ^{13}C-labeled methyl group from SAM in precorrin-4 (shown in gold) appears to trigger the ring contraction step. Both of these processes are catalyzed by the single enzyme, CobJ.