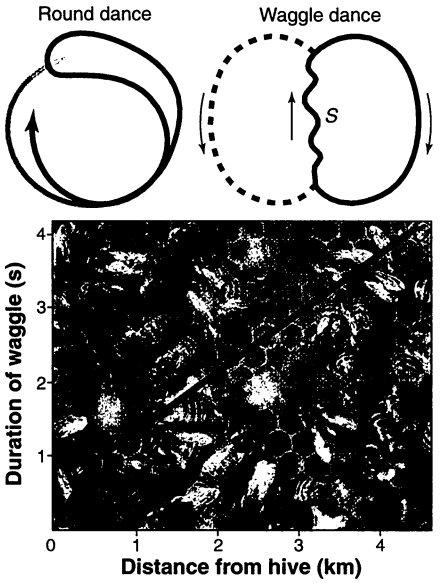

Flight of the honeybee. The round and waggle dances are performed by foraging bees on an area of vertical comb close to the entrance of the hive to signal the availability of food. When food is close to the hive, foragers perform a round dance, which gives no indication of the direction in which the food is located. For sites more distant than about 50 m, the direction of food with respect to the sun's azimuth is signaled by the angle of the waggle component (*S*) relative to gravity, and the distance of the food from the hive is signaled by the duration of the waggle component. Duration increases nonlinearly with distance.

sent an analysis of the dances that bees perform after flying through the tunnel (*2*).

The 11-cm-wide and 6.4-m-long tunnel was lit by skylight through the top. It was placed a few meters from the hive. Honeybees typically do not perform the waggle dance when the feeder is closer than 50 m from the hive. Instead, they signal through the round dance that food is nearby (see the figure). Bees returning from a feeder at the entrance to the tunnel performed the expected round dance. The feeder was then placed 6 m into the tunnel. When the tunnel had horizontal stripes that generated minimal image motion, bees continued to perform a round dance. In contrast, if the tunnel walls were covered with a random texture generating abundant image motion, bees performed a waggle dance that indicated a greatly magnified distance between hive and feeder of about 200 m. Flying a short distance through a narrow tunnel thus turns out to be equivalent to flying a distance 30 times further over open ground. Srinivasan *et al.* infer from these data that in the outdoor environment in which the bees were tested, features generating image motion were about 170 cm away from the bees' eyes. As the perceived image motion depends strongly on details of the terrain, the bees' measuring tape will be route dependent. This shortcoming does not matter if the principal use of the measuring tape is to gather and transmit information about a particular route.

The slope of the relation between waggle duration and distance becomes less steep with distances greater than about 500 m (see the figure). The shallower slope at greater distances might result either from saturation of the system that integrates optic flow, or because bees fly higher over longer journeys (*5*) and so are at a greater mean distance from objects generating optic flow. The great advantage of measuring the waggle dance of bees that have flown through the tunnel is that the optic flow in the tunnel is well defined. It thus becomes possible to test for the linearity of the integrator, provided, of course, that workshop and bees cooperate in building and flying the much longer tunnels that would be needed.

Different insects have to contend with different ecological problems so that image motion is unlikely to be the only cue that insects use for measuring distance. Because bees fly and are subject to unknown winds, ground speed and distance are most reliably controlled and measured through image motion. But insects that walk on the ground and are not subject to passive transport by wind can do better. Rather than relying on optic flow, with its unavoidable uncertainties, walking insects can, with advantage, use proprioceptive information or some kind of step counting to monitor the speeds and distances that they travel. Indeed, desert ants keep a precise record of the distance that they walk to and from their nest, and they seem to make little if any use of optic flow in monitoring these distances (*6*).

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PERSPECTIVES: BIOSYNTHETIC PATHWAYS

Biosynthesis Meets Bioinformatics

David E. Cane

Natural products continue to be a fertile ground for chemical and biochemical inquiry and serve as an invaluable source of some of the most widely used agents in human medicine, such as the antibiotics erythromycin, tetracycline, and penicillin, the anticancer agent taxol, and the cholesterol-lowering drug mevinolin, not to mention plant extracts used widely in dietary supplements and traditional medicine. Studies of the biosynthesis of natural products have revealed the origins of many of these biologically important and chemically complex substances and have been an invaluable tool for the discovery of new biochemical reactions. Traditionally, these studies have relied primarily on incorporation experiments with isotopically labeled precursors and on isolating new enzymes catalyzing individual transformations in multistep biosynthetic pathways. Two recent publications (*1*, *2*) illustrate the continuing role of biosynthetic studies in unearthing new biochemistry and highlight the increasing role of molecular biology and genomics in this quest.

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Until about 10 years ago, mevalonic acid was widely regarded as the common precursor of the more than 30,000 naturally occurring terpenoids. Inhibition of mevalonic acid biosynthesis by fungal-derived metabolites such as mevinolin has become a widely used and effective method for the therapeutic control of serum cholesterol levels. It therefore came as a surprise when the research groups of Rohmer in Strasbourg and Arigoni in Zürich independently discovered that certain bacteria, algae, and higher plants can use an entirely different pathway for the biosynthesis of the universal isoprenoid building block isopentenyl diphosphate (IPP) [for a review, see (*3*)]. The discovery of this mevalonic acid–independent pathway initiated intensive investigations aimed at identifying the relevant enzymes and metabolic intermediates. The pathway is now known to begin with the conversion of the glycolytic intermediates pyruvate and glyceraldehyde-3-phosphate (**1**, G-3-P) to the five-carbon sugar 1-deoxyxylulose phosphate (**2**, dXP) in a thiamin diphosphate (TPP)–dependent reaction (*4*, *5*) (see panel A in the figure). Interestingly, dXP is also a precursor for thiamin and vitamin B_6 in bacteria such as *Escherichia coli*.

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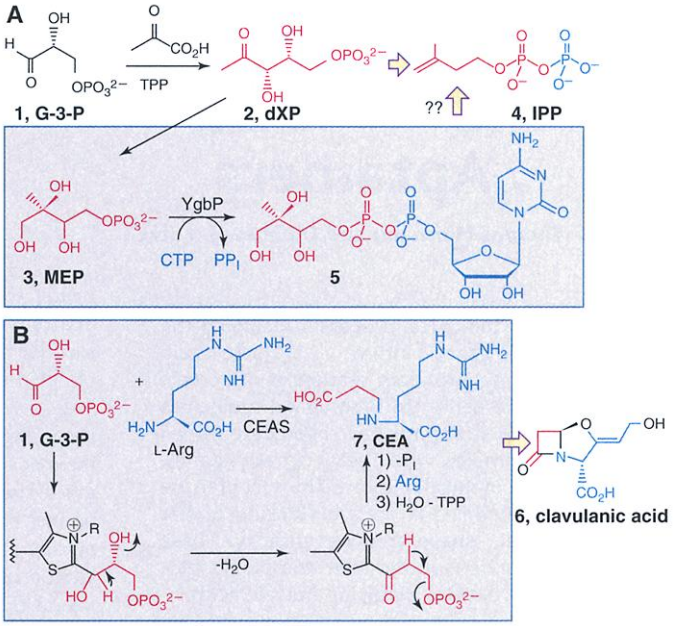
In 1998, Kuzuyama *et al.* showed that reductive rearrangement of dXP gives 2-*C*-methylerythritol-4-phosphate (**3**, MEP) (*6*). The genes encoding both dXP synthase and dXP reductoisomerase have now been detected in numerous bacterial and plant species, and it has been reported that inhibitors of MEP formation may be effective antibacterial or antimalarial agents (*7*).

Conversion of MEP to the common isoprenoid precursor IPP (**4**) formally requires addition of a phosphate group to generate the characteristic diphosphate function, followed by a series of as-yet unknown reductive steps. Rohdich *et al.* have now identified the next enzyme in the pathway and the likely source of the diphosphate function in the IPP (*1*). By incubating labeled samples of MEP with crude cell-free extracts of *E. coli*, they observed a cytidine triphosphate (CTP)–dependent reaction that gave rise to a new product, the sugar diphosphonucleotide **5** (see panel A in the figure).



Mining the treasure trove. (**A**) The mevalonate-independent route to IPP and isoprenoids. Rohdich *et al.* (*1*) have recently shown that the YgbP gene product catalyzes the CTP-dependent conversion of MEP to the diphosphonucleotide conjugate **5**. (**B**) The first step in clavulanic acid biosynthesis. Khaleeli *et al.* (*2*) have found that CEA synthase (CEAS) catalyzes the addition of the TPP ylide to the aldehyde carbonyl of G-3-P to form an adduct that undergoes dehydration and tautomerization to an acyl-TPP intermediate. Elimination of phosphate, followed by conjugate addition of L-Arg and hydrolytic release of TPP, will generate CEA, the first committed intermediate of the pathway.

The usual practice at this stage would have been to purify the relevant enzyme to homogeneity and then use deduced sequence information to clone the corresponding structural gene. Instead, Rohdich *et al.* took a conceptually novel and experimentally daring approach. They searched the nucleic acid and protein databases for enzymes known to catalyze the formation of analogous, relatively rare cytidine diphosphate conjugates. They identified a suitable candidate from *Haemophilus influenzae* that turned out to be similar to a second, unannotated *H. influenzae* gene, termed *ygbP*, whose homologs in the genomic sequences of a wide variety of bacterial and plant species correlated perfectly with the demonstrated presence of other genes of the dXP to IPP pathway. They then expressed and purified the corresponding *E. coli* YgbP protein and showed that this enzyme is a homodimer of 26-kD subunits that indeed catalyzes the CTP-dependent formation of **5** from MEP. The search is now on for additional intermediates between **5** and IPP.

The clavulanic acid biosynthetic pathway has also been the target of intense research. Clavulanic acid (**6**) is a potent inhibitor of β-lactamases, enzymes that hydrolyze penicillins and cephalosporins and thereby provide resistance to these important antibiotics (*8*). Since 1992, the majority of the enzymes and the relevant structural genes responsible for clavulanic biosynthesis in the Gram-positive soil bacterium *Streptomyces clavuligerus* have been identified (*9*, *10*). The identity of the three-carbon building block that combines with arginine to give the first committed (*11*) intermediate of the pathway, N^2-(2-carboxyethyl)arginine (**7**, CEA), however, has remained a mystery (*12*–*15*).

Townsend and co-workers at Johns Hopkins have now solved this puzzle by an ingenious series of experiments that began with the recognition that the first open reading frame in the biosynthetic gene cluster contains an apparent thiamin diphosphate–binding site (*2*). The importance of this observation was initially obscure because there were no known TPP-dependent reactions that could account for the formation of CEA.

In a traditional enzymological study, the discovery of new enzymes usually starts with the recognition of a biochemical transformation, followed by the identification of the enzymatic activity capable of mediating that transformation. Townsend and coworkers turned this paradigm for enzyme discovery on its head (*2*). They expressed the recombinant protein for CEA synthase in *E. coli*, systematically screened for substrates, and identified G-3-P (**1**) as the cosubstrate that combines with arginine in the TPP-dependent reaction (see panel B in the figure). They propose that formation of CEA involves an internal redox reaction, followed by a β-elimination and addition that results in the formation of the characteristic C–N bond. Their study has thus not only elucidated the first step in the clavulanic acid biosynthetic pathway: Townsend and co-workers have also discovered an entirely new reaction type mediated by the intensively studied thiamin cofactor.

The two studies show how many investigations of biosynthetic pathways will be performed in the future. The recognition that the genes for many biosynthetic pathways are clustered in microorganisms has already led to the discovery of dozens of new biosynthetic genes with as-yet unknown biochemical function. At the same time, genomic sequencing programs have revealed several thousand presumptive structural genes of unknown function. This treasure trove of new enzymes is likely to grow exponentially in the next few years, revealing hundreds or even thousands of novel biochemical reactions for the biosynthetic chemist to mine, while holding the promise of rational genetic manipulation of biochemical pathways, including the controlled biosynthesis of new natural products.

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