Source of a scent. *The Soul of the Rose*, painting by John William Waterhouse (1908).

BIOCHEMISTRY

The flowering of a new scent pathway in rose

The monoterpene-based scent of roses is generated by an unusual biosynthetic route

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Floral scent is an important trait of ornamental roses that has provided sensual pleasures for humans since antiquity. However, most modern rose cultivars used for cut flowers have little fragrance as a result of breeding preferences for traits such as color and longevity. Restoring scent attributes by breeding or biotechnological means (*1*) requires a detailed understanding of the biosynthesis of rose scent. Rose fragrance consists of hundreds of volatile compounds with diverse biosynthetic origins whose amounts vary among the different rose varieties. Genomic approaches over the past 15 years have identified several genes and enzymes involved in rose scent production (*2*), but these efforts have not succeeded in elucidating the biosynthetic steps in the formation of geraniol and other monoterpenes that constitute one of the major groups of rose fragrances. On page 81 of this issue, Magnard *et al.* (*3*) describe the discovery of an unexpected enzymatic pathway to these scent compounds.

The biosynthesis of monoterpenes, the 10-carbon representatives of the enormous class of terpenoid natural products, usually requires the enzymatic action of terpene synthases. As one of the most celebrated groups of catalysts in nature (*4*), terpene synthases convert the 10-carbon prenyl diphosphate intermediates—in most cases, geranyl diphosphate—to a highly diverse range of cyclic and acyclic products by a carbocation-mediated reaction mechanism that is initiated by the ionization of the diphosphate group. Such terpene synthase activities have been previously associated with the formation of sesquiterpenes, 15-carbon terpenes, in rose scent (*5*). However, the enzymatic machinery leading to the biosynthesis of the 10-carbon monoterpenes, such as geraniol, has remained a mystery. Geraniol, an alcohol, has been demonstrated to be made in basil from geranyl diphosphate via a terpene synthase that simply cleaves the diphosphate moiety and allows the resulting carbocation to be quenched by water (*6*). Although using a terpene synthase to carry out such a simple reaction might be considered a waste of its catalytic potential, the ubiquity of terpene synthases in monoterpene formation has led to the belief that this is the sole route of geraniol biosynthesis in all plants.

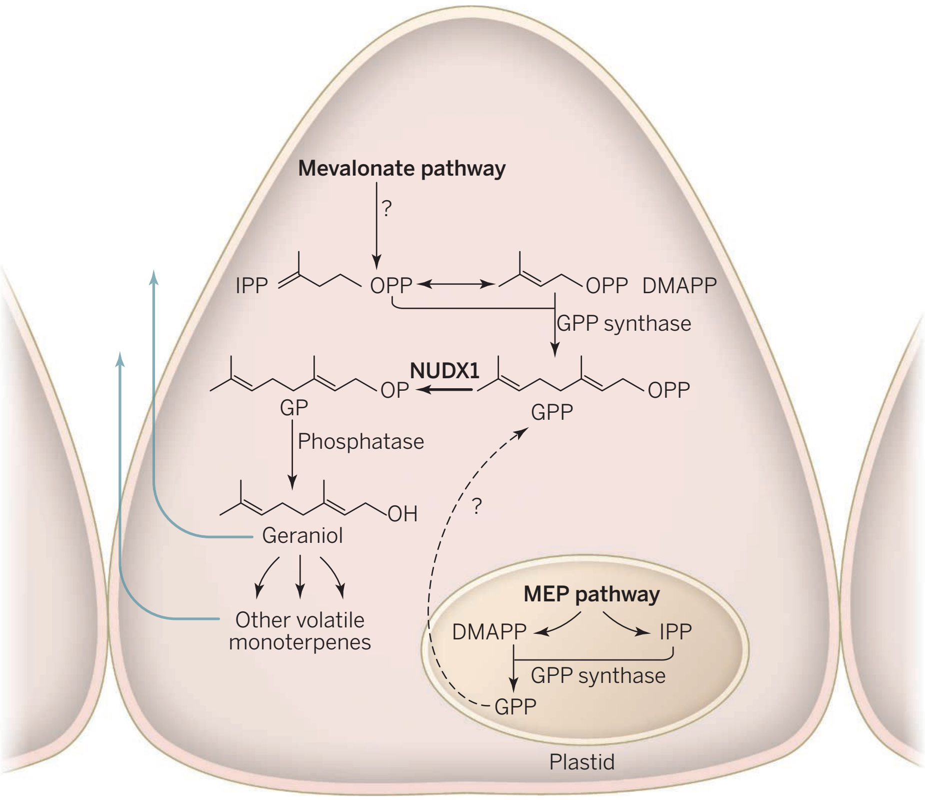
Surprisingly, Magnard *et al.* demonstrated that rose flowers use an alternative route to produce geraniol by employing a diphosphohydrolase of a completely unexpected enzyme family (see the figure). Roses lack the full contingent of modern genetic and genomic tools available for model organisms such as *Arabidopsis thaliana*; instead, the authors had to painstakingly accrue evidence for the role of this enzyme in scent production. To sniff out how geraniol is formed in roses, they first compared a rose variety with a typical rose scent to one with low scent that produced very small amounts of geraniol and other typical monoterpene scent constituents. The gene with highest differential expression in the scented versus unscented varieties did not encode a geraniol-forming terpene synthase or any other obvious enzyme of monoterpene biosynthesis, but instead was a gene of the Nudix family, which usually encodes enzymes that cleave nucleoside diphosphates from a variety of other chemical moieties (*7*). Expression of this gene correlated with the presence of geraniol and other monoterpenes in a survey of cultivars with different scent profiles. Moreover, it colocalized with a quantitative trait locus (QTL) for geraniol formation in a full-sib family of hybrid lines derived from a cross between two genotypes with different scent compositions. In addition, this Nudix hydrolase gene was found to be expressed almost exclusively in petals, and when knocked down by stable and transient RNA interference, the result was a reduction in monoterpene levels. After heterologous expression in *Escherichia coli*, the encoded protein was found to have a clear role in geraniol formation by converting geranyl diphosphate in vitro to geranyl monophosphate, which was in turn found to be hydrolyzed to geraniol by a rose petal–derived phosphatase activity. Expression of the Nudix protein in *Nicotiana benthamiana* further supported its role in geraniol biosynthesis in planta.

The authors' success in elucidating this novel biosynthetic route to geraniol in rose flowers raises the thorny question of why such an unusual monoterpene biosynthetic pathway evolved in the first place. One answer may come from enzyme kinetics. With a Michaelis-Menten constant (K_M) for geranyl diphosphate that is two orders of magnitude lower than that of the geraniol-forming terpene synthase known from basil, the Nudix hydrolase may be a much more efficient catalyst than a terpene synthase for forming this simple monoterpene. This may be particularly advantageous in roses, where monoterpene scent compounds are produced in relatively large amounts.

Another factor that may have led to the recruitment of the Nudix protein for monoterpene formation is its cytosolic location. Monoterpenes are thought to be manufactured principally in plastids, using substrates from the methylerythritol 4-phosphate pathway (*8*), but the lack of photosynthesis in rose petals may result in diminished flux through the methylerythritol 4-phosphate pathway, so that terpene formation must rely on the mevalonate pathway located in the cytosol. In fact, rose petals were one of the first plant tissues shown to readily use the mevalonate pathway in monoterpene formation (*9*). The cytosolic location of the Nudix protein requires the provisioning of geranyl diphosphate in this cellular compartment by synthesis in situ or possibly by transport from plastids, the usual site of geranyl diphosphate formation in plants. It will be interesting to find out how roses accomplish this feat, one very seldom documented in plant metabolism.

Other unanswered questions revolve around when the pathway to geraniol originated in roses and how widely distributed it is in other plants. The recruitment of Nudix hydrolase into this pathway may have been a recent event in the evolution of roses, or this type of enzyme may be much more widely involved in monoterpene formation in plants. There are now an increasing number of examples in the plant kingdom where the same types of natural products are produced by different pathways in different plant lineages, and thus arose independently (*10*, *11*). The value of monoterpenes in flowers—for example, as pollinator attractants (*12*) or as defenses against pathogens—may have led to the repeated evolution of different biosynthetic pathways for them.

The value of rose monoterpenes for humans is driven by the enormous popularity of roses in the floral industry and as sources of essential oils for natural fragrances. The discovery of a Nudix hydrolase involved in the formation of geraniol now provides a reliable molecular marker for a major group of floral scent compounds in roses that can be exploited to enhance fragrance in these iconic flowers. ■



An unexpected source of scent. The formation of geraniol and other geraniol-derived monoterpenes typical of the rose proceeds from geranyl diphosphate (GPP) to geranyl monophosphate (GP), catalyzed by NUDX1. GP is hydrolyzed to geraniol, and this product and other downstream monoterpene metabolites are then volatilized (blue arrows). The pathway is presumably situated in the epidermal cells of rose petals (depicted are conical cells of the adaxial epidermis). The subcellular compartmentalization of the pathway is not fully understood. Although NUDX1 is localized to the cytosol, its substrate GPP might be produced from the 5-carbon precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), formed in the cytosol or in plastids. OPP, pyrophosphate. MEP, methylerythritol 4-phosphate.

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