RESEARCH PAPER ANALYSIS

A CLONED TYROSINASE TURNS *E. COLI* BLACK

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REASONS

Getting *Escherichia coli* to overproduce interesting proteins is one of biotechnology's chief endeavors. But most of that activity has gone into proteins of potential pharmaceutical interest; relatively little has been directed at manipulating bacteria to produce other kinds of useful compounds. In this issue of *Bio/Technology*, Guy della-Cioppa and his colleagues at Biosource Genetics (Vacaville, CA) report the bacterial overproduction of a cloned tyrosinase gene from a Streptomycete. Depending on the substrate supplied, the *E. coli* host secretes a variety of melanin pigments into the growth medium.

Melanin is an irregular polymer composed of indoles, benzthiazoles, and amino acids. Its synthesis begins with the enzymatic oxidation of tyrosine to dihydroxyphenylalanine (DOPA) quinone. Further polymerization is non-enzymatic, and the chemical reactivity of quinones (and the substrate tolerance of tyrosinase) can turn melanins black, brown, yellow, red, or violet.

As a broad-band absorber of electromagnetic radiation, melanin is an ideal material for photoprotective applications. These uses include topical sunscreens and a coating for either opaque or transparent plastics (to absorb light and delay photodegradation). The variety of colors available from melanins also makes them attractive for applications in the cosmetic industry, where natural materials and colors are desirable.

Until now, melanin has only been available in research quantities from two sources: extracted from natural materials (squid ink sacs or bovine

retinas) or made synthetically by oxidizing DOPA. Neither route has been able to provide material in sufficient quantities or with sufficient reproducibility to be industrially useful.

To coax *E. coli* into the synthesis of DOPA quinones, the Biosource scientists introduced a plasmid containing the *mel* locus of *S. antibioticus*, which is capable of forming dark melanin pigments. The *mel* locus contains two open reading frames that encode a putative protein (ORF438) and a tyrosinase. While the function of ORF438 remains unclear (the most attractive suggestion is that it provides a metallothionein-like function and delivers copper to the apotyrosinase), it appears to be essential for high-level melanin production in the *E. coli* host. In the absence of ORF438 protein, clones with high levels of catalytically active tyrosinase were nonetheless poor melanin producers. The cloned genes were placed behind the phage T7 promoter. In cells containing a second plasmid expressing a T7 polymerase, such cloned genes are selectively and actively transcribed. A temperature-inducible promoter from phage lambda con-

trolled expression of the polymerase, and adding copper and tyrosine to the growth medium, followed by a temperature shift, resulted in the production of melanin pigments.

In cells that naturally produce melanin, the DOPA quinones produced by the tyrosinase are quite reactive, and thus a variety of different chromophores may find their way into the final heteropolymer. The Biosource scientists demonstrated this in their bacterial system by using tyrosine analogs in the induction protocol to produce melanins with unique pigmentations. Incorporating ferric chloride into the agar plates produces an intense black melanin due to the reaction of the ferric ion with catechols. Thus, the system described here provides opportunities to develop color-modified melanins, and to screen other classes of precursors for incorporation into "*E. coli* melanins." Additionally, screening for melanin production in the absence of supplemented tyrosine may be used to identify amino acid overproducing mutants, as well as providing new opportunities for protein engineering of the tyrosinase itself. —Harvey Bialy

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