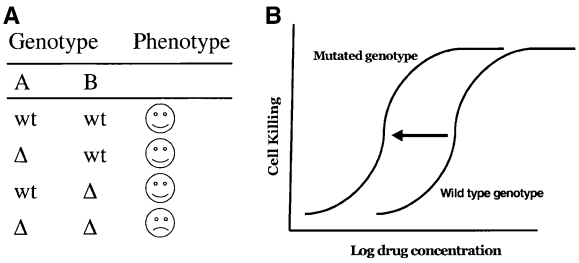

Figure 1. A Schematic Representation of the Traditional Synthetic Lethal Strategy Used in Yeast Genetics Studies

(A) A and B represent different genes. When both normal genes are present, the yeast are viable, signified by the smiling face. When either gene A or B are altered or deleted (indicated with Δ), the organism remains viable; when both genes are mutated, the lethal phenotype, signified by the frowning face, occurs.

(B) In a synthetic lethal chemical screen, the lethal phenotype occurs only when the organism has the altered gene or genes and is exposed to a specific chemical agent. Thus, the concentration/dose-response curve is shifted to the left in the cells that have the altered or deleted gene(s). In some cases, the small molecule might inhibit function of the product of a second gene—for example, gene B—that is essential for survival only when the product of gene A is mutated or deleted.

cused on exploiting apoptotic death as a target for anticancer agents, a good case can be made that the resulting immunological recruitment associated with selective nonapoptotic death of tumor cells might actually be a desirable attribute for any drug. Thus, understanding how erastin selectively kills cells would seem to be a worthy undertaking. Another unanswered question is why only partial resistance to camptothecin was seen when small interfering RNA to topoisomerase 1 was introduced into TERT/LT/ST/RAS^{V12} cells. Perhaps there are other as yet unknown targets for the camptothecins that exist. We also do not know how important the transfected gene expression stoichiometry is for the ultimate sensitivity to particular agents. Nonetheless, Dolma et al. [5] nicely illustrate the power of the synthetic lethal approach for exposing agents with both unique and potentially rich pharmacological properties. It seems likely that many others will emulate their approach.

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Novel Carotenoids Genetically Engineered in a Heterologous Host

Carotenoids are commercially important pigments that are essential for human health. Diverse carotenoids have been identified, but availability has impeded evaluation of their pharmaceutical potential. Molecular techniques have been developed to produce specific and novel carotenoids with improved biological activities.

Over the last few years, molecular procedures have been developed to engineer metabolic pathways. These include directed evolution by gene shuffling to alter the catalytic properties of enzymes, and combinatorial biosynthesis, i.e., coexpression of genes encoding different enzymes from related pathways. Both approaches have successfully been applied to carotenoid biosynthesis. The color of carotenoids is very helpful for the screening and selection of transformants. Typical carotenoids consist of 40 carbon atoms with an extensive conjugated double bond system. They may also carry substituted cyclic ionone end groups. These structural features are targets for modification and for mixing substituents in the same molecule.

Compounds can be synthesized in a modular way by combining genes that comprise different branches of a biosynthetic pathway, which are isolated from different organisms. Pioneering work following this approach was carried out on the synthesis of novel polyketide antibiotics [1]. This strategy only works, however, when the substrate specificity of a key biosynthetic enzyme is such that it does not need to recognize the entire substrate molecule but only certain regions of the molecule that are suitable for conversion. This is generally also the case in carotenoid biosynthesis, and therefore this combinatorial approach was very successful in generating numerous carotenoids including novel structures not found before [2]. Among the carotenoids produced by combinatorial biosynthesis were carotenoids with improved biological activity [3].

Highly homologous carotenogenic genes from related organisms may encode enzymes with modified substrate and/or product specificities [4], but this is a rare occurrence. One experimental way of acquiring enzymes with extended catalytic properties for the synthe-

sis of novel carotenoids is gene shuffling. Stemmer first described this technique in 1994 [5], and it involves the recombination of two or more DNA segments modified by mutagenesis or originating from different organisms into a full-length gene. The library of reassembled genes is then screened for functional diversity. By random shuffling of phytoene desaturase genes from two *Erwinia* species, a recombined gene was obtained encoding an enzyme which introduced six instead of four double bonds into the phytoene molecule, yielding 3,4,3′,4′-tetradehydrolycopene [6]. In addition, a lycopene cyclase was generated with several altered amino acids, which, unlike the nonmodified enzyme, was able to convert 3,4-didehydrolycopene into the corresponding monocyclic product torulene.

In the May issue of *Chemistry & Biology*, Lee et al. [7] presented an extensive study on the generation of novel carotenoids by using the genes of the modified desaturase and cyclase mentioned above and coexpressing them in *Escherichia coli* as a heterologous host together with wild-type carotenogenic genes. The wild-type carotenogenic genes originated from various species that accumulate different carotenoids as end products. Various combinations of these genes resulted in carotenoids of different chain length, altered degrees of desaturation, and substitutions by oxygen groups at individual positions of the molecule. A keto group was introduced at position 4 of torulene by the ketolase CrtO from the cyanobacterium *Synechocystis*. Alternatively, torulene was hydroxylated at position 3 by the hydroxylase CrtZ from *Erwinia*, and this hydroxy group was further glucosylated by CrtX from the same bacterium, or the β-ionone end group was converted to an aromatic ring using the *crtU* gene from *Brevibacterium linens*. 3,4,3′,4′-Tetradehydrolycopene was oxygenated at positions 2 and 2′ to phillipsiaxanthin (which is 3,4,3′,4′-tetradehydrolycopene-2,2′-dione) by the carotenoid 2-ketolase CrtA from the photosynthetic bacterium *Rhodobacter*. Surprisingly, CrtA was able to oxygenate unsubstituted acyclic carotenoids, like lycopene and others, at position 2 in *E. coli*. In purple bacteria, the source of the *crtA* gene, 1-hydroxy-3,4-dehydrocarotenes are the only substrates for this reaction. Similar to the modification of C_{40} carotenoids, C_{30} carotenes were cyclized or a keto group introduced at position 2. It should be pointed out that these and most of the other carotenoids synthesized in this study were novel.

Researchers have made tremendous advances in this field, from generating the first heterologous carotenoid by expressing a gene cluster from *Erwinia* in noncarotenogenic *E. coli* in 1990 [8, 9], to the first combination of carotenogenic genes from different organisms in 1993 [10], and, finally, to Lee and colleagues' detailed and systematic exploitation of combinatorial biosynthesis using genes mutated by gene shuffling for the provision of special precursors [7]. When gene shuffling and combinatorial biosynthesis are complementary, there is a huge potential for the creation of unique and diverse carotenoids, provided that appropriate wild-type genes are available. In the future, mutagenesis by error-prone PCR, which has already been used to generate a type of phytoene synthase able to form C_{30} and C_{40} products [11], will most likely be employed to extend the potential of combinatorial biosynthesis of carotenoids.

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