sufficient to stabilize an overall dimeric structure for the hybrid proteins, despite the absence of interactions between equivalent NRPS modules. The authors envision a structure in which a dual-track assembly line is preserved in these hybrid proteins by the protrusion of pairs of monomeric NRPS loops from a dimeric PKS core, in much the same way as has been proposed for the optional β-ketoacyl reduction, dehydration, and enoylreduction domains of PKS modules themselves [4]. However, NRPS and PKS modules are not always connected covalently in hybrid assembly lines. For example, the hybrid NRPS/PKS proteins responsible for the biosynthesis of myxothiazol, epithilone, and yersiniabactin contain NRPS modules that appear to function in *trans* with adjacent PKS modules on separate polypeptides [1]. In the case of exclusively PKS modular proteins, it has been proposed that fidelity in the intermodular transfer of intermediates to the appropriate downstream polypeptide depends both on the specificity of the interaction between the acyl carrier protein domain of the upstream (donor) polypeptide and the downstream (acceptor) β-ketoacyl synthase domain and on the presence of complementary linker regions at the N and C termini of the interacting polypeptides [12]. Whether similar mechanisms could be operative in facilitating interactions between NRPS and PKS modules located on separate polypeptides and in the maintenance of a dual-track assembly line through these junctions remains to be determined.

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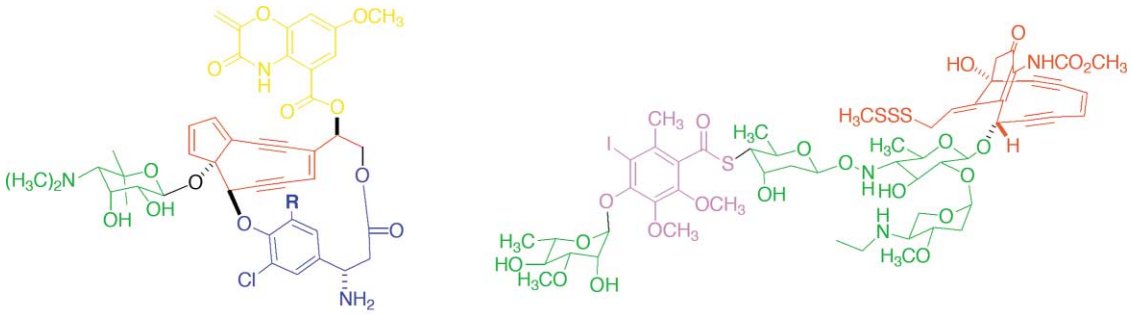
# Toward Bioengineering Anticancer Drugs

**The biosynthetic route for enediyne production remained mysterious until two independent groups recently reported the genes that orchestrate enediyne synthesis in two different microorganisms. These discoveries lay the foundations for engineering this pathway to generate improved anticancer drugs.**

The enediyne class of antitumor agents are complex natural products whose unique structure, mechanism of action, and potent cytotoxicity have earned the interest of chemist and biologists. The total synthesis of members of the class has been achieved [1], and elegant research based upon fundamental work done by Robert Bergman in the 1970s revealed the way in which enediynes cleave DNA [2]. Recently, a new anticancer drug consisting of one member of the class conjugated to a cancer cell-recognizing antibody has been approved for use in acute myelogenous leukemia patients [3]. Despite these advances, a fundamental question concerning the biosynthetic origin of this group of highly unsaturated natural products remained unresolved. Now, two groups working with two different enediyne-producing organisms have reported the biosynthetic genes responsible for the construction of these natural products. Their results indicate that the enediynes share a common polyketide biosynthetic pathway. This work lays the foundation for bioengineering this pathway in order to produce improved anticancer drugs.

The enediynes contain at their core two acetylenic groups flanking a double bond or incipient double bond within a nine- or ten-membered ring chromophore. The key to the extreme cytotoxicity of this group of natural products is the ability of this unstaturated core to undergo a so-called Bergman cyclization to produce a reactive diradical intermediate. The interaction of the diradical with double-stranded DNA results in the oxidative cleavage of the DNA and ultimately cell death. Most of the nine-membered ring enediynes are produced as complexes with specific proteins that serve to stabilize the chromophore, whereas the more stable ten-membered enediynes are found free from stabilizing proteins. Biosynthetic studies of both nine- and ten-membered enediynes have been carried out by feeding specifically labeled biosynthetic building blocks, such as acetate, to enediyne-producing organisms [4]. These labeling experiments demonstrated that both nine- and ten-membered enediyne chromophores are constructed of head-



**1** (R = OH)
**2** (R = H)

**3**

Structure of the Enediynes C-1027 (**1**) from *Streptomyces globisporus* and Calicheamicin $\gamma_1^I$ (**3**) from *Micromonospora echinospora* spp. *calichensis*

The enediyne core structures, shown in red, are biosynthesized from homologous polyketide synthase (PKS) genes in the two producing organisms. A different PKS gene is responsible for the synthesis of the orsellinic acid portion (pink) of calicheamicin. Other biosynthetic genes involved in elaborating the unusual sugars (green), the β-aminoacid unit (blue), and the benzoxazolinate group (yellow) were also identified. Disruption of a hydroxylase gene involved in the synthesis of the β-amino acid portion of C-1027 led to the isolation of the deshydroxy C-1027 analog **2**.

to-tail coupled acetate units. Such head-to-tail linking of acetate units could be explained by a biosynthetic scheme involving a precursor unsaturated fatty acid which is cleaved and cyclized to afford the enediyne chromophore [5]. Alternatively, a polyketide route to the enediyne chromophore can be postulated, in which a polyunsaturated intermediate is cyclized into the enediyne chromophore core. In the nine-membered enediynes, each of the two aceylene carbons are derived from an intact acetate unit. In contrast, the acetylene carbons in ten-membered enediynes arise from carbons of two different acetate building blocks. From this observation, it was expected that the biosynthetic routes to nine- and ten-membered enediynes would be different, for example with the nine-membered enediynes involving the fatty acid route and the ten-membered enediynes the polyketide route.

Shen and his group, working with the nine-membered ring enediyne C-1027-producing organism *Streptomyces globisporus* [6], and Farnet, Thorson, and coworkers, working with the ten-membered ring enediyne calicheamycin $\gamma_1^I$-producing organism *Micromonospora echinospora* spp. *calichensis* [7], have shown that both of these enediynes are produced through similar polyketide synthesis pathways. Based on the known propensity of secondary metabolite biosynthesis genes to cluster together along with genes conferring self-resistance, Shen and his group were able to locate genes involved in the biosynthesis of C-1027 that were near the gene encoding the specific protein that stabilizes the chromophore and provides self-resistance. They sequenced over 85 kilobases (kb) of DNA and by disrupting genes at either end were able to define the 5′ and 3′ ends of the entire 75 kb biosynthetic gene cluster. Shen's group found a single polyketide synthase (PKS) gene within the cluster, along with the expected genes required for the biosynthesis of the modified sugar, β-amino acid, and benzoxazolinate portions of the natural product. The *Streptomyces* enediyne PKS gene consists of six separate domains encoding ketoacetyl synthase, acyltransferase, acylcarrier protein, ketoreductase, dehydratase, and a carboxy-terminal region. This multidomain structure places it in the type I PKS family, but because it does not encode a separate domain for each biosynthetic step, the *Streptomyces* enediyne type I PKS must be employed in an iterative sense in order to construct the entire enediyne core.

Using a shotgun sequencing approach, the groups led by Farnet and Thornson sequenced the entire gene cluster for the biosynthesis of calicheamicin $\gamma_1^I$ in *Micromonospora*. The calicheamicin gene cluster contains two PKS genes, one gene for the biosynthesis of the orsellinic acid portion of the natural product and another gene for the enediyne core. Surprisingly, the enediyne PKS gene from *Micromonospora* and that from *Streptomyces* are highly homologous, with identical domain organization. In both the *Streptomyces* and *Micromonospora* gene clusters, there are additional genes that appear to be involved in the biosynthesis of the enediyne core. Based upon sequence similarity to other iterative type I PKS genes, Farnet and Thornson propose that the enediyne PKS leads to a common cyclododecylpolyene skeleton that is elaborated to either the nine- or ten-membered enediynes [7].

The extraordinary reactivity and cytotoxicity of the enediynes has sparked considerable interest in the pharmaceutical industry, yet the lack of tumor specificity of these compounds has complicated their use in cancer chemotherapy. One approach that has been successfully employed to increase the specificity of these agents is their conjugation to cancer cell-targeting antibodies [3]. Alternative approaches involve designing novel triggering mechanisms that may lead to enediynes that only undergo diradical cyclization in cancer cells [8]. A more recent approach involves the redesign of the enediyne core to provide both selective triggering and more dis-

criminating diradical intermediates [9]. The uncovering of the biosynthetic pathway to the enediynes opens up another route to improve upon these natural products by engineering new enediynes with the potential for increased selectivity. The finding that both nine- and ten-membered enediynes are biosynthesized through a common polyketide pathway indicates that it may be possible to bioengineer this pathway to produce completely novel enediynes [10]. However, the bioengineering of novel enediyne cores will require much more work to determine the way in which the enediyne PKS, together with other biosynthetic enzymes, work in concert to effect the construction of the enediyne chromophore.

In contrast, bioengineering of novel enediynes based upon manipulating the genes involved in the synthesis of groups attached to the enediyne core, such as the unusual sugars and aromatic groups, should proceed rapidly. In fact, Shen and coworkers report the preparation of a novel C-1027 analog by disrupting a specific hydroxylase gene involved in the biosyntheses of the β-amino acid portion of the natural product [6]. Interestingly, the resulting deshydroxy C-1027 (**2**, see Figure) analog is more stable than C-1027 itself. The availability of bioengineered enediynes will provide additional insights into these fascinating natural products and may lead to improved drugs to fight cancer.

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# Converting Solution Macromolecular Thermodynamic Properties into Gas-Phase Mass Spectrometry Observations

**Solution dissociation constants and changes in free energies associated with ligand binding to proteins have been measured in the gas phase using mass spectrometry.**

Can a protein, nucleic acid, or other macromolecular complex retain a memory of its solution state as solvent and counterions are removed in vacuo? At first glance, the notion seems improbable [1]. However, since the early 1990s, noncovalent complexes of proteins, nucleic acids, and their ligands have been moved intact from solution into the gas phase using electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). The solution characteristics of these complexes, such as their dissociation constant ($K_D$), have been measured in the gas phase using ion abundances from the various species, and these ESI-MS results are in good agreement with solution values over a wide range (nM to mM) [2–4]. Additional information on ligand stoichiometry, ligand binding sites, and activation energy for gas-phase dissociation is available directly or through gas-phase dissociation of ions from the complex or free macromolecule [5, 6].

The solution exchange rates of hydrogen atoms for deuterium (HDX) in peptides, proteins, and nucleic acids can be monitored using mass spectrometry [7–10]. Solvent-exposed amide hydrogen atoms will exchange with deuterons, and each incorporated deuteron increases the mass of the molecule by 1 Da, easily measured by modern mass spectrometers. The measured difference between the theoretical maximum incorporation and the experimental result quantitatively reflects the protection from exchange. Various experimental strategies (quenching, digestion, etc.) can be used with MS to measure HDX rates for surface amide residues that exchange rapidly or for slowly exchanging amides sequestered in the core of a protein [11]. MS HDX studies have been used to establish the folding of proteins, to observe changes in folding induced by various perturbations, and to map the locations of amides protected from exchange by ligand binding. MALDI-TOF mass spectrometry is well suited to measurement of HDX, since exchange is quenched by addition of the acidic matrix substrate.

Powell, Ghaemmaghami, and coworkers recently have described a general mass-spectrometry-based assay (called SUPREX) for quantitation of noncovalent complexes of proteins in solution [12]. Rather than vary the concentration of ligand and directly observe the ratio of free and bound protein, they have estimated the free energy of ligand binding and dissociation constant by