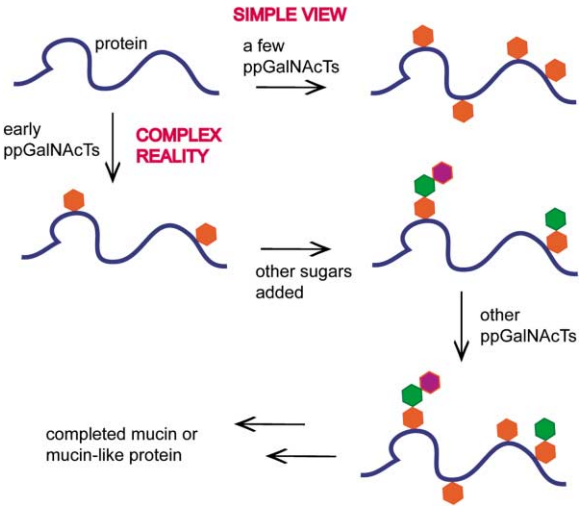

Figure 1. Proposed Mechanisms for the Modification of a Polypeptide by Isoforms of pGalNAcTs to Form Highly Glycosylated Proteins

used to design selective inhibitors of the enzymes involved in mucin biosynthesis. Selective inhibitors of each isoform of ppGalNAcT are valuable not only as research tools but as potential cancer therapeutics. Cell surface mucin expression is often altered in cancer cells and influences tumor metastasis [13]. In the March 2004 issue of *Chemistry & Biology*, the Bertozzi and Tabak groups reported screening of a 1338-member uridine-based library to find inhibitors of mouse ppGalNacTs [14]. Two compounds from the library could affect mucin-type *O*-glycosylation but not *N*-linked glycosylation in cells; however, the two library members did not show significant selectivity among ppGalNAcT isoforms. An understanding of the individual substrate specificities now provides the platform to build isoform-selective inhibitors of the enzymes that initiate carbohydrate chains on the mucin protein backbone.

Nicola Pohl
Department of Chemistry and
the Plant Sciences Institute
Gilman Hall
Iowa State University
Ames, Iowa 50011

Selected Reading

1. Pratt, M.R., Hang, H.C., Ten Hagen, K.G., Rarick, J., Gerken, T.A., Tabak, L.A., and Bertozzi, C.R. (2004). Chem. Biol. *11*, this issue, 1009–1016.
2. Gabius, H.-J., Siebert, H.-C., Andre, S., Jimenez-Barbero, J., and Ruediger, H. (2004). Chembiochem *5*, 740–764.
3. Davis, B.G. (2000). Chem. Ind. (London) 134–138.
4. Tong, L., Baskaran, G., Jones, M.B., Rhee, J.K., and Yarema, K.J. (2003). Biotechnol. Genet. Eng. Rev. *20*, 199–244.
5. Drinnan, N., and Ramsdale, T. (2003). In Molecular Pathomechanisms and New Trends in Drug Research, G. Keri and T. Istvan, eds. (London: Taylor & Francis), pp. 178–190.
6. Apostolopoulos, V., Plebanski, M., and McKenzie, I. (2003). In Immunobiology of Carbohydrates, S.Y.C. Wong and G. Arsequell, eds. (New York: Kluwer/Plenum), pp. 292–301.
7. Bertozzi, C.R., and Kiessling, L.L. (2001). Science *291*, 2357–2364.
8. Hassen, H., Bennett, E.P., Mandel, U., Hollingsworth, M.A., and Clausen, H. (2000). In Carbohydrates in Chemistry and Biology, Volume 3, B. Ernst, G.W. Hart, and P. Sinay, eds. (New York: Wiley-VCH), pp. 273–292.
9. Ten Hagen, K.G., Fritz, T.A., and Tabak, L.A. (2003). Glycobiology *13*, 1–16.
10. Hang, H.C., Yu, C., Pratt, M.R., and Bertozzi, C.R. (2004). J. Am. Chem. Soc. *126*, 6–7.
11. Ten Hagen, K.G., and Tran, D.T. (2002). J. Biol. Chem. *277*, 22616–22622.
12. Schwientek, T., Bennett, E.P., Flores, C., Thacker, J., Hollmann, M., Reis, C.A., Behrens, J., Mandel, U., Keck, B., Schafer, M.A., et al. (2002). J. Biol. Chem. *277*, 22623–22638.
13. McDermott, K.M., Crocker, P.R., Harris, A., Burdick, M.D., Hinoda, Y., Hayashi, T., Imai, K., and Hollingsworth, M.A. (2001). Int. J. Cancer *94*, 783–791.
14. Hang, H.C., Chong, Y., Ten Hagen, K.G., Tian, E., Winans, K.A., Tabak, L.A., and Bertozzi, C.R. (2004). Chem. Biol. *11*, 337–345.

Chemistry & Biology, Vol. 11, July, 2004, DOI 10.1016/j.chembiol.2004.07.003

Novel Mechanism for Priming Aromatic Polyketide Synthases

In this issue of *Chemistry & Biology*, a novel priming mechanism is proposed for aromatic polyketide biosynthesis, with an iterative type I polyketide synthase generating a starter unit primed for a type II polyketide synthase [6]. This novel priming system participates in hedamycin biosynthesis, a DNA alkylating agent.

Many natural products are bioactive, and polyketides constitute one of the most important families of natural products [1, 2]. Polyketides are widely distributed in plants, fungi, and bacteria and exhibit a wide range of biological activities of interest to the pharmaceutical industry (antibiotics, anticancer, antifungals, or immunosuppressive agents) and the agrochemical industry (insecticides or antiparasitic agents) [3]. From simply looking at the chemical structures of polyketides, there are no common structural features to suggest that these compounds all belong to the same family. However, when the various biosynthetic pathways for generating polyketide are considered, it is clear that they share a common thread: all polyketide biosynthesis involves the assembly of carbon chains from acyl precursors in a series of reactions catalyzed by a complex enzymatic system, the polyketide synthase (PKS). The reaction begins when PKS is primed by a starter molecule and then

continues with repetitive decarboxylative condensation of CoA analogs of simple carboxylic acids.

Based on their protein architecture, PKSs have been classified into three distinct families (type I, type II, and type III), but increasing evidence supports the view that PKSs have much greater diversity [4]. Type I PKSs, the modular PKSs, are large multifunctional polypeptides; examples are the erythromycin, rapamycin, or avermectin PKSs. Type II PKSs are multienzyme complexes of single proteins and usually work as iterative heterodimers resembling type II fatty acid synthases from bacteria and plants; the actinorhodin, tetracenomycin C, and granaticin are classical examples of this subfamily. Finally, a more recently discovered subfamily in bacteria, the type III PKSs, are iterative homodimers; a type III PKs is involved in the biosynthesis of the diffusible red-brown pigment produced by the erythromycin producer.

Aromatic polyketides are usually synthesized by type II PKSs. In most cases, biosynthesis of bacterial aromatic polyketides is initiated by priming acetate as starter unit, but some aromatic PKSs can also use other starter units such as benzoate, salicylate, malonamate, or short-chain fatty acids [5]. Although the mechanism of attachment of starter units is not well understood, one can speculate that activation and transfer of these nonacetate starter units is mediated by CoA ligases or a ketosynthase (KSIII) and an acyltransferase.

In the July issue of *Chemistry & Biology*, Jon Thorson and colleagues [6] report an alternative priming system for the biosynthesis of an aromatic polyketide. The pluramycin family of antitumor antibiotics was first discovered more than 50 years ago by Umezawa and coworkers and was found to have antimicrobial and anticancer activity [7]. For many years, pluramycins remained chemical evolutionary oddities composed of chemical features borrowed from diverse groups of natural products. These antibiotics are structurally complex DNA-reactive agents consisting of a planar 4-*H*-anthra(1,2-b)pyran chromophore with either carbohydrate or epoxide moieties attached at the corner of the planar chromophores, depending on the pluramycin type. The pluramycin family has the ability to intercalate into the DNA molecule and interact with both grooves of the DNA helix. Furthermore, those pluramycins displaying epoxides also have the ability to cause DNA alkylation [8].

Thorson and colleagues isolated and characterized the first biosynthetic gene cluster for a pluramycin-type compound, hedamycin (Figure 1). A region of approximately 45 kb from the chromosome of the hedamycin producer *Streptomyces griseoruber* ATCC15422 was cloned and sequenced, and 32 open reading frames were identified, many of which the authors suggest have a role in hedamycin biosynthesis. Sequence analysis of the gene cluster showed the presence of the classical type II PKS components: a KS_{α}-KS_{β} heterodimer and an ACP but also a second catalytic ketosynthase (KSIII) and an AT, usually an indication of nonacetate priming in PKSs. Two genes were also found that encode modules of a type I PKS. The authors propose that one of these genes (HedT) could act as a loading domain, while the other (HedU) could act iteratively to elongate one acetyl-CoA starter unit with two malonyl-CoA extender units, thus synthesizing the unusual hexenoate starter until after several ketoreduction and dehydration steps.

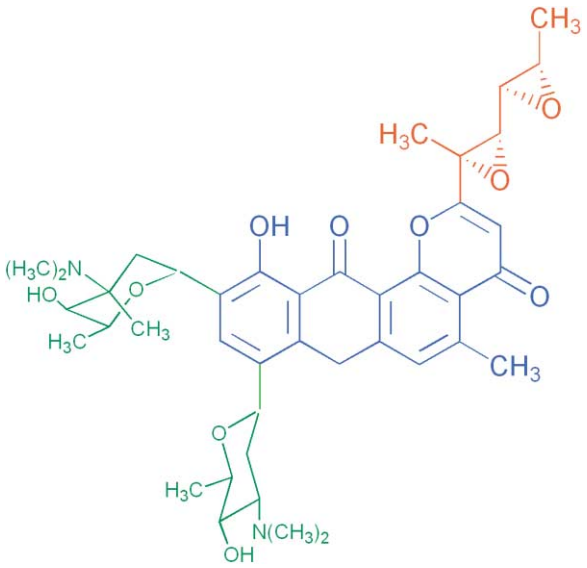

Figure 1. Structure of Hedamycin

Red, hexenoate starter unit; blue, aromatic polyketide moiety; green, deoxysugars.

Iterative usage of a type I PKS (here to synthesize the starter unit) goes against the dogma for these multidomain enzyme complexes. However, over the past few years, evidence has accumulated to support the iterative use of single modules of type I PKSs. This process, known as "stuttering," occurs with some PKSs as an aberrant process producing low levels of side products [9, 10], but some type I PKSs have probably evolved to repeatedly use some modules during polyketide chain extension as a programmed event [11–15]. Consequently, there is great interest in the Thorson groups' observation of an iterative type I PKS initiating biosynthesis of the polyketide chain. Their report provides the first description of this novel mechanism, which uses a type I PKS acting iteratively for the generation of the starter unit that subsequently primes the type II PKS system.

Another notable structural peculiarity of hedamycin is the *C*-glycosidic attachment of two different sugars to the aglycon. The glycosyltransferases responsible for the sugar transfer have been identified within the hedamycin cluster. Most bioactive compounds are glycosylated through *O*-glycosidic linkages; *C*-glycosidation is found less frequently in natural products. The unique regio- and stereoselectivity of the two identified hedamycin glycosyltransferases make them especially good candidates for engineering novel *C*-glycosides through combinatorial biosynthesis.

José A. Salas
Departamento de Biologia Funcional e Instituto Universitario de Oncología del Principado de Asturias (I.U.O.P.A)
Universidad de Oviedo
33006 Oviedo
Spain

Selected Reading

1. O'Hagan. D. (1991). The Polyketide Metabolites (Chichester, UK: Ellis Horwood).
2. Staunton, J., and Weissmann, K.J. (2001). Nat. Prod. Rep. *18*, 380–416.
3. Hopwood, D.A. (1997). Chem. Rev. *97*, 2465–2497.
4. Shen, B. (2003). Curr. Opin. Chem. Biol. *7*, 285–295.
5. Moore, B.S., and Hertweck, C. (2002). Nat. Prod. Rep. *19*, 70–99.
6. Bililign, T., Hyun, C.-G., Williams, J.S., Czisny, A.M., and Thorson, J.S. (2004). Chem. Biol. *11*, this issue, 959–969.
7. Maeda, K., Takeuchi, T., Nitta, K., Yagishita, K., Utahara, R., Osato, T., Ueda, M., Kondo, S., Okami, Y., and Umezawa, H. (1956). J. Antibiot. (Tokyo) *9*, 75–81.
8. Hansen, M.R., and Hurley, L.H. (1996). Acc. Chem. Res. *29*, 249–258.
9. Wilkinson, B., Foster, G., Rudd, B.A., Taylor, N.L., Blackaby, A.P., Sidebottom, P.J., Cooper, D.J., Dawson, M.J., Buss, A.D., Gaisser, S., et al. (2000). Chem. Biol. *7*, 111–117.
10. Hardt, I.H., Steinmetz, H., Gerth, K., Sasse, F., Reichenbach, H., and Höfle, G. (2001). J. Nat. Prod. *64*, 847–856.
11. Gaitatzis, N., Silakowski, B., Kunze, B., Nordsiek, G., Blöcker, H., Höfle, G., and Müller, R. (2002). J. Biol. Chem. *277*, 13082–13090.
12. Mochizuki, S., Hiratsu, K., Suwa, M., Ishii, T., Sugino, F., Yamada, K., and Kinashi, H. (2003). Mol. Microbiol. *48*, 1501–1510.
13. Olano, C., Wilkinson, B., Moss, S.J., Braña, A.F., Méndez, C., Leadlay, P.F., and Salas, J.A. (2003). Chem. Commun. (Camb.) *21*, 2780–2782.
14. He, J., and Hertweck, C. (2003). Chem. Biol. *10*, 1225–1232.
15. Olano, C., Wilkinson, B., Sanchez, C., Moss, S.J., Sheridan, R., Math, V., Weston, A.J., Braña, A.F., Martin, C.J., Oliynyk, M., et al. (2004). Chem. Biol. *11*, 87–97.

Chemistry & Biology, Vol. 11, July, 2004, DOI 10.1016/j.chembiol.2004.07.002

Ribozyme Diagnostics Comes of Age

Biosensing ribozymes could soon be used to diagnose viral infection. The Kossen group from Sirna Therapeutics have developed a sensitive, high-throughput means of screening for hepatitis C virus, using their target activated half-ribozyme technology, as reported in the June issue of *Chemistry & Biology* [1].

A little over two decades ago, the first demonstrations of catalytic RNAs (ribozymes) were reported in the literature [2, 3]. These ribozymes were the cleavage-ligation catalyzing Tetrahymena ribosomal RNA intron [3] and the RNA component of the endoribonuclease RNase P, which catalyzes the site-specific cleavage of the 5′ precursor segment of transfer RNAs [2]. Subsequent to these important findings, several other smaller, naturally occurring ribozymes with RNA cleavage and ligation activities have been described [4], and most recently the RNA component of the large ribosomal subunit has been identified as the ribosomal transpeptidase [5]. The spectrum of the catalytic capabilities of RNA was greatly enhanced following the development of techniques for in vitro evolution of RNAs with new capabilities ranging from ATP hydrolysis to polymer biosynthesis [6]. An important development for evolving biologically and chemically useful ribozyme functions is the addition of allosteric activation functions to ribozymes, which can be mediated by binding of a variety of ligands ranging from small organic molecules through proteins or nucleic acid oligomers [7, 8]. Allosteric activation is the key to generating biosensing nucleic acids that can be used to monitor any of a number of biological or chemical processes. Ribo-reporters could in essence become inexpensive replacements for antibodies and other methods currently in use for diagnostic testing.

Last year, a group from Sirna Therapeutics reported the development of a target activated ribozyme capable of detecting zeptomole (10^{-21}M) quantities of hepatitis C viral RNA in solution [9]. The key to such sensitive detection properties is that the ribozyme component parts have absolutely no activity in the absence of the cognate substrate, which differs from previously published allosteric ribozymes whose rates of activity are dictated by the rate differences of ribozyme function in the absence and presence of substrate or allosteric modifier. The Sirna group developed a "half-ribozyme" approach in which the target RNA itself serves to complete the ribozyme structure. In this ribozyme reaction scheme, the multiple turnover class 1 ligase motif developed by Bartel and colleagues [10] was truncated to form a bimolecular substrate RNA, which interacts with the target sequence to allow ligation of one of the substrate RNAs containing a 3′ cis-diol with a 5′ triphosphate containing substrate RNA oligo (Figure 1). This half-ribozyme has no ligase activity in the absence of target RNA and displayed an observed rate increase of 4,000,000-fold when bound to the target.

This exquisitely sensitive ribozyme reporter has been further analyzed for detection of HCV sequences and high-throughput assays, as reported in last month's *Chemistry & Biology* by Kossen and coworkers [1]. In this study, naturally occurring variants of HCV that contain mismatched pairings to the half-ribozyme were analyzed for their effects on ribozyme-mediated ligation of the substrate RNAs. By extending the base pairing of the ribozyme to target the mismatched sequences, these were accommodated without significant kinetic impairment of target-dependent ligation rates. The variant HCV sequences represent greater than 80% of the GenBank HCV 5′UTR entries, with one of the sequences representing 66%. Thus, the half-ribozyme assay can be generally applied to the majority of HCV clinical samples.

In order to make this assay suitable for clinical applications, the Sirna investigators collaborated with investigators from Thermo Electron, Corp., Point of Care and