

to the cell type. The cells' individual identity is robustly maintained thereafter by heritable (but potentially reversible) chemical modifications both to the genes themselves and to proteins that are bound to the DNA. These modifications are referred to as 'epigenetic' because they regulate the all-important genetic networks without altering the actual genetic constitution of the cells.

So how did Takahashi and Yamanaka break through these formidable barriers that maintain the differentiated state of specialized cells and convert the cells into reprogrammed ES-like cells? Essentially, their approach was to initiate a new transcriptional network. To do this, they isolated 24 genes that are apparently necessary for the unique properties of ES cells, and introduced extra copies of all these genes together into cultured fetal and adult mouse cells. A very small fraction of cells showed ES-like characteristics. Remarkably, the authors eventually found that only four genes — *Oct3/4*, *Sox2*, *c-Myc* and *Klf4* — are sufficient for this conversion (Fig. 1). The first two are well known as genes that encode gene-regulatory proteins that are necessary to maintain ES-like characteristics<sup>3,4</sup>. The *c-Myc* and *Klf4* proteins support the self-renewal of ES cells<sup>4</sup>, and *Klf4* also augments the levels of *Oct4* (ref. 5).

One key gene-regulatory protein — Nanog — is notably absent from the list. Nanog can enhance reprogramming<sup>6</sup>, and, together with *Oct3/4* and *Sox2*, constitutes the core set of gene-regulatory factors in ES cells<sup>3,4</sup>. Nanog was detected in most of the reprogrammed cells, however, and so the introduced *Oct3/4* and *Sox2* may have stimulated its activity in these cells<sup>7</sup>. Indeed, the combined effects of the four genes identified must induce extensive changes in the overall transcriptional network in the cells.

A detailed 'fingerprint' of the gene expression pattern in reprogrammed cells confirms similarities between them and the ES cells, but significant deviations are also evident<sup>1</sup>. Nevertheless, the reprogrammed cells display the crucial capacity for self-renewal and differentiation into many types of tissue that is characteristic of ES cells (Fig. 1). Furthermore, when introduced into a host embryo, the reprogrammed cells in the resulting chimaeric fetuses differentiate into many types of tissue. Unlike the case with ES cells, however, none of the chimaeras developed to adulthood, which suggests imperfections in the reprogramming. Notably, the target cells' own copies of the *Oct3/4* and *Sox2* genes were not significantly expressed after the reprogramming, and the cells instead relied on the activity of the newly introduced genes to maintain their ES-cell properties. Moreover, the cells' own *Oct3/4* gene showed significant DNA methylation<sup>1</sup>, which is an epigenetic hallmark of an inactive gene and unlike the situation in ES cells. The epigenetic modifications in the reprogrammed cells thus seem to differ somewhat from

those in ES cells, which have their own unique epigenetic characteristics essential for their properties and plasticity<sup>8,9</sup>.

So what of the future? It seems that there are at least two major requirements for efficient reprogramming of adult cells. One of these is clearly to kick-start a new transcriptional network through the introduction of crucial factors such as *Oct3/4* and *Sox2*. But before this, erasing at least some of the existing epigenetic modifications from the specialized cells<sup>10</sup> might make it easier to set up the required changes in the transcriptional network. If this erasure could be achieved, it might make the procedure more efficient, such that the introduced genes might be needed only transiently to achieve full reprogramming of adult cells.

In principle, the technique devised by Takahashi and Yamanaka<sup>1</sup> should work with human cells because the transcriptional factors needed to maintain human ES cells are apparently similar to those in mice<sup>3,4</sup>. The environmental factors and cues needed for the derivation of mouse and human ES cells are different, however, and their role during cell reprogramming has not been fully worked out. But if successful, the procedure could eventually be used to generate reprogrammed stem-like cells from adult human body cells for use in therapy. Reprogrammed cells from patients with complex diseases, such

as diabetes, would also be a valuable resource for research. They could be used to generate differentiated tissues to see how different mutations and genetic backgrounds influence the progression of the disease — information that might lead to prevention or a cure. Before this can happen, however, we must address the gaps in our knowledge, so that we can derive fully reprogrammed cells efficiently. This approach may eventually eliminate the need to use early embryos for deriving stem cells — an enticing objective, but one that will require extensive research on both mouse and human ES cells. ■

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## CHEMICAL BIOLOGY

# A sweet exchange

Christopher T. Walsh

**Many drugs isolated from microorganisms have complex molecular structures, making it difficult for chemists to modify them. But it seems that enzymes can provide a short cut to drug variants.**

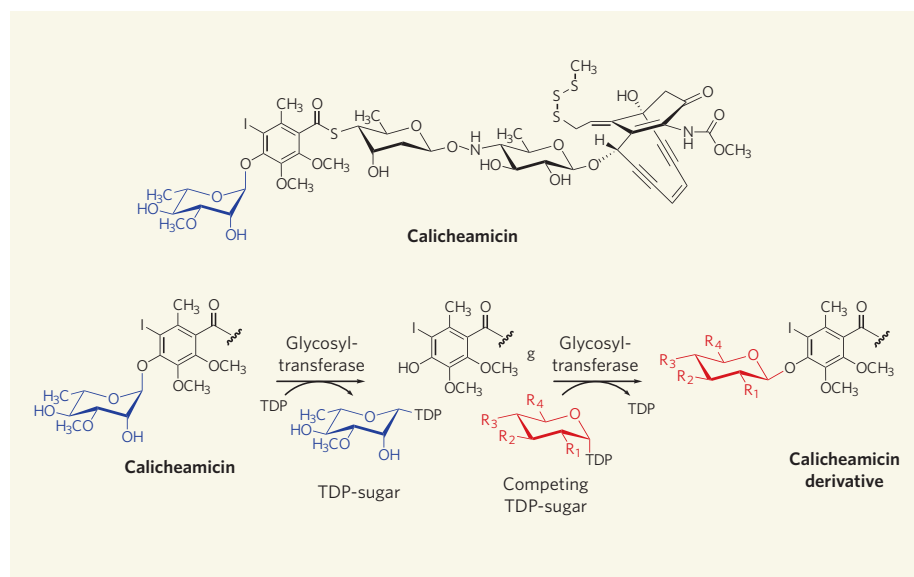
Naturally occurring compounds — referred to as natural products by chemists — are excellent starting points for making new medicines. Many drugs hail from natural products isolated from microorganisms: for example, the erythromycin and vancomycin classes of antibiotic, the daunomycin and adriamycin classes of anticancer drug, and the so-called 'enediynes warheads' that have been attached to therapeutic antibodies, which are also used in anticancer therapy.

Nature makes these molecules biologically active by building in conformational constraints, which set specific architectures into the molecular 'scaffolds'. The peripheries of the scaffolds are then decorated with chemical groups that are recognized by biological receptors. Dedicated tailoring enzymes modify the nascent natural-product scaffolds at distinct locations, bringing in methyl groups, acyl groups and a range of unusual sugars that become key recognition elements for biological targets. Chemists dearly want to introduce

these rare sugars into natural-product scaffolds at will, varying the point of attachment in the hope of making improved drugs, but making these sugars is extremely difficult. This problem may now be solved, as Zhang *et al.* report in *Science*<sup>1</sup> that sugars can be enzymatically harvested from natural products and readily swapped between scaffolds.

The sugars attached to natural products serve many functions. Because they contain hydroxyl (OH) groups, sugars are hydrophilic, so their incorporation into a natural product can make the overall molecule water-soluble. The arrays of hydroxyl groups might also act as hydrogen-bonding elements for specific interactions with biological macromolecules<sup>2</sup>.

The appended sugars can be widespread in the natural world — for example glucose and mannose, which belong to a family of sugars known as D-hexoses. But most often they are specialized, such as L-vancosamine in vancomycin, desosamine in erythromycin or L-daunosamine in daunomycin. These sugars



**Figure 1 | Enzymatic swap of sugars between antibiotic scaffolds.** As reported by Zhang *et al.*<sup>1</sup>, a glycosyltransferase (GT) enzyme catalyses the removal of a sugar (blue) from a molecule of the anticancer drug calicheamicin, in the presence of deoxythymidine diphosphate (TDP), producing a TDP-sugar as a side-product; R<sub>1</sub>–R<sub>4</sub> represent general chemical groups. If this reaction is carried out in the presence of a competing TDP-sugar, the GT will attach that sugar (red) to the drug scaffold, effecting a net sugar-exchange reaction. In this way, variants of calicheamicin are produced that would be extremely difficult to make by chemical synthesis.

differ from D-hexoses by lacking a particular hydroxyl group (deoxyhexoses) or by lacking a hydroxyl and gaining an amine (NH<sub>2</sub>) group (aminodeoxyhexoses). The variations in chemical structure create a variable hydrophilic or hydrophobic balance in the resulting sugar, providing a unique chemical code that can be read by target proteins.

The enzymes that synthesize these specialized sugars are encoded by DNA that lies close to the gene clusters that encode the scaffold-producing enzymes. This proximity allows sugar synthesis to be regulated so that the sugars are produced only when the scaffolds are ready for them. D-Hexoses provide the raw material from which the rare sugars are prepared. The hexoses are converted into reactive intermediates, known as deoxythymidine diphosphohexoses (TDP-sugars), which are then acted on by dedicated glycosyltransferase (GT) enzymes. The GTs transfer sugars in the form of TDP-hexoses to specific sites in the natural-product scaffold, so creating an active final product. For example, in the biogenesis of vancomycin, six enzymes are needed to convert TDP-glucose to TDP-vancosamine, whereupon two GTs attach the sugar to the vancomycin scaffold. Frustratingly for drug developers, vancosamine, like desosamine in erythromycin and daunosamine in daunomycin, is not readily available by synthetic routes<sup>3,4</sup>.

It had long been assumed that GT-catalysed reactions are irreversible: TDP-sugars react with a scaffold to yield an end-product, with TDP produced as a side-product. But Zhang *et al.*<sup>1</sup> have serendipitously discovered reversible reactions with a GT known as CalG1. While using this enzyme to modify the core

of the antitumour agent calicheamicin, they found that an appended sugar within the drug could be transferred back to TDP. When they attempted this transfer in the presence of a particular TDP-deoxyglucose, the sugar in calicheamicin was replaced with that deoxyglucose: a net sugar-exchange reaction had occurred (Fig. 1). This is an unexpected and extremely useful observation.

Zhang *et al.*<sup>1</sup> found that a variety of hexoses may be installed on the calicheamicin scaffold using this GT-mediated sugar exchange. They then extended the process by harvesting exotic sugars from other natural products and appending them to modified calicheamicin scaffolds in one reaction. In this way, they prepared a library of 70 calicheamicin analogues. Creating a library of this size by traditional chemical synthesis would be extremely time-consuming. Even more impressively, Zhang *et al.*<sup>1</sup> identified a second enzyme, CalG4, which acts at a different part of the calicheamicin scaffold; CalG4 removes a different sugar (an aminopentose) from this position in the scaffold, and also catalyses sugar-exchange reactions. Combinations of sugar-exchange reactions using CalG1 and CalG4 could presumably give libraries with high structural diversity, by mixing pairs of hexoses and aminopentoses on the calicheamicin core.

Perhaps most encouragingly, the authors<sup>1</sup> expanded the scope of the sugar-exchange reactions by using GTs associated with other natural products. The enzyme GtfE decorates the scaffold of the antibiotic vancomycin with D-glucose. Incubations of GtfE and CalG1 together in the presence of TDP allow the first enzyme to remove a modified glucose

(azidoglucose) from a variant of vancomycin, transiently generating TDP-azidoglucose. This short-lived intermediate is then used by CalG1 to transfer azidoglucose to a calicheamicin scaffold with a good overall yield (albeit on a very small reaction scale of 5 nmol of calicheamicin). This constitutes a simple enzymatic swap of sugars between completely different natural products. It is early days, but if these reactions can be run on a larger scale, GT-mediated sugar exchange might provide a short cut for shuffling biologically active sugars around natural-product scaffolds. This could be useful for finding drug molecules for many therapeutic areas.

Evaluating the generality and utility of this approach still presents several challenges. The first is to test a larger set of GTs as catalysts for sugar-exchange reactions. More than a hundred microbial GTs are noted in genome databases. If many of these enzymes can indeed be used in this way, a door opens to a mix-and-match strategy for creating new sugar forms of therapeutic natural products.

A second challenge is to obtain a ready supply of the rare deoxyhexose and aminodeoxyhexose sugars that are required for running the reactions. These sugars can only be obtained from their parent natural products, which are invariably generated from large-scale fermentations of producer microorganisms. Most of these natural products are not routinely available, but if Zhang and colleagues' method is widely adopted, this might put a premium on fermentation scale-ups.

Many enzymes exist that decorate natural products with chemical units such as methyl groups, acyl groups and occasionally phosphoryl groups<sup>5</sup>. These enzymes should also be examined for the purpose of transferring chemical groups between natural products. For example, the net transfer of acyl groups between natural-product scaffolds by acyltransferase enzymes would, in combination with sugar swaps, produce building-blocks for libraries of molecules with potential therapeutic activities.

By following up a chance observation of unexpected enzyme activity, Zhang *et al.* have developed a tool for selectively modifying exceedingly complex molecules. If this is a truly general process, then the long-held dreams of many carbohydrate chemists have been surpassed. ■

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