specificity for the α-fucosynthases. Using β-L-fucosyl azide as the donor, the SsD242S mutant was able to take 16 mono- and disaccharides (so far tested) as acceptors for transglycosylation. All the reactions proceeded in an absolute stereo-specific control, leading to the formation of α-linked fucoside derivatives. The regio-selectivity seemed to be dictated by the structures of the acceptor substrates. Thus, various fucose-containing oligosaccharides with α-1,2-, α-1,3-, α-1,4- and/or α-1,6-fucosylated linkages could be formed, depending on the acceptors (Figure 2). Another interesting feature of the fucosynthase-catalyzed transglycosylation is the formation of branched trisaccharides. Notably, nonlinear trisaccharides were observed in the fucosynthase-catalyzed reactions, implicating a novel acceptor recognition mode for the new α-fucosynthases. It should be pointed out that the ability of the new α-fucosynthases to use the stable β-fucosyl azide for transglycosylation is of particular importance for practical synthesis. A recently reported α-1,2-fucosynthase that was evolved to use the labile β-fucosyl fluoride could only give a very low (6%) yield, because of the spontaneous hydrolysis of the β-fucosyl fluoride during transglycosylation (Wada et al., 2008). The discovery of the new α-fucosynthases thus opens a new avenue to the practical synthesis of biologically interesting fucose-containing oligosaccharides.

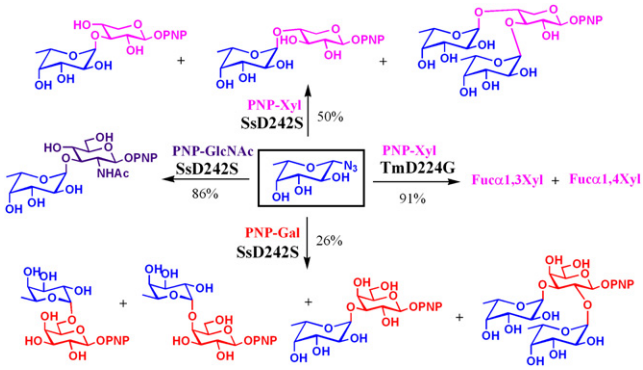

Figure 2. Synthesis of Fucose-Containing Oligosaccharides by Fucosynthase-Catalyzed Transglycosylation

In summary, the new α-fucosynthases described in this study constitute the first α-fucosynthases derived from retaining α-fucosidases. The remarkable acceptor substrate promiscuity of these new α-fucosynthases provides a unique opportunity to discover various new α-fucosynthases with tailored specificity and/or enhanced activity by directed evolution, as exemplified by a recent report for evolving endo-glycoceramidase (Hancock et al., 2009). Most importantly, the new α-fucosynthases represent the first α-glycosynthases that can use β-glycosyl azide as donor substrate for transglycosylation. It is likely that other glycosynthases can be evolved to take corresponding glycosyl azides for transglycosylation, which clearly indicates an exciting direction to expand the repertoire of glycosynthases.

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Tools to Tackle Protein Acetylation

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In the recent issue of *Molecular Cell*, Neumann et al. dissect the effect of H3K56 acetylation on chromatin structure using a novel method for generation of acetylated proteins. This is a valuable addition to the toolkit for those interested in unraveling how posttranslational modifications regulate protein function.

In the nucleus of eukaryotic cells, DNA is tightly associated with proteins forming a structure known as chromatin. These protein/DNA associations allow high compaction of the DNA, and e.g., at the same time, ensure its accessibility for the transcription and replication machinery. The building blocks of chromatin are nucleosomes, which consist of a protein component, built as an octamer of four core histones (H2A, H2B, H3 and H4), around which ~147 base pairs of DNA are

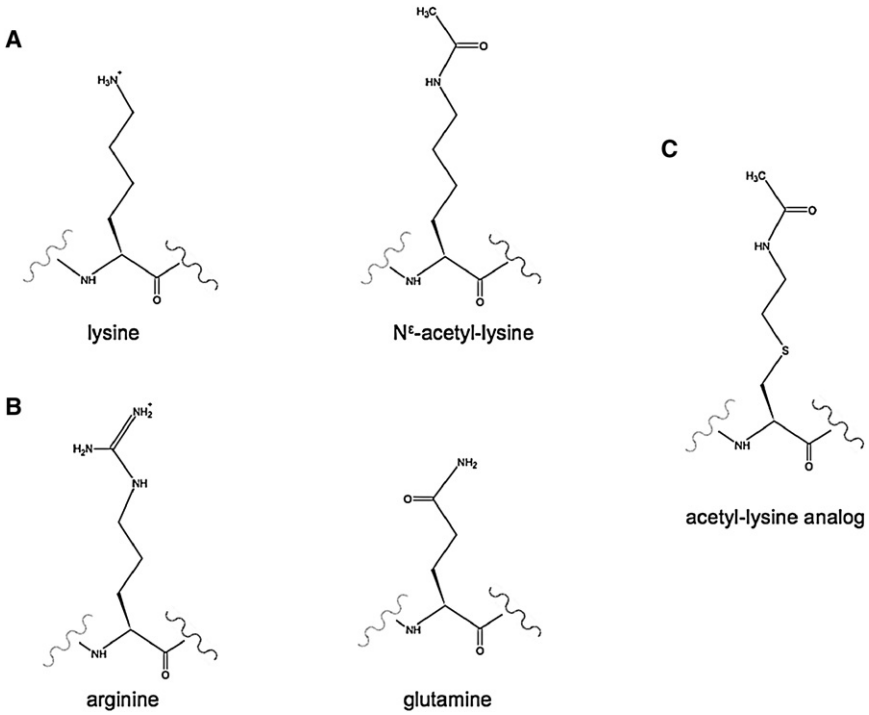

Figure 1. Mimicking Lysine Acetylation
(A) Structure of lysine and N^{ε}-acetyl-lysine.
(B) Structure of arginine and glutamine, amino acids used to mimic the unmodified and acetylated lysine, respectively.
(C) Structure of acetyl-lysine analog (Guo et al., 2008).

wrapped. Histones are subject to several covalent posttranslational modifications, including methylation, phosphorylation, and acetylation (Kouzarides, 2007). Modifications of histones, sometimes referred to as a "histone code," have been extensively studied and can therefore serve as an excellent model to address how posttranslational modifications govern protein function.

Acetylation of lysine residues in histones has been found to be involved in the regulation of all DNA dependent processes. In addition to histones, many other proteins are modified via lysine acetylation; a recent mass spectrometry screen has identified 3600 acetylation sites on 1750 proteins (Choudhary et al., 2009). There are two main mechanisms proposed for the function of lysine acetylation: (i) it neutralizes the basic charge of the lysine, which can lead to changes in protein structure and/or interactions; and (ii) it can serve as a signature to recruit "effectors" that specifically recognize the acetylated protein, such as chromatin-remodeling enzymes.

Proteins containing acetyl-lysine at defined sites are important tools needed to unravel the functions of lysine acetylation. Unfortunately, such proteins have been very difficult to obtain thus far. One way to produce them is by using lysine (K) acetyltransferases (KATs) to acetylate recombinant proteins; however this approach has several pitfalls. First, the KAT for the given modification needs to be known; second, it is difficult to control the degree of modification as the enzymatic reaction is hardly ever complete; and third, most KATs are not specific for a single site.

Many researchers have chosen a different strategy that uses an acetyl-lysine mimic by substitution of the modified lysine by a glutamine, often using simultaneously a lysine-to-arginine substitution as a mimic of the unmodified state. While this method is widespread due to its simplicity and the possibility to apply both in vivo and in vitro, the acetylation mimic is far from perfect because of differences in the structure of glutamine and N^{ε}-acetyl-lysine (Figures 1A and 1B).

To overcome these limitations, native chemical ligation has been applied to produce acetylated proteins (He et al., 2003). In this strategy, a peptide containing a modified amino acid is chemically synthesized and then ligated to the remainder of the protein of interest, which is recombinantly produced containing an engineered N-terminal cysteine residue. This approach results in a homogenously modified protein and has been proven useful in multiple studies, such as the one performed by Shogren-Knaak et al. (2006) that demonstrated the role of acetylation of histone H4K16 in chromatin structure. However, chemical ligation has some drawbacks as well: it is difficult to introduce modifications located in the central part of proteins, and it requires advanced chemical skills.

Another breakthrough came with the introduction of analogs of modified lysines. Two groups have reported methods to chemically generate methyl-lysine analogs based on the cysteine thiol group reactivity (Simon et al., 2007; Bernardes et al., 2008). While an acetyl-lysine analog could, in principle, also be generated on the cysteine platform, to our knowledge this has not been reported yet. Recently, Guo et al. (2008) have succeeded in creating an acetyl-lysine analog starting with phenylselenocysteine. The main advantage of these strategies is that the analog can be incorporated at any site in the protein, and this approach has been already applied successfully in several studies. However, the replacement of the γ-carbon of the lysine with a sulfur (Figure 1C) can change the properties of the side chain, because the C-S bond is slightly longer than the C-C bond, has a slightly smaller bond angle around that center, has two lone pairs instead of two hydrogen substituents, and reduces the pK_a of the amine by 1.1 units.

Last year, Neumann et al. (2008) published an innovative strategy to generate acetylated proteins by genetically encoding acetyl-lysine. In their inspiring work, they demonstrated the site-specific incorporation of acetyl-lysine in recombinant proteins produced in *Escherichia coli* by an orthogonal N^{ε}-acetyllysyl-tRNA synthetase/$tRNA_{CUA}$ pair. This pair was derived from *Methanosarcina barkeri* MS pyrrolysyl-tRNA synthetase and its cognate amber suppressor tRNA introduced into *E. coli*, and subjected to several rounds of selection for incorporation of acetyl-lysine in response to the amber codon. This technology represents a straight-forward method to generate high amounts of native

proteins acetylated at any chosen site. The only limitation at the current state might be the difficulty to obtain proteins acetylated at multiple sites since the stop codon suppression by $tRNA_{CUA}$ can be incomplete, resulting in truncated proteins. Recently, the same group succeeded in producing lysine methylated proteins using an analogous system, thus also providing this new tool now to the methylation community (Nguyen et al., 2009).

In the current issue of *Molecular Cell*, Neumann and colleagues (2009) go an important step further and report a proof-of-principle application of their new method to investigate the effects of histone H3 acetylation on K56 (H3K56ac) on chromatin structure and compaction. H3K56ac is a modification conserved from yeast to humans, localized in the globular core of H3. It has been shown that H3K56ac is involved in DNA repair and replication, chromatin assembly, and regulation of transcription (reviewed in Downs (2008)). Because H3K56 is in close contact to the DNA at the entry-exit point of the nucleosome, the concept that its acetylation affects chromatin compaction has been previously made in the literature. Now, using their elegant approach, Neumann et al. (2009) reconstituted "designer" octamers, nucleosomes, and nucleosomal arrays specifically acetylated at H3K56. They employed then single molecule FRET (fluorescence resonance energy transfer), sedimentation velocity analysis, and mononucleosome repositioning assays to show that, surprisingly, H3K56ac has no direct effect on the compaction of chromatin. However, they found that it increases DNA breathing at the entry-exit point of the nucleosome, slightly changing the nucleosome structure. Interestingly, while H3K56ac did not enhance the binding of nucleosomes to the chromatin remodellers SWI/SNF, RSC or Brdf1, it did have a slight positive effect on nucleosome redistribution by RSC and SWI/SNF. Taken together, this work demonstrates, using histones as a model, that the new method has the potential to revise prevailing dogmas regarding the effects of lysine acetylation.

The novel technology will have a vast variety of applications. It will undoubtedly advance the histone acetylation field by allowing in vitro assays such as chromatin compaction, remodeling, and binding partner studies. However, we should not forget that there are 1750 or more acetylated nonhistone proteins in the cell (Choudhary et al., 2009), which likewise await functional characterization.

Orthogonal aminoacyl-tRNA synthetase/tRNA pairs have also been developed in *Saccharomyces cerevisiae* (Chin et al., 2003) and mammals (Sakamoto et al., 2002), which, in principle, could allow to genetically encode lysine modifications in eukaryotes in vivo. Thus, in the future, the method developed by Chin and colleagues can provide us with straightforward synthetic biology tools to unravel the molecular mechanisms and functions of a wide range of protein post-translational modifications in vitro and in vivo.

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