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SYMPOSIUM

Exploration of the Central Dogma at the Interface of Chemistry and Biology

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Ever since the term "central dogma" was coined in 1958, researchers have sought to control information flow from nucleic acids to proteins. Talks delivered by Drs. Anna Pyle and Hiroaki Suga at this year's Chemical Biology Symposium at Yale in May 2010 applauded recent advances in this area, at the interface between chemistry and biology.

The term "central dogma," coined by Dr. Francis Crick in 1958, refers to information flow from DNA to messenger RNA (mRNA†) via transcription, followed by mRNA translation into protein. The 13th annual Chemical Biology Symposium at Yale University in May 2010 highlighted recent progress in the field, enriching our understanding of the central dogma and paving the way for future research at the interface between chemistry and biology. Two exciting talks at the symposium — one by Dr. Anna Pyle (Yale University) on self-splicing group II intron RNA and the other by Dr. Hiroaki Suga (University of Tokyo) on non-natural peptide synthesis — extended our understanding of this dogma.

Most eukaryotic genes are interrupted by large, non-coding segments known as introns. Introns are transcribed along with coding regions but are spliced out before protein synthesis begins. The task of removing these non-coding sequences from precursor RNA falls primarily to a large complex of proteins and RNA known as the spliceosome. Self-splicing introns, however, can release themselves from the RNA molecule without the assistance of the spliceosome. Once removed, these introns have the potential to reinsert into the

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†Abbreviations: mRNA, messenger RNA; NAIM/NAIS, Nucleotide Analog Interference Mapping/Suppression; tRNA, transfer RNA; DBE, dinitrobenzyle; ARSs, aminoacyl-tRNA synthetases; mF^{lac}, p-methoxyphenyllactic acid; C-P-H^{OG}, cysteine-protein dipeptide sequence followed by a glycolic acid sequence.

genome, altering its structure and organization. By self-cutting and pasting, these "genetic parasites," as Dr. Pyle called them, may not only facilitate evolutionary change over time but also may emerge as promising gene therapy vectors in the future.

To decipher the three-dimensional molecular architecture of group II introns as well as their catalytic mechanism, Dr. Pyle's team conducted detailed biochemical studies over many years, including site-specific photocross-linking and Nucleotide Analog Interference Mapping/Suppression (NAIM/NAIS) [1,2]. The group accumulated an enormous amount of data regarding how the RNA is likely structured, including the interaction network among domains, a map of atoms critical for splicing, and the binding positions of metal ions in the active sites. Finally, Dr. Pyle's team successfully crystallized the first group II intron, purified from *Oceanobacillus iheyensis* [3], an alkaliphilic eubacterium inhabiting seabed mud at a depth of 1 km. The crystal structure of the *O. iheyensis* intron reveals an elaborate interaction network fully consistent with decades of biochemical and genetic studies. Distant domains on the molecule are interdigitated by tertiary structural elements, including a z-anchor, a ribose zipper, a major groove triple helix, and an α-α' kissing loop. Additionally, two magnesium ions sit inside the intron's catalytic center in a configuration consistent with a two-metal-ion mechanism of catalysis. Thus, Dr. Pyle's studies greatly elucidate an unconventional aspect of the central dogma.

For years, chemical biologists have been working to incorporate non-natural amino acids — structurally modified amino acids with different physicochemical and biological properties — into proteins. The aim of this research is to better understand normal cellular processes, such as posttranslational modification, as well as develop peptides for therapeutic use against targets. At the symposium, Dr. Suga presented his work on genetic code reprogramming, a method of addressing this aim. Suga's group reassigns codons for natural amino acids to non-natural amino acids using a flexizyme-based *in vitro* translation system. Flexizyme is a ribozyme that charges transfer RNA (tRNA) with amino or hydroxy acids esterified with a 3,5-dinitrobenzyle (DBE) group. Virtually, any amino acid can be charged onto any desired tRNA since the aminoacylation reaction is independent of the type of side chain.

A second component of the experimental system is a reconstituted, cell-free translation mixture derived from *Escherichia coli*. In this mixture, natural aminoacyl-tRNA synthetases (ARSs) are removed so their cognate tRNAs will not be charged. Those uncharged tRNAs, called vacant codons, are then reassigned to any desired non-natural amino acid using the flexizyme system. To maximize the variety of amino acids in the system, researchers artificially divide the codon box by taking advantage of redundancy in the genetic code (e.g., the codons GUU, GUC, GUA, and GUG all encode the single amino acid valine). For example, GUU and GUC would be reprogrammed for p-methoxyphenyllactic acid (mF^{lac}), while the GUA and GUG triplets would still encode valine [4].

One proof-of-concept of Dr. Suga's system is the synthesis of backbone-cyclized peptides [5]. A DNA template encoding a linear precursor peptide, composed of a cysteine-protein dipeptide sequence followed by a glycolic acid sequence (C-P-H^{OG}), was *in vitro*-transcribed and -translated. Expression of linear peptides bearing the C-P-H^{OG} sequence resulted in self-rearrangement into a C-terminal diketopiperadine-thioester. A cyclized peptide was thus generated non-enzymatically, with enhanced structural rigidity, protcolytic stability, and membrane permeability. Remarkably, all processes, including transcription of the DNA template, translation of the peptide, and peptide cyclization, take place in one reaction tube. Based on this method, Dr. Suga's group has built a library of backbone-cyclized peptides for rapid screening of peptides or substrate analogs for inhibition of functionally important enzymes [6].

Dr. Pyle's work on group II introns has clarified their structure and the mechanism of their removal from the RNA template before natural protein synthesis. Meanwhile,

Dr. Suga is steering information from mRNA toward non-natural peptides. These new discoveries at the interface of chemistry and biology are furthering our understanding of the central dogma, as well as allowing us to manipulate it for therapeutic use.

REFERENCES

1. Fedorova O, Pyle AM. A conserved element that stabilizes the group II intron active site. RNA. 2008;14(6):1048-56.
2. De Lencastre A, Hamill S, Pyle AM. A single active-site region for a group II intron. Nat Struct Mol Biol. 2005;12(7):626-7.
3. Toor N, Keating KS, Taylor SD, et al. Crystal structure of a self-spliced group II intron. Science. 2008;320(5872):77-82
4. Ohta A, Murakami H, Higashimura E, Suga H. Synthesis of polyester by means of genetic code reprogramming. Chem Biol. 2007;14(12):1315-22.
5. Sako Y, Goto Y, Murakami H, et al. Ribosomal synthesis of peptidase-resistant peptides closed by a nonreducible inter-side-chain bond. ACS Chem Biol. 2008;3(4):241-9.
6. Kawakami T, Ohta A, Ohuchi M, et al. Diverse backbone-cyclized peptides via codon reprogramming. Nat Chem Biol. 2009;5(12):888-90.