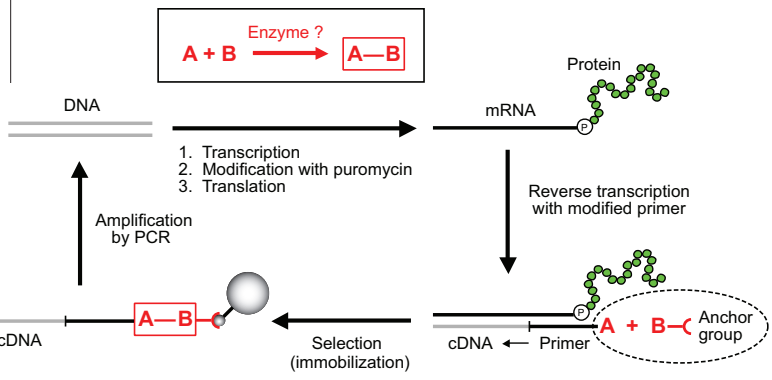
A New Evolution

Enzymes exquisitely catalyze an impressive range of chemical reactions, many of which can be quite challenging to accomplish through chemical methods alone. The ability to create enzymes with novel activities would provide additional tools with valuable research and therapeutic applications, but thus far, this has only been achieved when extensive knowledge of the mechanism of the reaction is in hand. Using messenger RNA (mRNA) display, Seelig and Szostak (*Nature* 2007, *448*, 828–831) now report the creation of a new enzyme capable of performing a previously unknown RNA ligase activity.

mRNA display technology and *in vitro* directed evolution were employed to create enzymes that could ligate a 5′-triphosphorylated RNA oligonucleotide to the terminal 3′-hydroxyl group of a second RNA strand. In mRNA display, proteins are covalently linked to their encoding mRNA, so a convenient decoding mechanism based on reverse transcription of the mRNA template exists for the identification of proteins of interest. Adding a clever twist to standard mRNA display methodology, the authors carried out the reverse transcription step by using a primer modified with the 5′-triphosphorylated RNA oligonucleotide substrate, and the result was a substrate–cDNA conjugate hybridized to the mRNA displayed protein. The attachment of a biotin moiety to the 3′-hydroxyl group of the second RNA substrate provided a method for identifying mRNA-displayed proteins capable of ligating the two substrates. When product is formed, it becomes covalently attached to the cDNA encoding the protein, and the product–DNA conjugate can be captured by streptavidin affinity chromatography. Subsequently, the cDNA of interest is amplified by using the polymerase chain reaction, and the entire cycle is repeated for further optimization. Using this strategy, the authors isolated several new enzymes with the desired RNA ligase activity. Structural and biochemical characterization of the most active clones revealed a dependence on zinc and pH, and evidence of a folded structure. This innovative application of mRNA display can be translated to the creation of other enzymes with both bond-making and bond-breaking activities. **Eva J. Gordon, Ph.D.**



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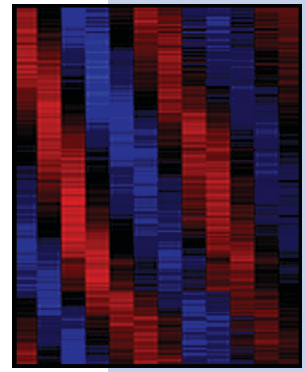
Hormones on the Clock



Reprinted from *PLoS Biol.*, *5*, Covington, M. F., and Harmer, S. L., The circadian clock regulates auxin signaling and responses in Arabidopsis, e222.

Ever since the first experiments investigating circadian rhythms in plants were documented in the early 1700s, scientists have studied numerous temporal and spatial regulatory mechanisms used by plants to control their growth, development, and response to their ever-changing environment. The circadian clock refers to the temporal regulation instilled by the ~24-h rhythm that organisms rely on to anticipate regular changes in their environment. In contrast, the auxin class of plant hormones, of which indole-3-acetic acid is the most prominent member, controls many spatial aspects of plant growth and development, including direction of growth and plant embryogenesis. Now, Covington and Harmer (*PLoS Biol.* 2007, *5*; e222) uncover an intriguing link between circadian rhythms and auxin signal transduction.

Genome-wide transcriptional profiling experiments provided initial evidence that several auxin-induced genes, but not genes induced by another plant hormone, brassinolide, are under circadian regulation, because a suspiciously high number of genes involved in auxin signaling exhibited circadian fluctuations in messenger RNA expression. The authors confirmed this observation by using a bioluminescence assay in which transgenic plants expressed the firefly luciferase gene under the control of an enhanced version of an auxin-responsive promoter. In this assay, plants were also found to have differential sensitivity to exogenous auxin at different times of day. Notably, both transcriptional and growth responses to exogenous auxin were found to be clock-regulated. This connection between circadian and auxin signaling pathways intimates the existence of an intricate web of temporal and spatial factors that influence plant growth, development, and environmental response. **Eva J. Gordon, Ph.D.**