more essential nutrients, which can eventually limit the growth response. A high-CO_2 environment could have exacerbated this growth-induced nutrient limitation, resulting in the negative NPP response (*2*).

The long-term response of nutrient-limited ecosystems to elevated CO_2 depends on the balance between processes that temporarily immobilize plant nutrients and those that release nutrients back into forms available to plants (see the figure). Decades or even centuries may be required for some of these processes to equilibrate after system perturbations such as those that are simulated in global change studies. Short-term, transient responses observed in experiments may thus not reflect the long-term, equilibrium response (*5, 8*).

This observation underscores a related problem for global change studies, especially studies that incorporate complex interactions between multiple variables: How does one interpret transient responses in light of the long time scales of many of the belowground processes? And how can information obtained by observing step changes in environmental factors—for example, an instantaneous doubling of the CO_2 concentration—be used to predict ecosystem responses in the real world of long-term, incremental changes in Earth's climate and atmospheric trace gas concentrations?

Answering both of these questions requires well-integrated computer modeling and observational investigations. Field studies, even ones that consider multiple factors, are insufficient for understanding the complex feedback responses that occur beneath the soil surface and determine the long-term system responses to global change (*8, 9*). Greater efforts must be made to understand the dynamics of nutrient cycles and to design experiments that target critical knowledge gaps. The results can then be incorporated into models to evaluate long-term consequences of incremental global changes.

Such integrated approaches in global change research are even rarer than the negative CO_2 production responses reported by Shaw *et al.* (*2*). But they will be required if we hope to achieve some predictive capability long before the results of our worldwide global change experiment materialize across the planet.

References

1. J. T. Houghton *et al., Climate Change 2001. The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, Cambridge, 2001).
2. M. R. Shaw *et al., Science* **298**, 1987 (2002).
3. S. J. E. Wand, G. F. Midgley, M. H. Jones, P. S. Curtis, *Global Change Biol.* **5**, 723 (1999).
4. B. G. Drake, M. A. Gonzàlez-Meler, S. P. Long, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 607 (1997).
5. P. C. D. Newton, H. Clark, G. R. Edwards, D. J. Ross, *Ecol. Lett.* **4**, 344 (2001).
6. S. Diaz, J. P. Grime, J. Harris, E. McPherson, *Nature* **364**, 616 (1993).
7. D. D. Ackerly, F. A. Bazzaz, *Global Change Biol.* **1**, 199 (1995).
8. J. H. M. Thornley, M. G. R. Cannell, *Plant Soil* **224**, 153 (2000).
9. D. R. Zak, K. S. Pregitzer, J. S. King, W. E. Holmes, *New Phytol.* **147**, 201 (2000).

PERSPECTIVES: CHEMISTRY

The Simplest "Enzyme"

Mohammad Movassaghi and Eric N. Jacobsen

How small can a highly active and stereoselective catalyst be? And what are the minimal functional and structural features required in a chiral catalyst? A series of recent reports on proline-catalyzed asymmetric reactions may be pointing to the ultimate answer to these practically and fundamentally important questions. The studies are all the more significant because they address some of the most challenging and useful reactions in organic chemistry.

Asymmetric synthesis is dedicated to the preparation of handed (chiral) compounds with defined three-dimensional molecular structure (stereochemistry). The importance of stereochemistry in chemical interactions is probably best appreciated in the context of drug-receptor interactions, because most biological targets are chiral entities. Hence, there is enormous pressure to devise viable and practical methods for preparing chiral compounds in pure form.

Nature is the principal practitioner of asymmetric synthesis. Living systems use enzymes to catalyze stereoselective reactions with very high fidelity. Enzymes exploit hydrogen bonding between the active site and substrate, together with nonbonded dipole-dipole, electrostatic, and steric interactions, to orient the substrate and stabilize the transition state, leading to high levels of stereoselectivity.

The challenge associated with organizing the key transition structure in a catalytic process, such that only a single enantiomer (handedness) of a chiral product is produced, appears formidable. It was therefore long assumed that complex supramolecular structures such as those found in enzymes were required for attaining high enantioselectivity. We now know, however, that synthetic small-molecule catalysts can approach and sometimes even match the enantioselectivity and reactivity characteristic of enzymes.

Since the first reports appeared in the late 1960s, a wide variety of chiral organometallic complexes have been identified as asymmetric catalysts (*1*). These catalysts not only effect useful reactions with high levels of enantioselectivity, but often do so with a wide variety of substrates. Such generality is highly unusual with enzymes. The 2001 Nobel Prize in chemistry was given to the leading figures in the field of asymmetric catalysis in recognition of these accomplishments (*2*).

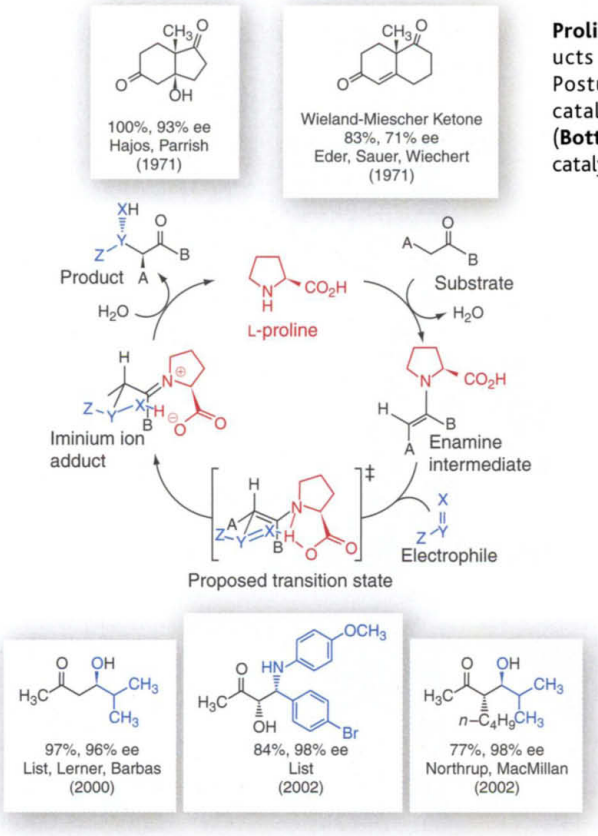
In the excitement over transition metal–based catalysts, a series of reports and patents from the early 1970s describing an enantioselective transformation that employed the natural amino acid proline as the catalyst (*3–6*) did not receive the attention they deserved. The chemistry was not ignored by organic chemists—the so-called Wieland-Miescher ketone prepared by proline catalysis has been used more or less continuously as a synthetic building block over the past 25 years (*7*). Yet, its broader implications for asymmetric catalysis were not appreciated until recently.

Recently, List *et al.* reported the intermolecular aldol addition reaction of acetone to various aldehydes catalyzed by proline (*8, 9*). The authors also used other ketones as the nucleophile component. Perhaps most noteworthy is the use of hydroxy acetone, which provides 1,2-diols as the aldol addition products with aldehydes with high stereoselectivity.

Proline has also been used to activate ketones and aldehydes as the nucleophilic component in various asymmetric conjugate additions and additions to imines. Most recently, MacMillan reported the use of proline to catalyze highly enantioselective cross-aldol reactions, with different aldehyde substrates serving as both donor and acceptor in efficient addition reactions (*10*). Such transformations have constituted a "Holy Grail" of sorts in the field of asymmetric catalysis because they provide operationally simple routes to useful products without generating any wasteful by-products.

The high levels of reactivity and enantioselectivity induced by proline in these reactions likely arise from a series of interactions similar to those involved in enzymatic catalysis. Proline catalysis, similar to other catalytic processes, involves organization and activation of the substrates, transition state stabilization, and product release to afford substrate turnover. In the postulated mechanism of proline-catalyzed

The authors are in the Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA. E-mail: jacobsen@chemistry.harvard.edu



Proline in action. (**Top**) Early products of proline catalysis. (**Center**) Postulated mechanism of proline-catalyzed aldol addition reactions. (**Bottom**) Recent products of proline catalysis. ee, enantiomeric excess.

aldol addition reactions (see the figure), the condensation of the secondary amino group of proline with a carbonyl substrate leads to formation of a nucleophilic enamine intermediate. This process mimics the condensation of the active-site lysine residue with a carbonyl substrate in type I aldolases (*11*). The adjacent carboxylic acid group of the enamine intermediate then directs the approach of the electrophile by formation of a specific hydrogen bond in the transition state structure. This provides both preorganization of the substrates and stabilization of the transition state structure, similar to the specific hydrogen bonds used in enzymatic catalysis. Upon electrophilic capture of the enamine derivative, the resulting iminium ion is hydrolyzed to release the product and the catalyst (proline). The handedness of proline is thus effectively relayed to the product, while the released proline can proceed to repeat the catalytic cycle.

Why did it take so long for chemists to appreciate and exploit the potential of proline-catalyzed asymmetric reactions? One factor was probably that researchers placed disproportionate emphasis on organometallic catalysts. Today, the vast majority of breakthroughs in asymmetric catalysis continue to rely on organometallic complexes, but recently there have been numerous exciting discoveries involving simple organic catalysts that are not much more complicated than proline (*12*).

Another factor was that researchers came to appreciate only recently how general small chiral catalysts can be. Many assumed that the early success with proline catalysis (*3*–*6*) must be highly limited in scope. This has proven not to be the case. But the most fundamental reason was probably that chemists could not believe that a molecule as simple as proline—a single natural amino acid—could possess all the properties necessary for activating normally unreactive substrates to useful asymmetric catalytic transformations. It is time to believe it.

References and Notes

1. E. N. Jacobsen, A. Pfaltz, H. Yamamoto, Eds., *Comprehensive Asymmetric Catalysis* (Springer, New York, 1999), vols. 1–3.
2. The Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry for 2001 to K. Barry Sharpless, William S. Knowles, and Ryoji Noyori for "the development of catalytic asymmetric synthesis."
3. Z. G. Hajos, D. R. Parrish, German Patent DE 2102623 (29 July 1971).
4. Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **39**, 1615 (1974).
5. U. Eder, G. Sauer, R. Wiechert, German Patent DE 2014857 (7 October 1971).
6. U. Eder, G. Sauer, R. Wiechert, *Angew. Chem. Int. Ed. Engl.* **10**, 496 (1971).
7. For example, the total synthesis of Taxol (*13*).
8. B. List, R. A. Lerner, C. F. Barbas III, *J. Am. Chem. Soc.* **122**, 2395 (2000).
9. B. List, *Tetrahedron* **58**, 5573 (2002).
10. A. B. Northrup, D. W. C. MacMillan, *J. Am. Chem. Soc.* **124**, 6798 (2002).
11. A. Heine *et al.*, *Science* **294**, 369 (2001).
12. P. I. Dalko, L. Moisan, *Angew. Chem. Int. Ed.* **40**, 3726 (2001).
13. S. J. Danishefsky *et al.*, *J. Am. Chem. Soc.* **118**, 2843 (1996).

PERSPECTIVES: MOLECULAR BIOLOGY

Untangling Checkpoints

Noriyuki Sagata

In eukaryotic cells, genotoxic stresses that damage the DNA or inhibit DNA synthesis result in activation of cell cycle checkpoints, leading to diverse cellular responses including cell cycle arrest, DNA repair, and cell death. These cellular responses help to prevent genomic instability, a principal cause of cancer. The cell cycle checkpoints activated by damaged or unreplicated DNA in turn activate signaling pathways that ultimately block the cyclin-dependent kinases (CDKs). CDKs together with their cyclin partners are key regulators of cell cycle progression. Inhibition of their activity delays or arrests the cell at specific phases of the cell cycle, enabling the DNA to replicate or be repaired (*1*).

In vertebrates, upstream elements of the checkpoint signaling pathways include the kinase ATM, a member of the phosphatidylinositol 3-kinase family, and its relative ATR. ATM and ATR phosphorylate and activate the effector kinases Cds1 (also called Chk2) and Chk1, respectively, which in turn block CDK activity (*1*). Typically, in response to DNA damage or unreplicated DNA, the cell halts just before mitosis. It is thought that Chk1 and Cds1 phosphorylate and inhibit Cdc25C, a phosphatase that directly activates the Cdk1–cyclin B complex, thereby preventing the cell from entering mitosis (*2*). Recent studies including a report by Zhao *et al.* (*3*) now reveal that Chk1 regulates the stability of Cdc25A, another member of the Cdc25 family, at multiple cell cycle checkpoints in vertebrate cells.

In contrast to Cdc25B and Cdc25C, the Cdc25A phosphatase is apparently important during the initiation and progression of S phase (the cell cycle phase when DNA is replicated). Cdc25A dephosphorylates and activates the Cdk2–cyclin E complex, a key kinase that promotes progression through S phase (*4*). The initial link between Cdc25A and the DNA damage and replication checkpoints came from the finding that Cdc25A expressed in certain human cell lines is rapidly degraded in response to ultraviolet (UV) light or drugs that block DNA replication. Furthermore, when overexpressed, this phosphatase abrogates checkpoint-induced arrest in S phase (*5*, *6*). The UV-induced degradation of Cdc25A required Chk1-like activity (*5*), and in mammalian cells, a block in DNA replication usually activates the ATR-Chk1 pathway (*1*). Thus, both UV-induced DNA

The author is in the Department of Biology, Kyushu University, Fukuoka 812-8581, Japan. E-mail: nsagascb@mbox.nc.kyushu-u.ac.jp