Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) is the key enzyme of photosynthesis in all plants and algae and in most photosynthetic bacteria. It is, of course, responsible for photosynthetic carbon fixation via the carboxylation of the five-carbon sugar ribulose-1,5-bisphosphate to yield two molecules of phosphoglycerate. At many levels, from the biochemical to the evolutionary, this important and plentiful enzyme (reputed to be the world’s most abundant protein) is also the best studied enzyme in plants. Indeed, the enzyme and its encoding genes have served as models for elucidating many fundamental properties of plant gene regulation, chloroplast-nuclear coordination, and protein import and assembly (Gutteridge and Gatenby, 1995), while the Rubisco large subunit gene rbcL, now sequenced in over 2000 plants, has become the gold standard of plant molecular systematics (Clegg, 1993).

Biochemically and physiologically, Rubisco poses a crucial problem: in addition to its useful carboxylation activity, Rubisco also possesses a competing oxygenation activity, which occurs at the same active site and leads to the wasteful loss of fixed carbon through the photorespiratory pathway (Hartman and Harpel, 1994). The L$_{8}$S$_{8}$ (8 large and 8 small subunit) form I Rubisco that is universally present in oxygenic phototrophs (plastids and cyanobacteria) exhibits on average a substantially higher specificity for CO$_{2}$ over O$_{2}$ than the more oxygen-sensitive L$_{2}$ form II Rubisco, which is restricted to certain anaerobic proteobacteria (some of which contain both types of Rubisco) (Jordan and Ogren, 1981; Tabita, 1994). But this biochemical adaptation, which genetic engineers seek to improve by tinkering with the sequence of the form I enzyme, is often not enough; many oxygenic phototrophs have evolved a variety of mechanisms to favor the carboxylation reaction and thereby reduce oxygenation and, hence, photorespiration. These include the CO$_{2}$-concentrating pumps of many aquatic plants, algae, and cyanobacteria (Badger and Price, 1992), and the C$_{4}$ and CAM metabolic pathways found in many plants that grow in hot and/or dry environments (Furbank and Taylor, 1995).

Another weakness of Rubisco, its low turnover number, is a boon for scientific investigation, as this presumably accounts for its amazing abundance. (There are an estimated 500 active sites of Rubisco for each CO$_{2}$ molecule in the chloroplast.)

How do plants and green algae coordinate the synthesis and assembly of such massive amounts of Rubisco from the thousands of identical rbcL genes (one per genome) present in the chloroplasts of a single leaf cell and the four to 24, nearly identical, small subunit (rbcS) genes present in the nucleus? Research on this question has elucidated much of our fundamental knowledge of how chloroplast and nuclear gene expression is regulated (especially in response to light induction), of how cytoplasmically synthesized proteins are imported into chloroplasts, and of how Rubisco assembly within the chloroplast is mediated by chaperonin 60, now recognized as a central and general agent of protein assembly in all organisms (Gutteridge and Gatenby, 1995).

Although one might naturally assume from all of this study that Rubisco’s grandest secrets have already been long revealed, this is clearly not the case. On pages 539–553 of this issue, Rowan et al. show that Rubisco in dinoflagellates exhibits all manner of surprising, even shocking, features, some of which were also revealed in a brief report last year by Morse et al. (1995). Breaking all rules, the dinoflagellate Rubisco is shown to be the form II, L$_{2}$-type enzyme found heretofore exclusively in anaerobic bacteria and to be encoded by nuclear-localized rbcL genes. Furthermore, the dinoflagellate Rubisco is encoded as a triple polyprotein by a surprisingly diverse gene family that contains noncanonical spliceosomal introns. Full understanding of the functional implications and underlying mechanisms of these features, and of their evolutionary significance, is sure to occupy many years of fruitful Rubisco labors and to vault dinoflagellates to unexpected heights of prominence as illuminating systems for cellular, physiological, and molecular inquiry.

Why study dinoflagellates in the first place? Although largely neglected by experimental and/or reductionist biologists, dinoflagellates are a fascinating group of richly diverse, abundant, and ecologically important protists. Phylogenetically, they appear related to two other intriguing but phenotypically dissimilar and far better studied groups of protists—the ciliates and the apicomplexans (parasites causing such diseases as malaria and toxoplasmosis). In appearance, dinoflagellates are unusual, often bizarre, being "armored" or "helmeted" with stiff cellulose plates present inside the plasma membrane, as opposed to the outside cell wall of plants and most algae. Their motion is also peculiar, a top-like spinning caused by the beating of two flagella within perpendicularly opposed grooves. Abundant in marine waters, dinoflagellates penetrate human consciousness chiefly for two reasons: Blooms of certain dinoflagellates are responsible, through production of powerful neurotoxins, for the infamous red tides that decimate fisheries and depopulate beaches. On the positive side, endosymbiotic dinoflagellates (most commonly Symbiodinium species) present in massive quantities within corals are largely responsible for the photosynthetic productivity that underlies the vast growth of
tropical coral reefs. But this is just the tip of the symbiotic iceberg; dinoflagellates occur as symbionts within a great variety of protists and animals, from sponges to mollusks, and in turn, they often harbor cyanobacteria and sometimes even other eukaryotic algae.

Rowan et al. is the culminating third in a series of papers from the laboratory of David Younglees at James Cook University (Queensland, Australia) on the Rubisco enzyme and genes from *Symbiodinium* dinoflagellates. Confirming an old report of the unusual instability of Rubisco in dinoflagellates (Bush and Sweeney, 1972), Whitney and Younglees (1995) first used the tight binding inhibitor [14C]carboxyarabinitol bisphosphate as a marker to enable purification of inactive Rubisco. Surprisingly, this lacked all traces of the enzyme and genes from *Symbiodinium* dinoflagellates further indicated similarity to the bacterial form II enzymes of two anaerobic proteobacteria, and vice versa, whereas neither antibody reacted with the form I large subunit of green and chromophytic algae. N-terminal sequencing of purified large subunit from two dinoflagellates further indicated similarity to the bacterial form II Rubisco (Whitney et al., 1995). Rowan et al. now provide a comprehensive molecular characterization of two Rubisco-encoding loci from *Symbiodinium*. Such study also provides important and convincing proof that their form II-like Rubisco is actually derived from the alga itself, copes with this O2 affinity than found in proteobacteria, or that the desired carboxylation reaction is elevated by a CO2-concentrating mechanism, such as the CO2 pumps mentioned above (Badger and Price, 1992). Novel mechanisms should also be considered. Might dinoflagellates employ a mechanism similar to any of those used by bacteria to prevent O2-inactivation of nitrogenase, such as compartmentalization of Rubisco in an O2-depleted microenvironment, or sequestration of O2 by a leghemoglobin-like protein?

Finding *rbcL* genes in the nucleus is also unprecedented but a little less surprising: there is mounting evidence for considerable variation in chloroplast gene content across taxa (Reith, 1995), and *rbcL* has been successfully reengineered into a functional nuclear gene in transgenic tobacco plants (Kanevski and Maliga, 1994). But the combination of finding *rbcL* genes of proteobacterial affinity in the *Symbiodinium* nucleus raises a major evolutionary question: where did they come from? The various possibilities, considered in some detail by Rowan et al., Morse et al. (1995), and Palmer (1995), are reasonably open to testing by comparative study and molecular phylogenetic analysis. I favor scenarios involving non-plastid-mediated transfer of a form II Rubisco gene to the dinoflagellate nucleus, either directly from a proteobacterium or indirectly from the dinoflagellate mitochondrion (which is itself of proteobacterial origin), followed by loss of the original form I *rbcL* and *rbcS* genes of plastid origin. Alternatively, nuclear form II Rubisco could have been transferred from the chloroplast, if either the cyanobacterial ancestor(s) of all plastids possessed both form I and II Rubisco or the dinoflagellate plastid is of separate, proteobacterial origin. In any event, it is clear that the dinoflagellate case represents just one of many promiscuous ways in which Rubisco genes have been passed around, both between organisms and among all three eukaryotic genetic compartments, making Rubisco a paradigm of evolution by lateral gene transfer (Palmer, 1995).

The sequence of one *Symbiodinium rbcL* locus, designated *rbcA* by Rowan et al., reveals an abnormally large open reading frame of over 1500 codons containing three tandemly repeated *rbcL* coding sequences separated by two in-frame, identical 69-bp "spacers." An *rbcL* transcript of correspondingly large size was detected, and the authors conclude that it probably specifies a polyprotein of three large subunits. This inference, and its corollary, that the presumptive polyprotein is proteolytically processed at the two spacer regions after import into chloroplasts, awaits confirmation. Given the overall rarity of polyproteins, it is extraordinary that the only other characterized chloroplast protein from dinoflagellates is also encoded by a nuclear polyprotein gene (Hiller et al., 1995), as are half of the six examined chloroplast proteins from the unrelated alga, *Euglena gracilis* (Houlne and Schantz, 1993).

A second *rbcL* locus in *Symbiodinium*, *rbcG*, encodes but a single large subunit polyepitope. The *rbcG* protein is exceptionally divergent. It is far less similar to form II Rubisco from α-proteobacteria (48% amino acid identity) than are the three essentially identical *rbcA* proteins (85% identity). More importantly, it deviates at half of the 19 predicted "active-site" residues (Hartman and Harpel, 1994), all of which are conserved in *rbcA*. But because *rbcG* exhibits typical codon usage for *Symbiodinium* genes and is maintained as an intact open reading frame despite extensive substitutional and deleterial
divergence, Rowan et al. argue that it is still an active gene but unlikely to encode a "normal" Rubisco. What role this bizarre Rubisco gene plays is completely unclear at the present time.

Finally, Rowan et al. report the first introns known from dinoflagellates, two in each of the four Rubisco coding sequences. Again breaking rules, seven of the eight introns do not contain GT/AG at their ends (all seven have A or C rather than T at the second nucleotide at the 5' end, and one also varies at its 3' end). No other eukaryote deviates more than rarely from the GT/AG rule (Jackson, 1991), and thus, it is likely that some aspects of spliceosomal splicing are significantly different in dinoflagellates compared with all other eukaryotes, including the related ciliates and apicomplexans.

In conclusion, the findings of Rowan et al., together with those of Morse et al. (1995), are both exciting in and of themselves and also portend many more fruitful dinoflagellate studies. These should range from general molecular biological inquiry into splicing mechanisms to specific physiological and biochemical study of how an "anaerobic" Rubisco functions in an aerobic environment, with side excursions into protein import and processing, Rubisco functional divergence, and evolution by lateral transfer. At the same time, one is hungry for any news about the dinoflagellate chloroplast genome; not a single gene sequence is yet reported, which is shocking at a time when entire chloroplast genomes have been sequenced from almost all other major groups of algae (see Plant Molecular Biology Reporter, vol. 13, no. 4). Given the many surprises offered by these genome sequences (see also Reith and Munholland, 1993, and Reith, 1995), one can only wonder as to what other mysteries the already surprising dinoflagellates hold in store. So turn up the spotlight on dinoflagellates, and stay tuned for more news on dinoflagellate Rubisco.

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