

might make large protein complexes, and therefore behave as a large cargo, so additional experiments are needed to look at smaller cargoes in mammalian cells.

The fate of the late cisternae also needs to be clarified. If the cisternal maturation model is correct, they should mature into secretory vesicles and other types of carrier, but this has yet to be confirmed by fluorescence microscopy. A related prediction is that blocking retrograde traffic should block cisternal maturation and send Golgi-resident proteins to the cell surface or the endosome. Surprisingly, Matsuura *et al.*² find that in a yeast mutant with a defect in COPI vesicle assembly, cisternal maturation is slowed about three-fold — but it still occurs. How are resident Golgi proteins being recycled in the absence of COPI function? Do resident Golgi proteins escape from the organelle under these conditions?

Even if cisternal maturation does occur, the details of the mechanism have yet to be defined. For instance, how is the polarized *cis-to-trans* distribution of resident Golgi proteins maintained? One suggestion is that it might be by the differential recycling of the Golgi proteins⁴. And what of other cell types? It is quite possible that in *S. cerevisiae*, the Golgi is formed *de novo* and then consumed during each round of secretory protein

transport. In mammalian cells, however, there is no evidence that Golgi membranes form *de novo* under physiologically relevant conditions during sequential rounds of secretion. Maybe these cells rely instead on a variety of trafficking modes commensurate with cargo size and Golgi organization. Whatever the answer may be, the images provided by Losev *et al.*¹ and Matsuura *et al.*² have certainly swung the scales heavily towards cisternal maturation, in yeast at least. ■

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enzyme Rubisco activase^{1,3}, when first CO₂ and then a magnesium ion bind to the active site. The substrate, ribulose biphosphate, then reacts with these to form an enediol intermediate, which engages with either another CO₂ or an O₂ molecule, either of which must diffuse down a solvent channel to reach the active site. Tcherkez and colleagues' achievement² is to have produced an explanatory mechanism for the trade-off usually observed between the specificity factor (that is, a ratio indicating selectivity for CO₂ over O₂, which ranges between 20 and 280; refs 1, 3) and k_{cat} (the rate of enzyme turnover, which varies between two and eight catalytic events per second).

Mutagenesis

Work leading up to this proposed mechanism⁴ involved site-directed mutagenesis in tobacco, which had the aim of destabilizing the enzyme active site and altering the reactivity of the enediol intermediate. Changing the binding of an amino-acid residue that encourages the addition of both CO₂ and O₂ dramatically increased the rate of oxygenase activity.

Such observations^{3,4} provided the key to the idea² that in the active site the enediol must be contorted to allow CO₂ to attack more readily despite the availability of O₂ molecules. The more the enediol mimics the carboxylate end-product, Tcherkez *et al.*² conclude, the more difficult it is for the enzyme to free the intermediate from the active site when the reaction is completed. When the specificity factor and selectivity for CO₂ are high, the impact on associated kinetic properties is greatest: k_{cat} becomes slower.

So, rather than being inefficient, Rubisco has become highly tuned to match substrate availability. Several other correlates are also explained by this relationship. For instance, Rubisco discriminates more against ¹³C than against ¹²C, the two naturally occurring stable isotopes in CO₂. But when the specificity factor is high, the ¹³C reaction intermediate binds more tightly, and so carbon isotope discrimination is higher (that is, less ¹³C is incorporated); in consequence, the carbon-isotope signals used to reconstruct past climates should perhaps now be re-examined. In contrast, higher ambient temperatures (30–40 °C) reduce the stability of the enediol, and Rubisco oxygenase activity and photorespiration rate increase.

These insights² into the mechanism of Rubisco catalysis are timely, because progress is being made in identifying the evolutionary origins of a wider range (forms I to IV) of Rubisco-like proteins⁵. The least effective of these forms have evolved, and now reside, in microorganisms in anaerobic sediments where catalysis does not have to compete with oxygen¹. One bacterium can express all three catalytically active forms (I, II and III), and switches between them depending on environmental conditions⁶. The evolutionary

PLANT BIOLOGY

Designs on Rubisco

Howard Griffiths

Rubisco is said to be both the most important enzyme on Earth and surprisingly inefficient. Yet an understanding of the reaction by which it fixes CO₂ suggests that evolution has made the best of a bad job.

Rubisco is the enzyme in photosynthesis that is responsible for the conversion of inorganic carbon, as CO₂, into organic compounds. The demanding initial catalytic step (or carboxylase reaction¹) precedes the photosynthetic reduction of reaction products using the energy trapped from sunlight. The acronym Rubisco actually stands for ribulose biphosphate carboxylase-oxygenase, because the enzyme also has a tendency to confuse O₂ for CO₂ as its substrate. Rubisco has the reputation of being slow and inefficient, but it is one of life's big successes: globally there is an estimated 5–10 kg Rubisco for every person on Earth, and each year it reacts with 15% of the total pool of atmospheric CO₂.

Active site

Work to 'improve' Rubisco and so increase crop productivity has usually foundered on the catalytic active site, which is highly conserved in different forms of the enzyme and

has generally proved to be intractable to genetic manipulation. But a fresh angle on such prospects comes from Tcherkez and colleagues², writing in the *Proceedings of the National Academy of Sciences*. They propose an explanation for the reaction mechanism that accounts for the selection of CO₂ in preference to O₂. The systematic evolution of enzyme kinetic properties seems to have occurred in Rubisco from different organisms, suggesting that Rubisco is well adapted to substrate availability in contrasting habitats.

It is curious that Rubisco should fix CO₂ at all, as there is 25 times more O₂ than CO₂ in solution at 25 °C, and a 500-fold difference between them in gaseous form. Yet only 25% of reactions are oxygenase events at this temperature, and carbon intermediates 'lost' to the carbon fixation reactions by oxygenase action are metabolized and partly recovered by the so-called photorespiratory pathway. Catalysis begins with activation of Rubisco by the

trade-offs observed by Tcherkez *et al.* are consistent with the occurrence of most of these enzymes. Alternatively, some higher plants and photosynthetic microorganisms have developed mechanisms to suppress oxygenase activity: CO₂-concentrating mechanisms are induced either biophysically⁷ or biochemically^{8,9}. There are systematic changes in Rubisco kinetics^{1,3}, consistent with Tcherkez and colleagues' views, that are thought to have evolved in the past 10 million years in plants related to sugar cane and maize.

Molecular techniques such as 'directed evolution' offer the possibility of manipulating part of Rubisco — the large subunit component — to select for catalytic improvements¹⁰, allowing access to 'sequence space' that is not available to conventional molecular manipulations. A sensible move would be to screen the kinetics of Rubisco enzymes from different plants that inhabit extreme environments^{1,3}. For instance, Rubisco has not been characterized in the so-called CAM plants, which use a form of photosynthesis (crassulacean acid metabolism) adapted for arid conditions. In these species, the enzyme is exposed daily to a range of CO₂ concentrations (0.01–2%) equivalent to those occurring throughout much of Earth's history⁹.

Other research avenues include manipulating the various components of Rubisco¹ and cell-specific targeting of chimaeric Rubiscos⁸. Potential pitfalls here are that the modified Rubisco would not only have to be incorporated and assembled by crop plants, but any improved performance would have to be retained by the plants. Finally, one suggestion is that we should engineer plants that can express two types of Rubisco — each with kinetic properties to take advantage of the degree of shading within a crop canopy¹¹. Such rational design¹⁰ would not only offer practical opportunities for the future, but also finally give the lie to the idea that Rubisco is intransigent and inefficient. ■

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MATERIALS SCIENCE

Relaxors go critical

R. E. Cohen

Relaxor ferroelectrics are fascinating and useful materials, but they seem to be heterogeneous, hopeless messes. Observing what they do under electric fields reveals critical behaviour that helps to make sense of them.

Piezoelectrics — materials that convert electrical energy into mechanical energy, and vice versa — form the alarms in our watches, the warning buzzers in our cars, and the transducers used in sonar and medical ultrasound. They are also being used for knifeless surgery and to build tiny pumps and motors for medical applications. On page 956 of this issue, Kutnjak and colleagues¹ report investigations of the thermodynamic properties and phase diagram of relaxor ferroelectrics with giant piezoelectric effects. These materials offer a response that is up to ten times greater than that of standard piezoelectrics, and they are revolutionizing acoustic imaging and surgical applications.

Ferroelectrics have a spontaneous polarization (a dipole moment per unit volume, or net charge flow per unit volume from a non-polar state) that can be switched in direction by applying an electric field. A prototypical ferroelectric is lead titanate (PbTiO₃, or PT). Ferroelectric behaviour arises as a result of competition between long-range forces between ionic charges in the material, which act to destabilize the nonpolar structure, and short-range, repulsive forces, which have a stabilizing influence. Covalency softens this repulsion and allows the atoms to have average off-centre displacements², and a net polarization. The piezoelectric behaviour of ferroelectrics arises from the coupling of strain and polarization when the polarization interacts with applied electric fields.

Relaxor ferroelectrics are solid solutions between a relaxor material and a ferroelectric such as PT. Relaxors do not have a polar ground state, and are heterogeneous³. They have disordered polarization, with small ordered 'polar nanoregions'^{4,5} that individually polarize⁶ below a temperature known as the Burns temperature⁷.

Kutnjak and colleagues study the relaxor ferroelectric system PMN-PT (in full, PbMg_{1/3}Nb_{2/3}O₃-PbTiO₃), which is an exemplar of a new class of relaxor ferroelectric with pronounced piezoelectric properties. In the normal, collinear piezoelectric case, electric field is applied parallel to the polarization, and the resulting strain is small. But in high-coupling relaxor ferroelectrics, the energy barrier for polarization rotation is low, so even a field applied obliquely can easily rotate the polarization (Fig. 1). It is the polarization rotation effect that gives rise to very large electromechanical coupling^{8,9}, with strains of up to 2% observed. Polarization rotations have been

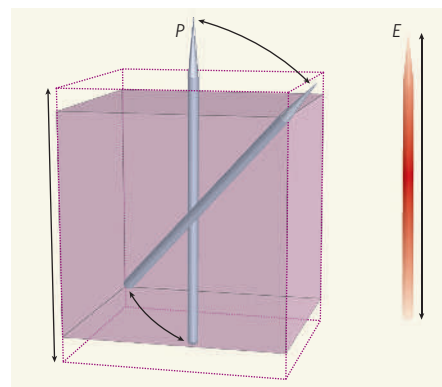


Figure 1 | The polarization rotation effect. When an electric field E is applied along the cube axis (vertical), the polarization P rotates from the cube diagonal towards the cube axis. With the polarization along the cube diagonal, the lattice strain is small (the lattice is close to cubic), but with the polarization along the cube axis the strain is large — there is a large piezoelectric effect. Kutnjak *et al.*¹ apply a field along the cube diagonal in a new-generation relaxor ferroelectric, and find that a first-order jump in polarization with temperature for small fields vanishes at a critical line that depends on temperature, electric field and the material's composition. The electromechanical coupling also peaks at this critical line.

proved experimentally from X-ray diffraction and optical studies^{10,11}.

Relaxor ferroelectrics are enormously useful because of their piezoelectric properties. But they also exhibit a cornucopia of mesoscopic and microscopic heterogeneities over a range of lengths and timescales. This diversity has so far made it difficult to systematize observations of them or even to determine their whole phase diagram — the jumping-off point for any materials study.

Kutnjak *et al.*¹ measured properties of PMN-PT as functions of temperature, electric field and composition. In the resulting phase diagram, they found a critical line under applied electric field. For low fields, there is a first-order transition where local dipoles formed by atomic displacements are ordered at low temperatures, and rotate or become disordered, as in a paraelectric material, for temperatures above the transition point. At this transition, there is a discontinuity in the net polarization. As electric field is increased, the discontinuity in polarization decreases until it is zero at the critical line. This finding complements recent evidence for critical behaviour in