protein disulfides by a cysteine thiol-disulfide exchange mechanism. The active-site disulfide in oxidized thioredoxin is itself reduced by thioredoxin reductase using electrons from nicotinamide adenine dinucleotide phosphate. Human and other mammalian thioredoxin reductases contain an active-site selenolthiol that mediates this reduction (*6*).

By inhibiting thioredoxin reductase in cultured T cells with the compounds dinitrochlorobenzene and auranofin (both target the active-site selenol in the reduced enzyme), Benhar *et al.* show that thioredoxin and thioredoxin reductase are the physiologically active principle. This was confirmed using RNA interference to reduce the expression of thioredoxin and thioredoxin reductase in cultured T cells. The authors also examined human macrophages treated with lipopolysaccharide and interferon-α, which induce nitric oxide production. They show that this treatment, combined with inhibition of thioredoxin reductase by auranofin, elicits robust S-nitrosylation of caspase-3. In human cells, thioredoxin 1 and 2 are the cytosol-nuclear and mitochondrial isoforms, respectively, and the products of two separate genes; the same is true for thioredoxin reductase. These results show that thioredoxin is the major denitrosylating agent in this cell type, and together with thioredoxin reductase, controls the amount of basal S-nitrosylation of caspases in response to Fas activation (*4*). Thioredoxin reductase may be rate-limiting for the overall effects.

Originally identified as a hydrogen donor for ribonucleotide reductase, thioredoxins have a long evolutionary history (*5*, *6*). Thioredoxin is a major reductant for keeping sulfhydryl groups on intracellular proteins in a reduced state, thus controlling redox states and signaling in the cytosol and nucleus. Thioredoxin also controls the redox state of thiols that are oxidized by hydrogen peroxide. As part of the cell's antioxidant defense, thioredoxin donates electrons to peroxiredoxins, enzymes that catalyze the breakdown of hydrogen peroxide. The enrichment of thioredoxin 1 at the plasma membrane (*6*), where enzymes that generate nitric oxide (nitric oxide synthases) are also concentrated, suggests that signaling by nitrosylation may be a localized phenomenon. Reactions between hydrogen peroxide, peroxynitrite, or nitric oxide donors and protein cysteine thiols may give rise to sulfenic acid, which is reduced by thioredoxin (*7*).

Previous results identified thioredoxin and thioredoxin reductase in denitrosylating the low molecular weight nitrosylating agent S-nitrosoglutathione in vitro (*8*) and proteins (including caspase-3) in human liver cells (*9*). Benhar *et al.* extend these findings by showing that both thioredoxin 2 in the mitochondria and thioredoxin 1 in the cytosol and nucleus are denitrosylases in the physiological context of apoptosis. The study also indicates the presence of a dynamic equilibrium, whereby nitrosylation and denitrosylation reactions occur (see the figure). The results are important because they identify a denitrosylase pathway in the cell and widen the range of thioredoxin functions to the control of apoptosis. Studying the turnover of thioredoxin and thioredoxin reductase in the context of nitrosylating and denitrosylating reactions should be a rich playing field for determining thiol redox control of membrane receptors, ion channels, and transcription factors (*6*, *7*).

The study by Benhar *et al.* also suggests that the effect of thioredoxin and thioredoxin reductase inhibitors as cancer treatments to control cell viability (*10*) may involve previously unknown effects on S-nitrosylation. Reduced thioredoxin is bound to proteins such as thioredoxin-interacting protein or apoptosis signaling kinase (*7*). These interactions should now be considered in relation to nitrosylation. Human cytosolic thioredoxin contains three structural cysteine residues that control thioredoxin activity (*6*) and potentially control transnitrosylation reactions (*11*). Furthermore, thioredoxin-dependent control of nitric oxide synthase may involve denitrosylation of the synthase (which would stimulate nitric oxide production) (*1*). We are only beginning to understand the links among thioredoxin, protein nitrosylation, and redox-controlled signaling (*3*, *9*, *10*).

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OCEAN SCIENCE

Marine Calcifiers in a High-CO_2 Ocean

Victoria J. Fabry

New results show that the response of marine organisms to ocean acidification varies both within and between species.

The oceans have taken up about one-third of the total carbon dioxide (CO_2) released into the atmosphere by human activities over the past 200 years (*1*). This addition of CO_2 to the surface ocean changes seawater chemistry, resulting in a decrease in pH and carbonate ion concentration, and an increase in the concentrations of bicarbonate ion and hydrogen ion. Ocean absorption of anthropogenic CO_2 also reduces the saturation state of seawater with respect to calcite and aragonite, two common types of calcium carbonate secreted by marine biota. Experiments with calcareous organisms indicate that calcification is strongly dependent on the carbonate saturation state of seawater (*2*–*6*), which suggests that ocean acidification will adversely impact calcifying taxa. Evidence is now accumulating that the acidifying effects of CO_2 on seawater may have diverse consequences for marine calcifiers.

Most studies on the effects of ocean acidification on calcification have focused on warm-water coral reef species (*2*–*4*). Only six species of living, planktonic calcifying organisms have been investigated—four coccolithophore species and two foraminiferan species. Similar to coral reef species (*4*) and several other taxa (*2*, *3*), two bloom-forming coccolithophores (including *Emiliania huxleyi*; see the first figure) (*5*, *6*), showed reduced calcification rates in response to elevated partial pressure of CO_2 (pCO_2) in seawater. However, two other coccolithophore

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species, which are important in terms of calcium carbonate export to sediments, did not conform to this pattern (*7*). Calcification in one species did not significantly change when seawater pCO_2 varied from 150 to 915 μatm (see the second figure, bottom right). In the other species, calcification rates measured over a similar range of pCO_2 levels resulted in a nonlinear curve (see the second figure, bottom left), with maximum calcification occurring at pCO_2 values that correspond to that of the present surface ocean.

Recently, Iglesias-Rodríguez *et al*. (*8*) confirmed that the response of calcifying organisms to CO_2-induced changes in seawater chemistry is not uniform and may be much more complex than previously thought. When the authors grew laboratory cultures of *E. huxleyi* at 750 parts per million by volume (ppmv) CO_2—almost twice the average value of today's surface ocean—rates of calcification doubled compared to calcification rates of cells grown at 280 ppmv (see the second figure, top right). This is in marked contrast to earlier work with laboratory cultures and field populations of *E. huxleyi* (*5*, *6*), which reported decreases in calcification rates under high-CO_2 conditions (see the second figure, top left).

The authors suggest that one reason their results differ from previous work with this species is that they manipulated the inorganic carbon system of seawater by bubbling with air containing different concentrations of CO_2. In contrast, several previous studies used hydrochloric acid to achieve target pCO_2 values (*5*, *6*). The former method more closely mimics ocean acidification because it increases the concentration of total dissolved CO_2, whereas adjusting the carbonate chemistry with acid holds the concentration of total dissolved CO_2 constant. Moreover, compared to the acid technique, the gas bubbling method results in a larger increase in the bicarbonate ion concentration, thought to be the substrate for calcification in *E. huxleyi* (*9*). Several earlier laboratory and mesocosm experiments bubbled with CO_2-enriched air, yet those studies reported reduced calcification rates in *E. huxleyi* in response to elevated pCO_2 (*6*). Part of the discrepancy in results may be due to other differences in experimental conditions. Factors such as nutrients, light, trace metals, temperature, and salinity, as well as their interactive effects with CO_2, can affect coccolithophore calcification in ways that are just beginning to be understood (*6*).

An alternative hypothesis that may explain the divergent results is that the traditional view of *E. huxleyi* as a single species is flawed. Increasingly, evidence suggests that this cosmopolitan species is composed of many varieties with genetically and morphologically distinct properties (*10*, *11*), which may account for the physiological variability observed in *E. huxleyi* with respect to calcification and other processes (*9*). Indeed, the high genetic diversity of *E. huxleyi* may confer ecophysiological plasticity that will allow it to persist and even flourish in the face of ongoing ocean acidification and global warming.

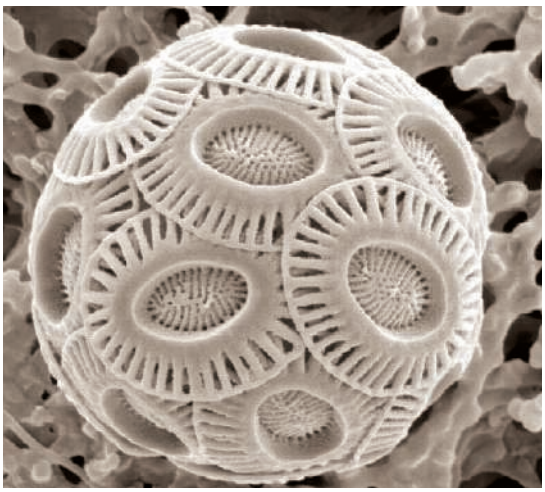
A taxonomically varied response in calcification to CO_2-induced ocean acidification is not limited to coccolithophores. Ries *et al*. (*12*) conducted experiments with a variety of calcareous benthic invertebrates and macroalgae over a range of carbonate ion concentrations and aragonite saturation states. Some organisms had increased rates of calcification in response to elevated pCO_2, while others showed decreased calcification rates, and still others displayed a nonlinear, parabolic response as a function of carbonate saturation state. Even closely related organisms—for example, tropical and temperate sea urchins—showed different responses.

Calcification mechanisms in calcium carbonate–secreting organisms are not sufficiently understood to explain the species-specific differences observed in CO_2-perturbation experiments. The energy dependence of calcification and mechanisms to prevent dissolution implies that there are physiological limitations such that net dissolution will exceed net calcification when CO_2 is raised above a threshold value. The results of Iglesias-Rodríguez *et al*. (*8*) and Ries *et al*. (*12*) suggest that such "tipping points" may vary considerably among calcifying species. A differential response of calcifiers to elevated CO_2 conditions may result in competitive advantages that could drive the reorganization of many planktonic and benthic ecosystems, which in turn could have significant ecological and biogeochemical implications.

Currently, persistent aragonite undersaturation of surface seawater in high latitudes is projected to occur as early as 2050 (*13*). Recent observations of seasonal aragonite undersaturation of surface waters in temperate, nearshore regions, however, suggest that some calcareous organisms may already be experiencing substantial, transient changes in seawater CO_2 chemistry (*14*, *15*). Given the importance of coastal and high-latitude regions to fisheries and other ecosystem services, a comprehensive understanding of the impacts of ocean acidification is urgently needed.

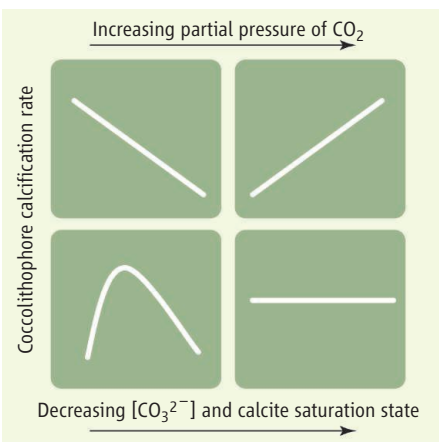
Future work should investigate additional species and life stages of calcifiers, examine the possible interactions of increasing CO_2 with other environmental variables, and explore the capacity of organisms to adapt to projected changes. Equally important is the development of internationally agreed-upon, standardized protocols for the control of seawater composition in manipulative experiments, as well as for the measurement of calcification rates. Without such protocols, it will continue to be difficult to compare results among laboratories, across taxa, between regions, or over time.

The diverse pattern of poorly understood biotic responses to ocean acidification found thus far makes it problematic to reliably predict the ecological and biogeochemical changes that will result from continued oceanic uptake of anthropogenic CO_2. As



Scanning electron micrograph of the coccolithophore *Emiliania huxleyi*.

PHOTO CREDIT: JEREMY YOUNG/NATURAL HISTORY MUSEUM, LONDON



Calcification response patterns of four coccolithophore species to elevated CO_2. (**Top left**) Decreased calcification (*5, 6*); (**top right**) increased calcification (*8*); (**bottom left**) nonlinear response (*7*); (**bottom right**) no change (*7*). Similar response patterns have been identified in benthic organisms (*12*).

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atmospheric CO_2 levels continue to rise, we are embarking on a global experiment with as yet uncertain long-term consequences for many marine calcifiers.

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MATERIALS SCIENCE

Stronger, Tougher Steels

Insights into the microstructure and brittle failure of steels is leading to a new generation of structural steels.

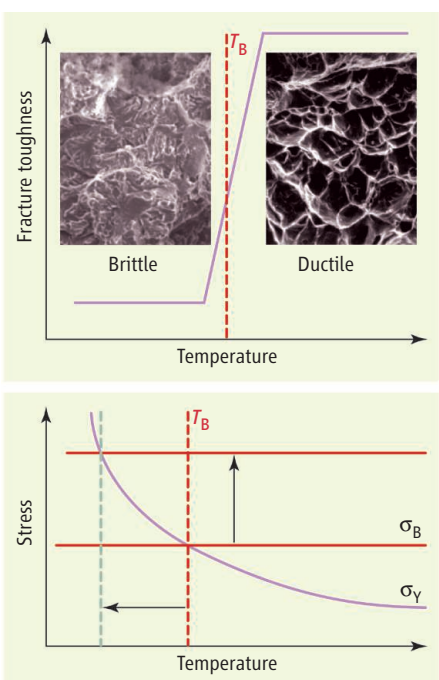
J. W. Morris Jr.

Steel is the workhorse of our infrastructure. Stronger, tougher steels are always needed to reduce weight and improve safety in transportation, enhance architectural flexibility in construction, and improve performance in heavy machinery. For structural steels to be both strong and tough (resistant to fracture), they must not be used at temperatures below the ductile-brittle transition temperature, T_B, at which the steel loses its toughness and fractures in a brittle mode (see the figure, top panel). This transition results from a competition between plastic deformation and brittle fracture at the tips of cracks or flaws in the steel. It can be controlled by techniques such as grain refinement that inhibit brittle fracture, or by techniques such as controlled delamination that facilitate plastic deformation. On page 1057 of this issue, Kimura *et al.* (*1*) show how these approaches can be combined to achieve low T_B and high toughness in an ultrahigh-strength low-alloy steel.

The ductile-brittle transition became infamous during the Second World War when hundreds of mass-produced American "liberty ships" cracked or split open in northern waters (*2*). This aquatic carnage led to the first systematic research on the ductile-brittle transition in steel and stimulated the development of fracture mechanics as a science.

After the war, the need for cold-weather and cryogenic structures—from pipelines, ships, and offshore structures for use in cold waters to the extremely low temperatures required for transporting and storing liquid helium—impelled research on methods to lower T_B. Initial successes used relatively expensive alloy additions, particularly nickel. As techniques such as transmission electron microscopy began to reveal the complex microstructures of high-strength steels, it became clear that the alloy additions modified the microstructure in ways that promoted ductile fracture (*3*). Since the 1970s, researchers have focused on thermal or thermomechanical processes that produce desirable microstructures in low-alloy steels, or processes that combine alloying and thermomechanical treatments to achieve high strength and toughness.

The source of the ductile-brittle transition can be understood from the Yoffee diagram (see the figure, bottom panel), an idealized representation of the relative likelihood of ductile or brittle fracture at the tip of a preexisting crack (*4*). As the applied stress is increased toward failure, the peak stress at the crack tip first reaches one of two levels: the effective yield stress (σ_Y) (*5*), at which significant plastic deformation occurs, or the brittle fracture stress (σ_B), at which the crack propagates in a brittle mode. The effective yield stress rises as the temperature drops, and the ductile-brittle transition temperature, T_B, is that at which σ_Y rises above σ_B.



Avoiding brittle fracture. (Top) At the ductile-brittle transition (T_B), the steel becomes brittle and most of its toughness is lost. **(Bottom)** The Yoffee diagram illustrates T_B at the intersection of the effective yield stress (σ_Y) and the brittle fracture stress (σ_B). Raising the brittle fracture stress (vertical arrow) causes a substantial decrease in T_B (horizontal arrow).

The Yoffee diagram suggests two ways to suppress the ductile-brittle transition: raising the brittle fracture stress or lowering the effective yield stress. Because a high yield stress is a desirable feature of a structural steel, most research has focused on the brittle fracture stress. Raising the brittle fracture stress (vertical arrow in the figure) is very effective in lowering T_B (horizontal arrow).

To raise the brittle fracture stress, it is first necessary to suppress intergranular fracture along grain boundaries. Intergranular embrittlement is usually a result of chemical impurities such as sulfur and phosphorus that accumulate between the grains. It can be controlled by purifying the steel to remove the offending species, or by introducing microalloy additions to "getter" them into mechanically innocuous precipitates. Suppressing intergranular fracture in steel lowers T_B but does not eliminate the ductile-brittle transition. The steel can also fracture by a cleavage mechanism in which the individual crystal grains break by separation on the face planes of the iron crystal; this transgranular cleavage mechanism must also be addressed.

The best way to raise σ_B for transgranular cleavage is to refine the "effective grain size," that is, to decrease the mean free path of a cleavage crack before a grain boundary or

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