Ca^{2+}-dependent gene transcription to activation of a specific type of VGCC? Recent work (*8*) suggests that the unique biophysical properties of L-type VGCCs enables them to discriminate between synaptic potentials (excitatory postsynaptic potentials) and action potentials. Clearly, such filtering would be useful for fine-tuning the coupling between Ca^{2+}-mediated gene transcription and different forms of neural stimulation (for example, orthodromic versus antidromic). Second, what steps lie between Ca^{2+} entry and gene transcription? The Dolmetsch work (*1*) implies that activation of the mitogen-activated protein kinase (MAP kinase) cascade is one such intermediate step. However, it is unclear how MAP kinase activation is connected to the rapid translocation of calmodulin to the nucleus after stimuli that open L-type VGCCs (*16*). Finally, does this general mechanism for specifying membrane-to-nucleus signaling apply to other types of VGCCs and Ca^{2+} binding proteins? When P/Q-type VGCCs interact with Ca^{2+}/calmodulin, their biophysical properties change in a manner reminiscent of the Ca^{2+}-dependent inactivation of L-type VGCCs (*17, 18*). The entry of Ca^{2+} through P/Q-type VGCCs may specify the transcription of unique sets of genes. Given that the sequencing of the human genome has identified more than 80 proteins related to calmodulin (*19*), many with functions as yet undetermined, a rich repertoire of potential interactions between this class of proteins and VGCCs remains to be explored.

References

1. R. E. Dolmetsch *et al.*, *Science* **294**, 333 (2001).
2. L. D. Hirning *et al.*, *Science* **239**, 57 (1988).
3. N. V. Marrion, S. J. Tavalin, *Nature* **395**, 900 (1998).
4. M. Tanabe, B. H. Gähwiler, U. Gerber, *J. Neurophysiol.* **80**, 2268 (1998).
5. K. Deisseroth, H. Bito, R. W. Tsien, *Neuron* **16**, 89 (1996).
6. Z. H. Sheng, R. E. Westenbroek, W. A. Catterall, *J. Bioenerg. Biomembr.* **30**, 335 (1998).
7. M. Sheng, M. A. Thompson, M. E. Greenberg, *Science* **252**, 1427 (1991).
8. P. G. Mermelstein, H. Bito, K. Deisseroth, R. W. Tsien, *J. Neurosci.* **20**, 266 (2000).
9. P. Brehm, R. Eckert, *Science* **202**, 1203 (1978).
10. R. D. Zühlke, H. Reuter, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3287 (1998).
11. B. Z. Peterson, C. D. DeMaria, D. T. Yue, *Neuron* **22**, 549 (1999).
12. N. Qin, R. Olcese, M. Bransby, T. Lin, L. Birnbaumer, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2435 (1999).
13. R. D. Zühlke, G. S. Pitt, K. Deisseroth, R. W. Tsien, H. Reuter, *Nature* **399**, 159 (1999).
14. X. M. Xia *et al.*, *Nature* **395**, 503 (1998).
15. G. S. Pitt *et al.*, *J. Biol. Chem.* **276**, 30794 (2001).
16. K. Deisseroth, E. K. Heist, R. W. Tsien, *Nature* **392**, 198 (1998).
17. A. Lee *et al.*, *Nature* **399**, 155 (1999).
18. C. D. DeMaria *et al.*, *Nature* **411**, 484 (2001).
19. J. C. Venter *et al.*, *Science* **291**, 1304 (2001).

PERSPECTIVES: CARBON CYCLE

Potential Impacts of CO_2 Injection on Deep-Sea Biota

Brad A. Seibel and Patrick J. Walsh

The potential for global warming has spurred the development of various strategies to control the concentrations of greenhouse gases, particularly CO_2, in the atmosphere. Technologies for carbon capture, storage, and sequestration to reduce greenhouse gas concentrations are receiving increasing attention (*1*). Because of its enormous volume, the ocean is an attractive site for possible storage of CO_2. First proposed nearly 25 years ago (*2*), CO_2 disposal in the ocean is now being actively explored (*3*, *4*).

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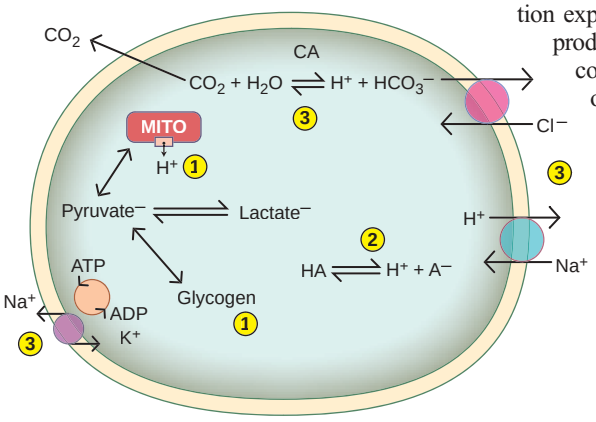
Recent modeling studies indicate that CO_2 must be released at great depths to avoid substantial outgassing (*5*). Direct studies of the biological consequences of CO_2 injection are in their infancy (*4*), but a large literature on the physiology of deep-living animals indicates that they are highly susceptible to the CO_2 and pH excursions likely to accompany deep-sea CO_2 sequestration. Microbial populations may be highly susceptible as well. The impacts of ocean sequestration on deep-sea biota and the biogeochemical cycles dependent on their metabolism are therefore of great concern. Increased CO_2 results in decreases in seawater pH. Primary responses of organisms to the consequent internal acid-base imbalance include metabolic production and consumption of acid-base equivalents, passive chemical buffering of intra- and extracellular fluids, and active ion transport (*6*, *7*) (see the figure). CO_2 and proton transport by extracellular respiratory proteins such as hemoglobin are also important for maintaining acid-base balance in some animal groups.



Regulation of intracellular pH in an animal cell. (1) Metabolic interconversion of acids and bases. (2) Buffering; HA represents a weak acid or base with a dissociation constant in the physiological pH range. (3) Transport of acids and bases across cell membranes; carbonic anhydrase (CA) catalyzes the hydration of CO_2 to yield H_2CO_3, which then dissociates to H^+, HCO_3^-, and CO_3^{2-} (an abbreviated reaction is shown). MITO, mitochondrion.

Failure to control pH within physiological limits due to sequestered CO_2 will have important consequences for the health of aquatic organisms, as has been demonstrated for the effects of acid rain on freshwater fish (*8*). Acid-base imbalances can lead to dissolution of exoskeletal components such as calcareous shells, metabolic suppression (a condition expected to retard growth and reproduction), reduced activity, loss of consciousness due to disruption of oxygen-transport mechanisms, and, if persistent, death.

Over the last 30 years, in situ and laboratory studies of the oxygen consumption rates of deep-sea animals have shown that deep-living animals—both fishes and invertebrates—have low metabolic rates (*9*). The metabolic rates of some deep-living animal species are nearly three orders of magnitude lower than those of their shallow-living relatives after correction for temperature differences (*10*). Metabolism in the deep sea appears to be reduced in part because selection for locomotory capacity is relaxed due to light-limitation on predator-prey interactions. It is reduced further by cold temperatures (*9*) and, in at least some instances, by limited food supply (*11*, *12*).

The reduction in metabolic rates with increasing depth is found to varying degrees in all phyla and all regions studied to date and extends to the deepest depths of the ocean (*9*). Microbial activity is also greatly reduced in the deep sea (*12*). Metabolic pathways in living cells are tightly regulated such that the production and consumption of metabolic end products are in balance.

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Species with low metabolic rates are therefore expected to tolerate only low concentrations of metabolic end products such as CO_2 and protons.

Under conditions of increased CO_2 outside the organism, CO_2 rapidly crosses biological membranes and is hydrated to carbonic acid, which subsequently dissociates to release protons into the cell (see the figure). Proton-elimination pathways may not be able to react fast enough to avoid proton accumulation in the cell. Passive buffering is then the only mechanism immediately available in the cell that can hold pH levels to values compatible with life functions (*13*).

In biological fluids, buffering is typically achieved by low molecular weight, nonbicarbonate buffers with dissociation constants close to physiological pH (for example, histidine-containing substances). Histidyl residues in proteins also contribute to buffering capacity (*14*). In line with the above suggestion that metabolic activity correlates with tolerance of acid-base disturbance, intracellular nonbicarbonate buffering capacities in deep-living fishes and cephalopods (squids and octopuses) are as much as 100 times lower than in comparable shallow-living species (*10*, *14*).

In the longer term (10 to 36 hours), ion-transfer processes at gas-exchange surfaces are required to eliminate H^+ and HCO_3^- accumulated during increased CO_2 conditions in the environment (*6*). Active ion transfer is usually mediated by carriers such as Na^+/H^+, Na^+/K^+, and Cl^-/HCO_3^- exchangers and pumps (see the figure) and is thus limited by the capacity and concentration of these carriers. Carbonic anhydrase also plays an important role by catalyzing the interconversion of CO_2 with HCO_3^- and H^+, thus generating counterions that facilitate CO_2 elimination (*6*) (see the figure).

Capacities for active ion regulation are greatly reduced in the gills of deep-sea fishes (*15*, *16*). Deep-sea animals (other than those inhabiting hydrothermal vents) also have substantially reduced carbonic anhydrase activities in gas-exchange tissue relative to shallower living species (*17*, *18*). This further limits their ability to eliminate protons from the body.

Reduced blood pH typically decreases the affinity of respiratory proteins (such as hemoglobin) for oxygen. Metabolic production of proton-equivalents thus facilitates unloading of oxygen at the tissues. Decreased respiratory protein–oxygen affinity following acid-base disturbance due to increased environmental CO_2 will, however, lead to a diminished capacity for oxygen uptake at the gill. In some organisms (e.g., mammals), the pH sensitivity of oxygen-transport proteins in the blood is positively correlated with metabolic rate, a finding that, if true for deep-sea organisms, would suggest insensitivity to pH excursions (*19*). But most deep-sea species have highly pH-sensitive respiratory proteins despite low metabolic rates (*20*, *21*).

A drop in arterial pH by just 0.2 would reduce bound oxygen in the deep-sea crustacean *Glyphocrangon vicaria* by 25% (*22*). A similar drop in arterial pH would reduce bound oxygen in the midwater shrimp *Gnathophausia ingens* by 50% (*20*). A drop in seawater pH by 0.5 diminished the effectiveness of oxygen uptake in this species (*23*), suggesting a very limited ability to protect extracellular pH during seawater pH excursions. Deep-sea fish hemoglobins are even more sensitive to pH (*21*).

Recent evidence suggests that small increases in CO_2 and the resultant reduced seawater pH may trigger metabolic suppression in a variety of organisms (*19*, *24*). Metabolic suppression is an adaptive strategy used by many aquatic organisms to survive temporary environmental energy limitation (such as oxygen deficiency or food deprivation). In the deep sea, this reversible response ranges from seasonal (*24*) to short-term metabolic suppression between food pulses (*11*). Many oceanic animals are suspected of suppressing metabolism during diurnal migrations into oxygen-deficient waters (*20*, *25*).

Metabolic suppression is achieved, at least in part, by shutting down expensive processes such as protein synthesis (*24*). Low pH has been shown to inhibit protein synthesis in trout living in lakes rendered acidic through anthropogenic effects (*8*). The ratio of oxygen consumed to nitrogen excreted decreased in an intertidal worm under increased CO_2 conditions, suggesting reduced protein synthesis during metabolic suppression (*19*). Metabolic suppression through reduced protein synthesis may be an important consequence of CO_2 sequestration, resulting in decreased growth and reproductive output.

The severity and extent of pH and CO_2 excursions that will result from deep-sea CO_2 sequestration will depend heavily on the injection method. In one recently modeled scenario, a CO_2-release rate corresponding to emissions from a single gas power plant reduced seawater pH by more than 0.1 within a volume of 0.5 km^3 (*5*). Other methods may result in more serious departures (more than one pH unit) over much larger areas (over 100 km from the injection site) (*26*).

We estimate that sequestration of CO_2 sufficient to stabilize atmospheric concentrations at 550 parts per million by volume (twice the pre-industrial level) would change the pH of the entire ocean, on average, by ~0.1 by 2100. This is a substantial fraction of the pH range of seawater. CO_2 concentrations and pH vary hourly or diurnally in some habitats (such as tidepools), but in the deep sea they have been stable for thousands of years, and organisms are highly attuned to this stability (*20*). For this reason, Haedrich (*27*) suggests that "any change that takes place too quickly to allow for a compensating adaptive change within the genetic potential of finely adapted deep-water organisms is likely to be harmful."

The available data indicate that deep-sea organisms are highly sensitive to even modest pH changes, a contention supported by preliminary in situ experiments (*28*). Small perturbations in CO_2 or pH may thus have important consequences for the ecology of the deep sea and for the global biogeochemical cycles dependent on deep-sea ecosystems. Experiments on CO_2 injection must carefully define the spatiotemporal extent of CO_2 and pH excursions from normal so that the acute and chronic effects of increased CO_2 on deep-sea, and perhaps global, ecosystems can be predicted from existing (and additional) data on the physiology and ecology of deep-sea organisms. Only then can the risks and benefits of deep-sea carbon storage be assessed appropriately.

References and Notes

1. R. A. Kerr, *Science* **292**, 1978 (2001).
2. C. Marchetti, *Climate Change* **1**, 59 (1977).
3. P. G. Brewer, *Oceanography* **13**, 84 (2000).
4. M. N. Tamburri *et al.*, *Mar. Chem.* **72**, 95 (2000).
5. H. Drange, G. Alendal, O. M. Johannessen, *Geophys. Res. Lett.* **28**, 2637 (2001).
6. P. J. Walsh, C. L. Milligan, *Can. J. Zool.* **67**, 2994 (1989).
7. D. White, *The Physiology and Biochemistry of Prokaryotes* (Oxford Univ. Press, New York, 1995).
8. I. J. Morgan, D. G. McDonald, C. M. Wood, *Global Change Biol.* **7**, 345 (2001).
9. J. J. Childress, *Tree* **10**, 30 (1995).
10. B. A. Seibel, E. V. Thuesen, J. J. Childress, L. A. Gorodezky, *Biol. Bull.* **192**, 262 (1997).
11. K. L. Smith Jr., R. J. Baldwin, *Mar. Biol.* **68**, 287 (1982).
12. J. D. Gage, P. A. Tyler, *Deep-Sea Biology: A Natural History of Organisms at the Deep-Sea Floor* (Cambridge Univ. Press, Cambridge, 1991).
13. N. Heisler, *Can. J. Zool.* **67**, 2923 (1989).
14. G. N. Somero, in *Transport Processes, Iono– and Osmoregulation*, R. Gilles, M. Gilles-Baillien, Eds. (Springer, Berlin, 1985), pp. 454–468.
15. S. K. Goffredi, J. J. Childress, *Mar. Biol.* **138**, 259 (2001).
16. A. H. Gibbs, G. N. Somero, *Mar. Biol.* **106**, 315 (1990).
17. R. P. Henry, H. L. Handley, A. Krarup, H. M. Perry, *J. Crustac. Biol.* **10**, 413 (1990).
18. R. E. Kochevar, J. J. Childress, *Mar. Biol.* **125**, 375 (1996).
19. H. O. Pörtner, A. Reipschläger, in *Ocean Storage of Carbon Dioxide. Workshop 2—Environmental Impact*, B. Ormerod, M. V. Angel, Eds. (International Energy Agency Greenhouse Gas R&D Program, Cheltenham, UK, 1996), pp. 57–81.
20. J. J. Childress, B. A. Seibel, *J. Exp. Biol.* **201**, 1223 (1998).
21. R. W. Noble *et al.*, *Biochim. Biophys. Acta* **870**, 552 (1986).
22. A. J. Arp, J. J. Childress, *Physiol. Zool.* **58**, 38 (1985).
23. T. Mickel, J. J. Childress, *Biol. Bull.* **154**, 138 (1978).
24. M. Guppy, P. Withers, *Biol. Rev.* **74**, 1 (1999).
25. J. C. Hunt, B. A. Seibel, *Mar. Biol.* **136**, 543 (2000).
26. D. Auerbach, J. Caulfield, H. Herzog, E. Adams, in *Ocean Storage of Carbon Dioxide: Workshop 2—Environmental Impact*, B. Ormerod, M. V. Angel, Eds. (International Energy Agency Greenhouse Gas R&D Program, Cheltenham, UK, 1996), pp. 41–55.
27. R. L. Haedrich, *J. Fish Biol.* **49**, 40 (1996).
28. J. Barry, B. Seibel, in preparation.