gests that dieldrin and toxaphene, at the lowest doses used, appeared to have induced the progesterone receptor, an estrogen-specific marker in mice, in a synergistic manner; no indication of this effect was seen when measuring uterine weight or uterine peroxidase activity. This suggests that some estrogen-dependent phenomena are better markers than others for revealing synergistic responses. Consistent with this idea is the observation that a combination of estradiol and 3,4,3′,4′,-tetrachlorobiphenyl synergistically induces pS2, an estrogen-regulated protein, but not another estrogen responsive marker, in the human breast cancer cell line, ZR-75-1 (*5*). Indeed, synergy observed in one cell line (ZR-75-1) was not seen in another (MCF-7) in the same study (*5*); this underscores the importance of cell type in determining estrogenic responses.

A mechanism underlying these synergistic effects remains to be determined. One of our working hypotheses is that under conditions in which the ER tends to exist as a monomer, the binding characteristics of two interacting molecules are different from that observed at high receptor concentrations. We contend that this low ER experimental condition better approximates ER concentrations found during early development [the ER content of uterine epithelial cells is low in fetal or newborn mouse (*6*) or rat (*7*), a period critical for estrogen-associated disorders (*8*)]. During these sensitive periods, chemical interactions resulting in synergy may occur at conditions in which critical ligand-receptor or receptor-receptor combinations occur.

Synergism between weakly estrogenic chemicals may not be universal, as Ramamoorthy *et al.* suggest. However, synergy in biological systems has a long history. Synergy has been observed between steroid hormones, different nuclear receptors (*9*), membrane and nuclear receptors (*10*), drugs and hormones (*11*), and temperature and hormone response (*12*). Synergistic interactions have also been observed between drugs and temperature (*13*) and weakly estrogenic compounds (*4*). Our discovery of synergy of natural and synthetic estrogens was made by observing the effects of these compounds on the sexual development of turtle embryos. We demonstrated synergy between a combination of two polychlorinated biphenyls (*4*), and, more recently, two steroidal estrogens (*14*). We also have recently reported that the binding of chemical mixtures to the estrogen receptor from the American alligator occurs in a synergistic manner (*15*). Our laboratory has shown that a combination of phytoestrogens produced a synergistic response in yeast (*16*). In addition, in cell culture studies of fish hepatocytes (*17*) as well as mammalian cells (*18*), mixtures of weakly estrogenic chemicals were shown to act synergistically in stimulating estrogenic responses appropriate to the species. These findings together suggest that the synergistic action of weak estrogens may be phylogenetically conserved and therefore fundamental.

We currently are evaluating the occurrence of synergistic interactions of chemicals with the ER in different yeast strains, mammalian cells, and biological systems. We have noted synergy in some yeast strains, but not others, as well as an apparent relationship to ER concentrations (*19*). We have likewise found a synergistic interaction between ovarian steroidal estrogens in both a yeast-based assay and the developing turtle (*14*). These latter studies both confirm and extend our previous report (*1*) and suggest a mechanism for synergy. We look forward to the continued clarification of this important issue.

John A. McLachlan
Steven F. Arnold
Diane M. Klotz
Bridgette M. Collins
Peter M. Vonier
Tulane-Xavier Center for
Bioenvironmental Research,
New Orleans, LA 70112, USA
Louis J. Guillette
Department of Zoology,
University of Florida,
Gainesville, FL 32611, USA

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Redox Stabilization of the Atmosphere and Oceans and Marine Productivity

Philippe Van Cappellen and Ellery D. Ingall provide a coupled biogeochemical box model to investigate whether negative feedbacks between the global cycles of phosphorus and oxygen might have stabilized the amount of atmospheric O_2 during the Phanerozoic (*1*). We have duplicated these results (*1*), but have found that slight modifications to the treatment of tectonic uplift and resultant weathering rates dramatically affect the outputs of the model.

Van Cappellen and Ingall set the rate of O_2 consumption during weathering to be proportional to the global rate of uplift. The rate of O_2 production is highly sensitive to marine reactive P availability through interactions with the carbon cycle. Van Cappellen and Ingall assume that the rate of P input to the oceans depends only on the size of the terrestrial lithosphere reservoir of this element and not on weathering rates. This assumption virtually decouples the rate of oxidative weathering from that of P transfer to the oceans on time scales of tens to hundreds of millions of years and accounts for the rapid depletion in atmospheric O_2 in the model after an increase in uplift rate (Fig. 1). It seems more likely that the flux of P to the oceans also depends on the rate of uplift. Today, refractory, detrital P phases account for less than 25% of the total solid-phase P in most marine sediments (*2*), and changes in total continental P weathering rates have apparently led to comparable changes in the chemical weathering of P phases over at least the last 100 million years (My) (*3*). When the model (*1*) is run with P and Fe oceanic inputs coupled to uplift rates, atmospheric O_2 is found to rise slightly rather than decrease dramatically in response to an increase in the uplift rate (Fig. 1.)

The output of the model (*1*) is also adversely influenced by the assumption that the C:P ratio in oxic sediments is much smaller (200) than in anoxic sediments (400 to 4000). Lower C:P ratios in oxic sediments are attributed to relative enrichment in P during organic matter remineralization by aerobic benthic bacteria (*4*), a

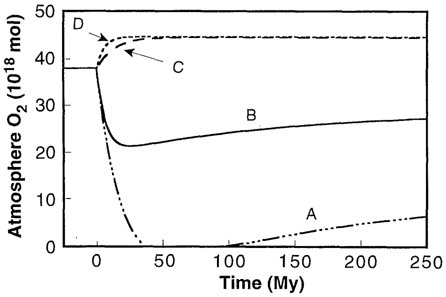

Fig. 1. Modeled change in atmospheric oxygen content in response to the tectonic forcing simulation. Curves (A) and (B) show output from the original model (*1*); curves (C) and (D) show output from our modified version of the model. At time = 0, the model shifts instantaneously from steady-state conditions to a tectonic forcing regime in which uplift and weathering rate coefficients are increased by 50% (in the original model, 50% enhanced uplift and weathering is simulated by multiplying k_{uplift} by 1.5; our modifications couple the kinetics of material transfer between "land" reservoirs and ocean reservoirs by multiplying not only k_{uplift}, but k_{25} and k_{1011} by the enhancement factor of 1.5). Curves A and D represent the results from model runs in which only Fe(III)-bound phosphorus burial rates are inversely related to concentrations of O_2; curves B and C represent results from model runs in which both Fe(III)-bound phosphorus and organic phosphorus burial rates are inversely related to concentrations of O_2. A value of 4000 was used for $C{:}P_{anoxic}$ in B and C model runs to show the maximum negative feedback tested. Decline in atmospheric O_2 in curves A and B is an artifact of the decoupling of oceanic inputs of P and Fe from weathering rates. Rise in atmospheric oxygen depicted in curves C and D results from enhanced oceanic P inputs that increase primary production. Increased primary production leads to a larger burial flux of reduced carbon and causes deep ocean water to become more anoxic, which in turn favors a shift toward burial of Fe and S in reduced phases. These enhanced burial fluxes of reduced species more than offset the enhanced oxidative weathering of reduced species, causing a slight increase in atmospheric O_2 content.

mechanism that deserves investigation. Currently available data for the C:P ratio of marine organic matter in modern marine sediments do not support this assumption (*5*). To test this hypothesis, studies are needed in which modern sediments, with spatially uniform accumulation rates and bottom-arriving compositions, undergo early diagenesis under widely differing conditions of bottom water oxygenation for diagenetically significant periods of time. Such studies should attempt to identify C:P ratios in the benthic microbial biomass and assess the control on total $C{:}P_{organic}$ exerted by the benthic biomass. To our knowledge, no published studies have effectively isolated the impact of bottom water O_2 content on the C:P ratio in buried marine organic matter in this manner.

One of the mechanisms proposed by Van Cappellen and Ingall for stabilizing atmospheric O_2, the Fe-P burial negative feedback mechanism, was first described by us in 1994 (*6*) and had received brief attention several years earlier (*7*). Other studies have also concluded that feedback between atmospheric O_2 and nutrient availability may have been important in regulating atmospheric O_2 (*8*).

Albert S. Colman
Department of Earth and Planetary Sciences,
Harvard University,
Cambridge, MA 02138, USA
asc@io.harvard.edu
Fred T. Mackenzie
Department of Oceanography,
University of Hawaii,
Honolulu, HI 96822, USA
fredm@soest.hawaii.edu
Heinrich D. Holland
Department of Earth and Planetary Sciences,
Harvard University

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Response: Central to our report (*1*) is the hypothesis that the redox-dependent burial of organic plus iron-bound phosphorus in marine sediments leads to a negative feedback mechanism stabilizing atmospheric O_2 on geologic time scales (*1*). We quantitatively tested the effectiveness of the feedback by simulating the response of the atmospheric O_2 content to a fairly large initial imbalance between the rates of consumption and production of atmospheric O_2. The imbalance was created by *deliberately* allowing the rate of oxidative weathering on land to increase after an uplift event, while delaying the increase in the rate of delivery of reactive P to the oceans by transient storage in the terrestrial reservoir [see reference 16 in (*1*)]. Thus, at the onset of the simulation, consumption of atmospheric O_2 greatly exceeded its production. The proposed feedback mechanism, however, rapidly restored the atmospheric O_2 balance by acting on the marine sedimentary burial of organic C plus iron sulfide. The effectiveness of the feedback does not depend on the mechanism causing the initial perturbation of the O_2 balance.

Colman *et al.* argue against the decoupling of the rate of O_2 consumption by oxidative weathering and the rate of reactive phosphorus delivery to the oceans that we used to produce the initial O_2 imbalance. As shown by their simulations, if the rates remain strictly proportional to one another, little effect of the feedback is observed because, under these conditions, the uplift event does not produce a significant imbalance in the rates of O_2 consumption and production. Although this result does not directly address the validity of the proposed feedback mechanism, it prompts us to more closely examine the possible causes for perturbations of the atmospheric O_2 balance.

We agree with Colman *et al.* that the flux of reactive P to the oceans depends on the intensity of chemical weathering and, hence, it must somehow be coupled to the rate of oxidative weathering. Nonetheless, the reactions and crustal constituents involved in oxidative weathering differ from those releasing P. Furthermore, P mobilized from primary minerals is extensively recycled on land, before being supplied, under variable chemical forms, to the oceans (*2*). Thus, the relationship between the reactive (bioavailable) P flux to the oceans and the rate of O_2 uptake by weathering depends on variables such as the lithology of drainage basins, continental topography, and climate. It is therefore unlikely that this relationship has

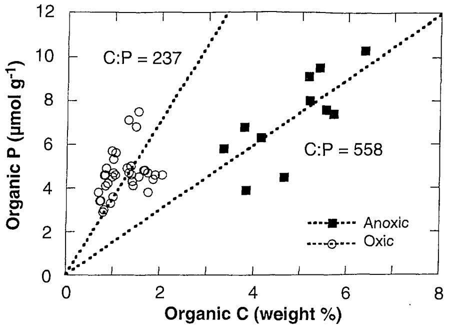

Fig. 1. Organic phosphorus plotted against organic C concentrations in modern Black Sea sediments. Anoxic sites correspond to microlaminated Black Sea abyssal plain deposits [Stations 9, 14, and 18A in (*8*)]. Oxic sites are from the basin margin and were overlain by oxic to suboxic waters at the time of coring [Stations 3, 4, 16, 16B, and 17 in (*8*)]. Molar organic C:P ratios are calculated from linear regression lines forced through the origin.

remained constant over geologic time.

Perturbations of the atmospheric O_2 balance can also be produced by forcings other than relative changes in the rates of oxidative weathering and reactive P supply to the oceans. A change in the intensity of ocean mixing, for example, would rapidly change the net rate of atmospheric O_2 production, by modifying the oceanic burial of organic carbon (3). However, it would not immediately affect the rate of O_2 uptake on the continents.

Thus, perturbations are likely to have affected the atmospheric O_2 balance during the Phanerozoic (4). Our modeling results suggest that the proposed feedback would have efficiently limited the impact of these perturbations on the atmospheric O_2 level. Whether the feedback acted alone or in concert with others remains to be determined (5).

According to Colman *et al.*, there is a lack of data from modern marine sediments supporting an inverse relation between the C:P ratio of buried organic matter and bottom water oxygenation. We proposed such a relation on the basis of a combination of data from modern marine and freshwater depositional environments, ancient shale sequences, wastewater treatment systems, and microbial studies (6). Additional evidence (Fig. 1) shows organic C:P ratios preserved in recent Black Sea sediments (7). The ratios are systematically higher for sites with permanently anoxic bottom waters. If the difference between oxic and anoxic end-members observed in the Black Sea were to be extrapolated to the entire ocean, our model would predict a strong stabilizing effect on atmospheric O_2 [figure 2C in (1)]. Although more studies are needed to isolate the effect of bottom water oxygenation from those of other environmental variables, the currently available evidence agrees with our hypothesis.

P. Van Cappellen
School of Earth and Atmospheric Sciences,
Georgia Institute of Technology,
Atlanta, GA 30332–0340, USA
E. D. Ingall
Marine Science Institute,
University of Texas at Austin,
Port Aransas, TX 78373–1267, USA

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Polyalanine Expansion in Synpolydactyly Might Result from Unequal Crossing-Over of *HOXD13*

Yasuteru Muragaki *et al.* (1) state that synpolydactaly, an autosomal dominant condition resulting in variable webbing and duplication of the digits, results from a polyalanine repeat expansion in the protein HOXD13. They found that the normal human HOXD13 contains 15 alanine residues near the amino terminus of the protein and, in three families segregating synpolydactaly, the disorder was associated with unusual *HOXD13* alleles that predict an expansion of the polyalanine tracts to 22, 23, and 25 residues, respectively. It is likely that the expanded polyalanine tract alters or changes the function of the mutant HOXD13, thereby leading to the disorder. However, Muragaki did not comment on the mutational mechanism that may lead to these abnormal alleles.

Recently, it has been stated that expanded polyglutamine tracts are responsible for a number of hereditary neurodegenerative diseases (2). These disorders are a result of the unstable expansion of the glutamine codon CAG, which is believed to result in an altered function of the mutant proteins. Because of these similarities, one could in-

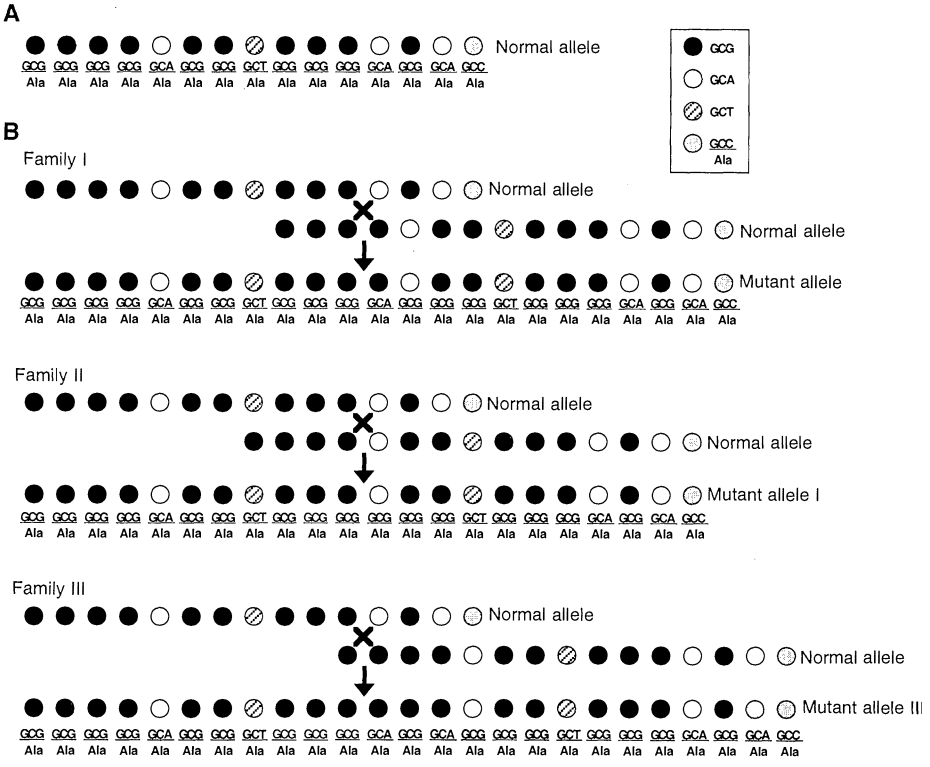

Fig. 1. (**A**) DNA sequence of the polyalanine tract of the normal human *HOXD13* gene. Each distinct alanine codon is represented by a unique circle. (**B**) Derivation of each of the three mutant *HOXD13* alleles found in synpolydactaly by unequal crossing-over of two normal alleles. Possible point of exchange is indicated by an X, and the resulting reading frame is shown below each mutant allele. Mutant alleles are numbered according to families I, II, and III of the report by Muragaki *et al.* (1).