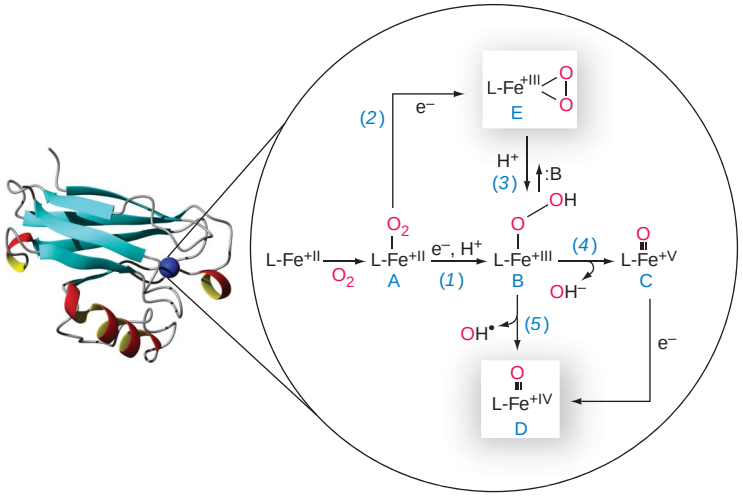
ure have been structurally characterized (*10*).

The widely accepted mechanism (*12*) of O_2 activation by heme iron enzymes (*10*–*12*) involves the initial formation of an Fe^{II}–O_2 (or Fe^{III}–O_2^-) species (intermediate A), which converts to an end-on Fe^{III}–OOH species (intermediate B) upon the addition of an electron (step 1 in the figure). Alternatively, a side-on peroxide (intermediate E) may form (step 2). The first observation of a side-on intermediate (A or E) by x-ray crystallography is reported by Karlsson *et al*. in this issue (*1*).



Mechanistic pathways available to iron in biology. Reduced iron (Fe^{II}) can react with oxygen to form potent biological oxidants. The molecular details of these reactions are revealed by probing the structure of metalloprotein intermediates or of synthetic analogs. Proposed mechanistic pathways and intermediates are shown, with those highlighted in this issue shaded. L, ligand.

A and E are the only intermediates that can activate both oxygen atoms of O_2 equally, thus favoring dioxygenase (chemistry in which both oxygen atoms are incorporated into a substrate) over monooxygenase chemistry. The structure reported by Karlsson *et al*. strongly suggests that naphthalene is oxidized by the nonheme enzyme naphthalene dioxygenase via a concerted mechanism involving intermediate A or E.

As shown by electronic structure calculations (*7*), a side-on peroxide is made more reactive by adding a proton (step 3). Synthetic analog chemistry shows that protonation will convert a side-on peroxide (intermediate E) to an end-on hydroperoxide (intermediate B) (*5*). Intermediate B is believed to act as the key catalytic oxidant in some systems (*3, 6, 11*). Addition of a proton to the distal oxygen of B polarizes the O–O bond, resulting in its heterolytic cleavage (step 4) to afford a high-valent Fe^{V}=O species (intermediate C). If the O–O bond is cleaved homolytically (step 5), the slightly less oxidized Fe^{IV}=O (intermediate D) forms. Intermediates C and D are the other key catalytic intermediates implicated in iron-catalyzed oxidation chemistry.

There has been some skepticism as to whether the high-valent iron-oxo species C and D could form without the support of a porphyrin ligand. If one compares the potential energy surface for a heme and a nonheme iron system undergoing step 4 of the scheme, this reaction appears not to be energetically favored for mononuclear nonheme iron systems (*6*).

Borovik and colleagues showed recently that an oxidized Fe^{III}–O can be stabilized in a mononuclear nonheme environment by hydrogen bonds (*15*). In this issue, Rohde *et al*. (*2*) report that Fe^{IV}=O can also form in a nonheme iron synthetic analog. Mechanistic studies imply that an Fe^{V}=O intermediate serves as the active catalyst in some nonheme iron systems (*3*). Whether Fe^{V}=O really forms and can be observed in a nonheme environment remains to be seen.

Although the mechanistic details of biological nonheme iron–promoted dioxygen activation are still being "ironed out," the observation of highly reactive intermediates reported in this issue (*1, 2*) provides clues regarding preferred pathways. The two studies show that both protein chemistry and synthetic analog chemistry play vital roles in unraveling the molecular-level details of these critical biological reactions.

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SIGNAL TRANSDUCTION

Imposing Specificity on Kinases

Mark Ptashne and Alexander Gann

The mitogen-activated protein kinase (MAPK) signaling cascades transmit signals from the cell surface to its interior. There are at least four MAPK cascades in yeast, and in response to a specific extracellular signal (different in each case), one of these cascades "fires." Each cascade contains three kinases, and sometimes a given kinase (for example, Ste11) is a component of more than one cascade (see the figure). Moreover, despite the fact that each cascade responds to a different signal, several of the cascades are triggered by a common kinase (Ste20). How do different combinations of kinases generate disparate responses? These kinases all have essentially the same active site, which phosphorylates serine or threonine residues in target proteins. How is specificity imposed on these enzymes, that is, how does each kinase choose its correct target protein? How difficult is it to evolve separate pathways using common elements?

On page 1061 of this issue, Park *et al.* (*1*) explore these matters by analyzing two MAPK cascades in yeast. One is involved in yeast mating, the other in the response to high salt concentrations in the medium (the osmolarity response). In each case, the three members of the respective cascade are attached to a protein scaffold—Ste5 in the mating cascade, Pbs2 in the osmolarity cascade. Park *et al.* show that even artificial, and rather loose, tethering of the appropriate kinases to a scaffold suffices for function. They also show that cells bearing a hybrid scaffold (constructed by the authors) elaborate an osmolarity response to a mating signal. These find-

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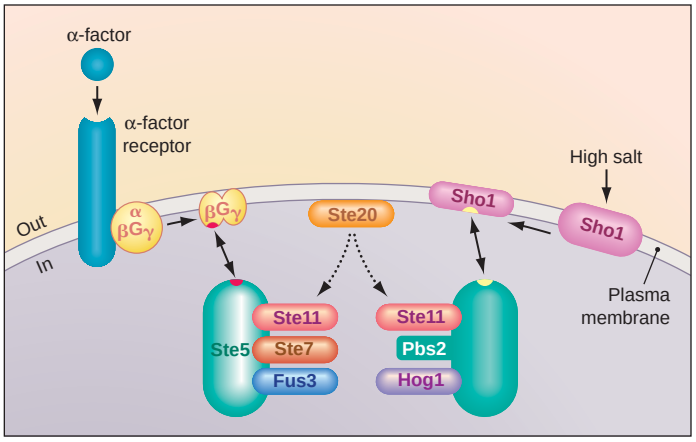
ings resonate with what we have learned about regulation of many other enzymes that work on macromolecules, a matter we return to below. We start by considering the cascade involved in yeast mating.

In broad outline, yeast foreplay goes like this: One yeast cell sends to another a peptide pheromone called α-factor (see the figure). The signal is received by a receptor that is linked, inside the cell, to a heterotrimeric guanine nucleotide–binding protein (G protein). In response to the signal, the G protein dissociates into two parts. One of these, the membrane-bound Gβγ, now exposes a site that binds to the Ste5 scaffold bearing the three inactive kinases that constitute the mating MAPK cascade. The scaffold is thereby recruited to the membrane where it encounters an active kinase already tethered there. This apposition triggers a chain reaction: Ste20 (the active, membrane-bound kinase) phosphorylates and thereby activates Ste11 (the kinase at the "top" of the scaffold in the figure); Ste11, in turn, activates Ste7; and Ste7 activates Fus3, the MAPK at the bottom of the scaffold. Fus3 then finds its targets (transcriptional activators, cytoskeleton organizers, etc.) that, in response to phosphorylation, produce the changes required to complete this early stage of mating.



Activation of two MAPK cascades in yeast. (**Left**) The mating cascade is activated when the cell's α-factor receptor receives the α-factor pheromone from an expectant partner. The receptor is associated with a G protein, and interaction with pheromone frees the Gβγ protein (also called Ste4/18). Gβγ (and in particular the β subunit) exposes a surface that binds to the scaffold Ste5. (**Right**) The osmolarity cascade is activated when the membrane protein Sho1 senses a high salt concentration in the medium. Under high-salt conditions, Sho1 evidently exposes a surface (indicated by the change in shape of Sho1) that binds to the scaffold Pbs2. (**Center**) Ste20 is an active kinase tethered to the membrane (*7*, *8*). Gβγ recruits Ste5 to the membrane, where Ste20 triggers the mating cascade. Sho1 recruits Pbs2 to the membrane, where Ste20 triggers the osmolarity cascade. The Pbs2 scaffold has two bound kinases and an intrinsic kinase domain, as indicated. Fus3 and Hog1 are called MAPKs, Ste7 and Pbs2 (the intrinsic kinase) are MAPKKs, and Ste11 is a MAPKKK. By extension, Ste20 is sometimes called a MAPKKKK.

Ste20, as we have noted, also triggers signaling by MAPK cascades other than the mating cascade. In particular, Ste20 triggers the osmolarity cascade as follows: Upon exposure to high salt concentrations, the membrane protein Sho1 binds to, and recruits to the membrane, the Pbs2 scaffold. When so positioned, its associated kinases are activated by Ste20, just as we saw for the mating cascade. Thus, the pathway that Ste20 stimulates is determined by apposition: One or another of these two scaffolds is recruited to the enzyme, and getting that recruitment right is essential for the specificity of signaling.

The kinases bound to the scaffolds illustrate this principle—imposition of enzyme specificity by apposition—in an even more pointed way. As seen in the figure, Ste11 is associated with the two illustrated cascades (mating and osmolarity). But in one case the substrate for this kinase is Ste7, and in the other the substrate is the intrinsic kinase of Pbs2. (Note that Pbs2 is a scaffold that bears, on a distinct domain, the Pbs2 kinase.) The choice of Ste11's substrate is determined by apposition on one or another scaffold: The scaffold Ste5 apposes Ste11 with Ste7, and the scaffold Pbs2 apposes Ste11 with the intrinsic kinase of Pbs2.

The picture drawn thus far is based on a wide array of genetic, biochemical, and cell biological experiments (*2*). One of these, published in 1998 (*3*), showed that artificially directing the Ste5 scaffold to the membrane sufficed for activation of the mating cascade. This result was obtained even in cells lacking the pheromone receptor and associated G protein, but depended on the presence of the membrane-bound kinase Ste20. We have here an example of a "bypass" experiment: The interaction ordinarily required for activation of the cascade (between the scaffold and Gβγ) was bypassed and was replaced with a simple membrane tether. In this configuration the mating cascade is "on" constitutively.

Park *et al*. describe another set of bypass experiments (*1*). They destroy, by mutation, individual kinase-scaffold interactions in the mating cascade and replace them with heterologous interactions. They find that artificial recruitment of the kinases to the scaffold, effected in this way, restores the response to α-factor. In their experiments, the scaffold is Ste5, the two kinases are Ste11 and Ste7, and the heterologous protein-protein interactions are mediated by PDZ domains (taken from mammalian proteins). The heterologous PDZ–PDZ interaction (between scaffold and kinase or between scaffold-bound kinase and the other kinase) restored the efficiency of mating (a measure of the activity of the cascade) by 3 to 4 orders of magnitude. [The experiment is particularly easy to appreciate by looking at figure 2 in the Park *et al.* paper, and we will not describe it further here. For a related experiment see (*4*)].

The results of Park *et al*. speak particularly to the question of how difficult it might be (conceptually, at least) to evolve one or another MAPK cascade. It has been suggested that the Ste5 scaffold itself undergoes important conformational changes upon encountering Gβγ and Ste20 at the membrane, and that there must be a rather complex relationship between a scaffold and its associated kinases [see, for example, (*5*)]. This may be the case: The bypass cascades of Park and colleagues work less well than do their wild-type counterparts. But the experiments of Park *et al*. show that evolution might have started with simple tethering of kinases to the scaffold, with the addition of elaborations thereafter. Mere tethering is sufficient to elicit biologically important signaling. Moreover, it is clear from these experiments that no very stereospecific arrangement of the kinases on the scaffold is required for this effect.

Park *et al.* carry matters one step further by performing a "specificity swap": They build a hybrid scaffold (which they call a "diverter") that responds to the mating pheromone α-factor by giving an osmoregulatory response. The experiment, which we now describe, shows that even a crude (and easy) cut-and-paste manipulation can change the biological specificity of a MAPK cascade. The diverter scaffold was constructed by fusing a mutant Ste5 scaffold that is defunct for the mating response (because it lacks the site that ordinarily binds Ste7) to a Pbs2 scaffold. The Gβγ-binding site on Ste5 now brings the intact osmolarity scaffold to the membrane, and the osmolarity cascade is activated. Because the Pbs2 scaffold they used

in this experiment lacks the Sho1 interaction site, the diverter gives no response to high salt.

The experiments and conclusions we have outlined are part of a larger picture. Many enzymes that work on macromolecules have, in each case, multiple substrates to choose from—enzymes that transcribe genes, splice RNA, destroy RNA (as in RNA interference), ubiquitinate, degrade, and acetylate (or deacetylate) proteins, and so on. (The typical RNA polymerase, for example, is capable of transcribing many different genes.) Regulation of all these enzymes, like the regulation of kinases described here, entails recruiting the enzyme to the proper substrate. Specificity can be imposed on these enzymes by simple binding interactions not involving the active site and, where explored, there are minimal stereospecific constraints on how that recruitment can be effected. In many cases, artificial binding interactions can replace normal ones (in bypass experiments), and hybrid molecules bearing swapped specificities are readily generated. Much of the diversity we find in the biological world is produced by such regulatory changes occurring over the course of evolution. Nature seems to have hit upon a simple strategy, used over and over again with many different enzymes, to generate those different patterns of regulation (*6*).

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GEOSCIENCE

Microbial Geoengineers

Lesley A. Warren and Mary E. Kauffman

Recent studies of deep-sea hydrothermal vents (*1*), highly contaminated, abandoned mines (see the figure) (*2*), and Earth's deep subsurface (*3*) underscore the ubiquitous presence of microbes in the geosphere. A session at the Fall meeting of the American Geophysical Union (AGU) (*4*) highlighted the close linkages between microbes and geochemistry.

Microbial activity is increasingly implicated in aqueous geochemical processes such as mineral precipitation and dissolution (*5*, *6*), contaminant degradation, sequestration or mobilization (*7*), fossilization, and weathering. These linkages are found in a broad spectrum of aqueous systems, including marine, freshwater, groundwater, and subglacial melt.

However, we are only just beginning to understand how microbes and geochemical processes interact. The geochemical reality of these interactions, which often occur at the micrometer scale, has only recently become quantifiable with high-resolution methods such as x-ray absorption spectroscopy (*8*). Perhaps more importantly, molecular biological techniques have revolutionized environmental microbiology by providing genetic snapshots of microbial diversity without requiring organisms to be cultured in the laboratory (*9*).

Unlike higher plants and animals, microbial evolution is one of metabolic diversity, rather than cell complexity and organism structure. Microbes are not restricted by geographic barriers (*10*), but their metabolic pathways are necessarily constrained by the available redox couples in the biosphere (*11*). However, microbes can use a wide range of electron acceptors other than molecular oxygen for respiration (such as carbonate, ferric iron, nitrate, and sulfate).

The geochemical influence of microbes therefore extends to all the major elemental cycles, particularly those relevant to life on Earth. Further, microbes are reaction accelerators, catalyzing otherwise slow redox reactions to kinetic rates that make them of geochemical interest. Thus, microbial influence on geochemical processes is not only widespread (*12*), but likely predictable, given a more systematic understanding of the mechanisms and controls involved.

Microbial metabolic or functional activities of geochemical interest, such as the oxidation or reduction of Fe and Mn, are often spread across the phylogenetic tree. Numerous genetically differentiated microbial strains can play the same geochemical role in differing environments (*10*), with potentially differing outcomes. Furthermore, many strains can switch metabolic pathways (for example, from sulfate to iron reduction), depending on the prevailing environmental conditions in which they find themselves. Thus, genetic characterization may not be geochemically sensitive.

There has been a rapid growth in the identification of novel microbial strains through phylogenetic analyses (mainly of 16*S* RNA oligonucleotide sequences) from natural systems. The geochemically relevant information gained from such identifications is, however, often limited. It is estimated that less than 10% of the microbes that exist in nature have been identified; of that small number, less than 1% have been successfully cultured.

Thus, despite a growing phylogenetic databank of environmentally identified microbial strains, the metabolism of many strains—and therefore how they might influence geochemical processes—remains unknown. The central issue, from a geochemical perspec-

Microbial geoengineering in action. Microbial slime streamers, a composite of microbes and minerals from Iron Mountain, California, are implicated in acid mine drainage and the cycling of Fe and S.

CREDIT: FROM (*15*)

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