washed with PBS and incubated again for 30 min in α-MEM with Epo or EMP1 to allow for efflux of unbound fMTX. Medium was removed and cells were washed with PBS, treated with trypsin, and suspended in 500 μl of cold PBS supplemented with 10% FBS to increase cell viability and kept on ice before cytometric analysis within 20 min.

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Hunting Behavior of a Marine Mammal Beneath the Antarctic Fast Ice

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The hunting behavior of a marine mammal was studied beneath the Antarctic fast ice with an animal-borne video system and data recorder. Weddell seals stalked large Antarctic cod and the smaller subice fish *Pagothenia borchgrevinki*, often with the under-ice surface for backlighting, which implies that vision is important for hunting. They approached to within centimeters of cod without startling the fish. Seals flushed *P. borchgrevinki* by blowing air into subice crevices or pursued them into the platelet ice. These observations highlight the broad range of insights that are possible with simultaneous recordings of video, audio, three-dimensional dive paths, and locomotor effort.

The process by which mammalian predators search for, locate, stalk, and subdue their prey has been the subject of considerable research efforts for terrestrial species (*1*). In contrast, less is known about the foraging behavior of marine mammals, primarily because they are so difficult to observe underwater. Direct observation of marine animal behavior with scuba, fixed-location cameras, remotely operated vehicles, and manned submersibles is limited by depth or duration. Often these technologies provide only fleeting glimpses of highly mobile species. Animal-borne time-depth recorders and acoustic tracking provide information on diving performance (*2*, *3*) but do not allow direct observation of animals at depth. As a result, our knowledge of the underwater behavior of marine mammals, especially deep diving species, is based primarily on indirect information provided by dive depth and duration statistics and estimated swim speeds. To provide a better understanding of marine mammal diving and hunting behavior, we developed an animal-borne video system and data recorder that enabled us to observe Weddell seals (*Leptonychotes weddellii*) foraging at depth and to compute their three-dimensional dive paths.

Weddell seals are large, marine predators that are highly adapted for hunting in shore-fast and pack ice habitats (*2*, *4*). To forage beneath the extensive, unbroken fast ice, these seals must locate, pursue, and capture prey in three spatial dimensions under low-light conditions and while holding their breath. Foraging is thought to occur in daily bouts consisting of up to 40 consecutive dives. These dives are usually to depths of 100 to 350 m (the maximum recorded dive depth is 741 m) and less than 25 min long. Analyses of partially digested prey, fish otoliths, and skeletal material obtained from stomach samples and feces indicate that Weddell seals consume a variety of prey, although local diets appear narrow (*2*, *5*). For example, Weddell seals in McMurdo Sound, Antarctica, forage primarily on small notothenioid fish (*Pleuragramma antarcticum*, *Pagothenia borchgrevinki*, and *Trematomus* spp.). They also capture large Antarctic cod, which grow to 165 cm in length and weigh up to 77 kg (*5*). However, virtually nothing is known about how Weddell seals find their prey, where they find it, and how they stalk and capture it.

We attached a small video system and data logger (*6*) to four adult Weddell seals (one male and three females) from October to December 1997 to study their hunting behavior in the fast ice environment of McMurdo Sound, Antarctica. The video system recorded images of the seal's head and the environment immediately in front of the animal. The data logger recorded time, depth, water speed, and compass bearing once per second, and flipper stroke frequency and ambient sound were recorded continuously on the audio channels. These data enabled us to compute the seals' three-dimensional dive paths and locomotor effort.

In 57.4 hours of underwater video and data recordings, we observed several encounters between seals and their known prey. Three of these encounters were midwater interactions with Antarctic cod. One dive by seal 4, a 462-kg female, provides details on how Weddell seals stalk large prey in three dimensions. This seal departed the breathing hole and descended at an average swim speed of 1.3 m s^{-1} to a depth of 51 m (Fig. 1A). Without changing horizontal direction (bearing), the seal then ascended to 33 m at 1.2 m s^{-1} and began a second, gliding descent at 0.7 m s^{-1}. We surmise that the seal visually located a cod and began to stalk it at 4 min 51 s into the dive and a depth of 53 m when the seal suddenly accelerated to speeds of almost 2 m s^{-1} with large swimming strokes. At this point, the seal was about 23 m from the cod. Before this (minute 4 of the dive), the seal had been gliding at a descent angle of 31° along a straight course, bearing 103°, which almost intersected the point of contact with the cod (Fig. 1, A and B). Instead of continuing directly toward the fish, the seal leveled its descent and veered 28° to the right with the sudden acceleration (Fig. 1, A and B). This bearing took the prey out of the seal's line of sight and increased the distance between them. At 5 min 39 s into the dive and a distance of 28 m from the cod, the seal accelerated through a looping turn and a 23° descent to 73 m, reaching speeds in excess of 2 m s^{-1} and bringing it beneath a very large (>1 m long) Antarctic cod (Fig. 1C). The seal extended its neck and struck the cod near the anal fin with its muzzle. The fish reacted vigorously with a powerful tail thrust and disappeared from view; it was not seen again. By attacking from below at the posterior part of the fish, the seal silhouetted the cod against the under-ice surface and remained out of sight. The seal did not appear to pursue the cod after the strike but continued descending to 85 m. At the bottom of the dive, the seal turned left and then ascended quickly to the ice hole at an average speed of 1.8 m s^{-1}.

Two other encounters with Antarctic cod were recorded: one by a 475-kg male (seal 2) and another by seal 4. The seals approached to within centimeters of the fish, from slightly below or horizontally, without eliciting a response. The cod encountered by seal 2 was at a depth of less than 20 m and strongly backlighted by the under-ice surface. As the seal approached, it extended its head with erect vibrissae toward the fish. The seal vocalized briefly as it swam over the fish. Seal 4 approached a cod against a dark background at a

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depth of 100 m. It moved anteriorly along and extremely close to the fish's dorsal fins. Neither encounter was aggressive.

In addition to these midwater encounters, we observed Weddell seals hunting for smaller fish in the subice zone. On one brief dive by seal 2, the seal's eyes scanned the water above as it swam to a depth of 40 m. During the slow ascent, the seal lunged forward and to one side or the other five times apparently in response to something it saw above. The video recording showed small fish darting under the ice. The seal ascended to within a few centimeters of the ice where two fish (identified from the video as *P. borchgrevinki*) could be seen in a crevice. The seal expelled a blast of air through its nostrils for 1 s (Fig. 2A) and one of the fish immediately swam out of the crevice (Fig. 2B). The seal attempted to catch the fish but failed and returned to the remaining fish. The seal prodded the ice with its muzzle and the second fish fled. During another dive, seal 2 blew air into the subice surface twice and then plunged its head into the soft platelet ice three times for 4 to 6 s at a time. Immediately after withdrawing its head the third time, the seal jerked its head to either side three times in a manner reminiscent of a mammal chewing on something hard or manipulating food. We observed no fish but surmise that the animal had captured a small prey item in the platelet ice. It is not likely that the seal was chewing on ice, as the platelet ice is too fragile to produce such head movement. Seal 4 also penetrated the platelet ice, but deeper (about 1 m) and for a longer period (30 s).

There is circumstantial evidence that vision is important for under-ice prey detection by Weddell seals (*7*). Our data also indicate that vision may be important for interactions with prey, at least at shallow depths. Both interactions between seal 4 and the cod involved backlighting. When the seal struck the cod from below, the under-ice surface was visible (Fig. 1C). Because the seal eye is more sensitive than our video camera (*8*), there was sufficient light for the seal to see the cod's silhouette. Erection of vibrissae when the seal was very close to its prey (cod and *P. borchgrevinki*; Fig. 2B) suggests that the recently

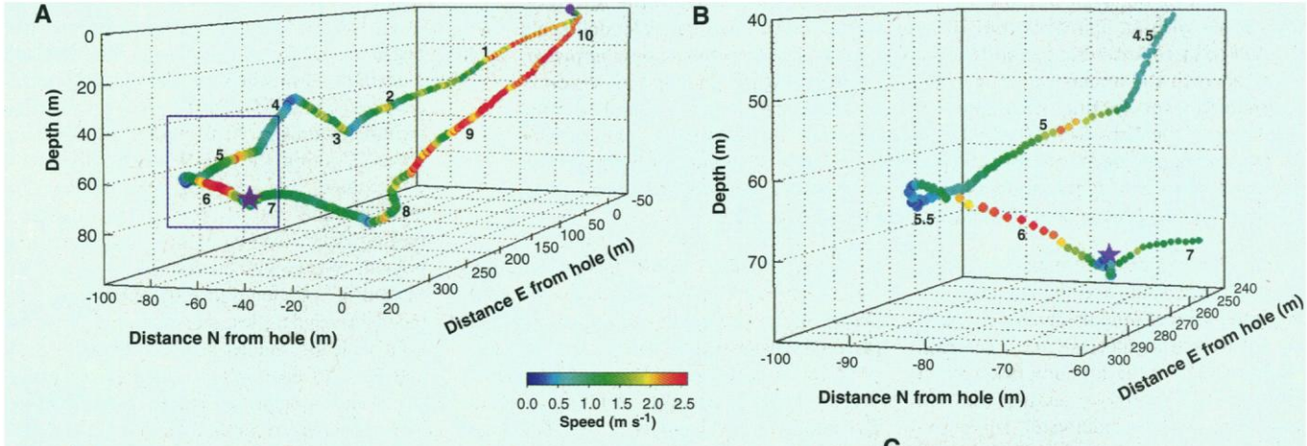

Fig. 1. (**A**) Swimming path during a 10 min 20 s dive in the course of which a Weddell seal made contact with an Antarctic cod. The solid circle marks the breathing hole and the star marks the point of contact between the seal and the cod. The numbers indicate elapsed time (in minutes) along the dive path. The total distance traveled was 760 m. The area enclosed by the rectangle in (A) is enlarged in (**B**). (**C**) The image is from the video recorded by a camera located on the seal's back. A second camera on the seal's head was not in use at the time. The cod is silhouetted against the under-ice surface as the seal approaches from below.

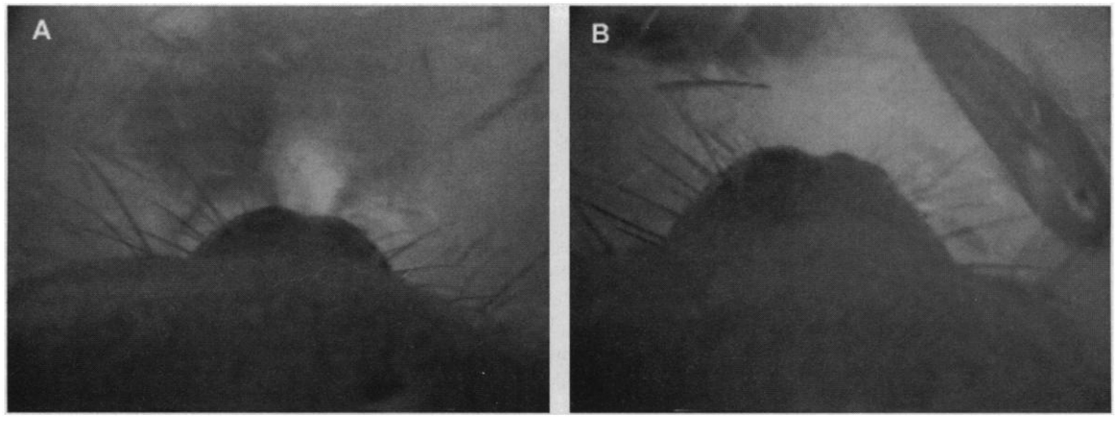

Fig. 2. (**A**) Seal 2 blowing air out of its nostrils and into crevices in the platelet ice where two *P. borchgrevinki* are hiding. The image was recorded with a head-mounted camera and shows the seal's forehead, muzzle, and vibrissae. (**B**) Moments after seal 2 finishes blowing air into the platelet ice, one of the *P. borchgrevinki* is flushed out and darts past the right side of the seal's head.

described hydrodynamic receptor system (*9*) may help guide seals during the final stages of an attack. Although Weddell seals produce a variety of underwater vocalizations (*10*), we recorded only nine calls during 139 dives, one of which occurred during a predator-prey interaction (*11*). Therefore, it seems unlikely that Weddell seals use active sonar to locate prey as some other marine mammals do. Weddell seals have excellent directional hearing (*2*) and may be able to detect and localize soniferous prey. However, we recorded no identifiable sounds from the fish during the interactions.

Although more recordings are needed to understand the importance of observations of Weddell seals interacting with Antarctic cod at depth, some preliminary conclusions can be made. First, not all encounters between this predator and its potential prey result in obvious aggression. In two interactions, the seals displayed curiosity but did not behave aggressively toward the cod. Nevertheless, we know that Weddell seals will capture Antarctic cod after being moved to an isolated ice hole. A few days after seal 1 was transported to the field site and before the camera and data logger were attached, it caught a cod (1.2 m long) and began consuming it in the ice hole by tearing off pieces. Another observation is that seals are able to approach to within a few centimeters of cod without startling the fish. Yet even in the absence of antipredator behavior, a seal may contact a cod without capturing it, as in the third interaction. We cannot be certain that the seal intended to catch the cod it struck, but the fish responded vigorously to the contact. This type of interaction may contribute to the scars that are common on the skin of large Antarctic cod (*5*).

Our records of seals attempting to feed on fish in the subice zone revealed previously unknown tactics for extracting prey from their refuge in the ice. Janssen *et al.* (*12*) reported *P. borchgrevinki* taking cover in subice crevices when Weddell seals approached to within 10 to 15 m and, noting previous observations of *P. borchgrevinki* tails in Weddell seal stomachs (*13*), suggested that the seals bite off the exposed tails of the fish in hiding. Blowing bubbles to flush *P. borchgrevinki* out of the platelet ice has to our knowledge never been observed or suggested. Platelet ice, which is composed of large, loosely packed ice crystals, can be more than a meter thick. In addition to biting exposed fish tails and flushing the fish out, seals may pursue the fish into the ice. This has been reported (*7*) and was seen during this study. These observations of Weddell seal foraging behavior, although preliminary, highlight the broad range of insights that are possible with simultaneous recordings of video, audio, three-dimensional dive paths, and locomotor effort.

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6. The underwater video system and data logger were manufactured by Pisces Design (San Diego, CA). The torpedo-shaped metal housing is 35 cm long and 13 cm in diameter and is pressure-rated to a depth of 1000 m. The miniature, low-light–sensitive, black and white video camera (minimum illumination = 0.05 lux; angle of view = 80° horizontal, 60° vertical) is encircled by an array of near-infrared light-emitting diodes (LEDs). These LEDs enable the camera to record images underwater in complete darkness to a distance of about 1 m. When additional ambient light is available, objects are visible at much greater distances. Because seals have a maximum visual sensitivity under low-light conditions in the spectral region of 496 nm, the near-infrared LED light source (λ_{max} = 850 nm) is believed to be invisible to them and their prey (*14*), as it is to humans. This prevents alterations in the normal behavior of the seals and their prey due to artificial light from the instrument. For some dives, the small (6 cm long by 6 cm in diameter) camera head with LEDs was detached from the main housing and mounted on top of the seal's head to obtain closeup images of the seal's eyes and muzzle and the area in front of the animal. The main housing contains an 8-mm video tape recorder (VTR; Sony EVO-220 VTR), rechargeable lithium ion batteries, and an on-board microcomputer (Onset Computer Corporation, Pocasset, MA) with 4 MB of RAM. The microcomputer controls the VTR and data acquisition from the transducers. The video system is activated by an external switch and can record for 6 hours. Transducers for pressure (Keller-PSI, Hampton, VA), water speed (Ultramarine Instruments, Seattle, WA), and compass bearing (KVH, Middletown, RI) are sampled once per second, and the data are stored on a PCMCIA card. A separate housing (17 cm long and 5.5 cm in diameter) for the gimbaled flux-gate compass is positioned behind the main housing and connected to it with a cable. Sound is recorded on one audio channel of the VTR with a hydrophone with a frequency response of 50 Hz to 16 kHz. Flipper stroke frequency is determined from the lateral motion of a small accelerometer (6 cm by 3 cm by 2 cm; Ultramarine Instruments) mounted near the base of the tail. The analog output from the accelerometer is converted into an audio frequency that is recorded on the other audio channel of the videotape. Weddell seals were captured with a purse-string net on the ice along the west shore of Hut Point Peninsula, Ross Island, and transported to the hut in a sled. Newly captured seals were weighed on a platform scale, placed in the hut, and immobilized to attach the video system and data logger. Seals were immobilized with an intramuscular injection of ketamine hydrochloride (3 mg/kg; Fort Dodge Laboratories, Fort Dodge, IO) and diazepam (0.1 mg/kg; Steris Corporation, Phoenix, AZ). After cleaning the fur in the middorsal area of the seal with acetone, a sheet of neoprene rubber (0.5 cm thick and 30 cm in diameter) was glued to the fur along the dorsal midline above the shoulders with neoprene rubber cement. The video housing rested in a molded, noncompressible foam cradle that was attached to the neoprene rubber and positioned overlooking the seal's head. The compass housing and accelerometer were glued to fur in a similar fashion. Once mounted on the animal, the entire video system and data logger were neutrally buoyant. Seals were allowed 18 hours to recover from anesthesia before being released into the breathing hole. All procedures were in accordance with Animal Use Protocols of Texas A&M University. The animals performed voluntary dives from an isolated ice hole located 10 km west of McMurdo Station and 200 m east of the McMurdo Ice Shelf (77.86°S, 166.22°E). This area of McMurdo Sound is about 600 m deep. A research hut was positioned over a 1.2-m-diameter hole drilled through 3-m-thick sea ice. Each instrumented seal was released into the ice hole beneath the hut for 4 to 5 days. The hole was located sufficiently far from other cracks so that the animals could not escape. The seals began performing dives similar to those of free-ranging seals within 24 hours of release (*2*). The main housing of the video system was removed from the seal every 6 to 12 hours while the animal rested in the ice hole, and the data were downloaded to a computer database. The batteries and videotape were replaced, and the main housing was reattached to the seal. Three-dimensional dive paths were computed from depth, compass bearing, and swim speed after correcting for ocean current direction and speed with standard "dead reckoning" methods (*15*). We assumed that the current speed and direction were uniform over the duration and depth of each dive. The three-dimensional dive paths were plotted with Systat (SPSS, Chicago, IL).
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A Copper Cofactor for the Ethylene Receptor ETR1 from *Arabidopsis*

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The ETR1 receptor from *Arabidopsis* binds the gaseous hormone ethylene. A copper ion associated with the ethylene-binding domain is required for high-affinity ethylene-binding activity. A missense mutation in the domain that renders the plant insensitive to ethylene eliminates both ethylene binding and the interaction of copper with the receptor. A sequence from the genome of the cyanobacterium *Synechocystis* sp. strain 6803 that shows homology to the ethylene-binding domain of ETR1 encodes a functional ethylene-binding protein. On the basis of sequence conservation between the *Arabidopsis* and the cyanobacterial ethylene-binding domains and on in vitro mutagenesis of ETR1, a structural model for this copper-based ethylene sensor domain is presented.

Small gaseous molecules act as signals for a variety of organisms. In many cases, signal perception involves the use of a transition metal cofactor that mediates the interaction between the signal and its proteinaceous receptor. For example, sensors for NO in animal cells and O_2 in bacteria use a heme moiety to achieve high-affinity binding of the signal (*1*). The plant hormone ethylene is effective at nanomolar concentrations, reflecting the presence of high-affinity receptors (*2*). Theoretical considerations indicated Cu(I) as a possible receptor cofactor (*3*, *4*). The opportunity to directly investigate the role of a metal cofactor in ethylene sensing has been provided by the cloning and characterization of the *ETR1* gene from *Arabidopsis* (*5*). The ETR1 protein forms a membrane-associated disulfide-linked homodimer both in plant tissues and when heterologously expressed in yeast (*6*). Expression of truncated forms of ETR1 in yeast indicated that the first 165 amino acids of the protein contain the sequences that are necessary and sufficient to bind ethylene (*7*).

To further delineate the ethylene-binding domain of the ETR1 protein and to facilitate the purification of the binding activity, a chimeric gene consisting of the coding sequence for the first 128 amino acids of the ETR1 protein fused to the glutathione S-transferase (GST) coding sequence [ETR1(1-128)GST] was constructed (*8*). Yeast cells expressing the fusion protein showed ethylene-binding activity equivalent to that detected in cells expressing full-length ETR1 (Fig. 1A). Introduction of an amino acid substitution into the fusion protein, corresponding to the *etr1-1* mutant allele [$Cys^{65} \rightarrow Tyr^{65}$ (C65Y)], resulted in the complete loss of binding activity by the expressed protein, as was previously observed for the full-length etr1-1 protein (*7*). Both the mutant and the wild-type fusion proteins formed membrane-associated disulfide-linked homodimers and were expressed at equivalent levels as determined by immunoblot analysis using antibodies to GST (*9*).

We assessed binding in membrane extracts of yeast expressing both the full-length ETR1 protein and the ETR1(1-128)GST fusion protein (*10*, *11*). Addition of 300 μM $CuSO_4$ to the isolated membranes led to a 10- to 20-fold increase in ethylene-binding activity (Fig. 1B); up to twice that observed in intact cells. The addition of copper had no effect on ethylene binding in native yeast membranes nor in membranes expressing the mutant etr1-1(1-128)GST fusion protein. Of other transition metals tested (Fig. 1C), only silver ions stimulated ethylene-binding ac-

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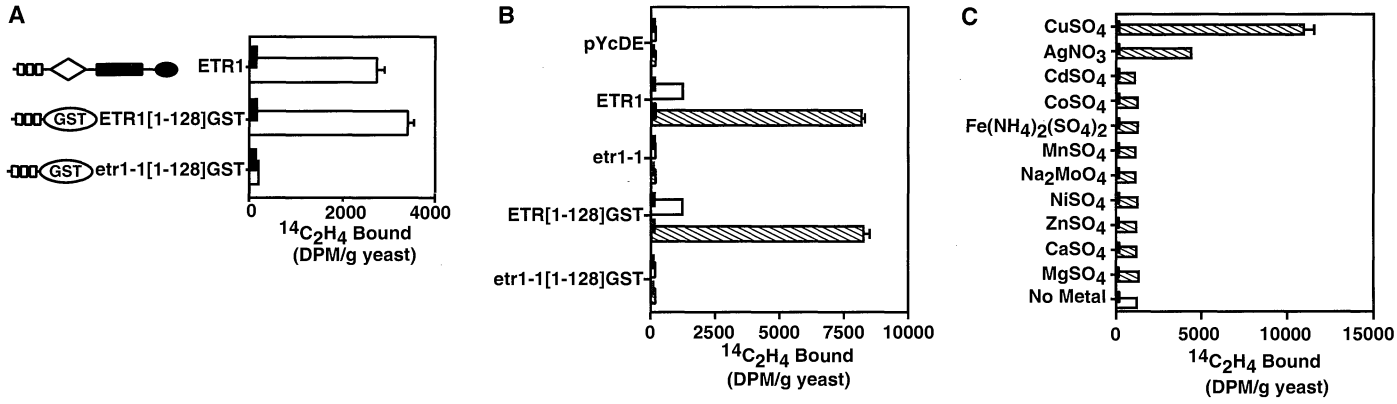

Fig. 1. Requirements for ethylene binding to the ETR1 protein expressed in yeast. Saturable ethylene binding is indicated as the difference in ^{14}C-ethylene between samples treated with ^{14}C-ethylene (0.1 μl/liter) (white or hatched bars) and identical samples treated with ^{14}C-ethylene (0.1 μl/liter) plus ^{12}C-ethylene (100 μl/liter) (overlapping black bars). DPM, disintegrations per minute. (**A**) Saturable ethylene-binding activity in yeast cells expressing different ETR1-derived constructs. Expressed proteins are depicted diagramatically, with the hydrophobic (small white squares), GAF (*19*) (white diamond), histidine kinase (black rectangle), receiver (black oval), and GST (white oval) domains indicated. (**B**) Effects of $CuSO_4$ addition to membranes extracted from yeast cells expressing the control vector (pYcDE) or the indicated ETR1-derived constructs. Ethylene-binding assays were performed with assay buffer alone (white bars) or with 300 μM $CuSO_4$ (hatched bars) (*11*). (**C**) Effects of $CuSO_4$ and other transition metals on ethylene-binding activity in yeast membranes expressing the ETR1 protein. Ethylene-binding assays were performed with assay buffer alone (white bar), or with 300 μM of the indicated metal salts (hatched bars).