the sporophytic to the gametophytic generation—for example, by eliminating recombination or the first meiotic nuclear division. Chemically induced *Arabidopsis thaliana* mutants (5, 6) exhibit autonomous inception of endosperm divisions in unfertilized ovules, albeit without full maturation. These and other findings imply that angiosperm reproduction can be genetically manipulated.

To harness apomixis, we will need to answer some fundamental biological questions: What factors determine the commitment of specific cells in the ovule to somatic, meiotic, or apomictic development? What are the respective roles of cell lineage and positional sensing in cell determination? How do gametes communicate during fertilization? What functional relations exist between cell cycle regulators and regulators of sexual and apomictic development? We will also need to understand reproductive development in yeast and ferns, in which mutants that mirror angiosperm recurrent apomixis have been identified (7). In angiosperms, relatively few reproductive genes have been identified and mapped, and little is known about the role of plant hormones in female reproductive development, although new techniques (8, 9) promise to reveal much about natural and induced apomictic reproduction.

The harnessing of apomixis will lead to large increases in agricultural production, perhaps even greater than those seen with the adoption of broadly adapted, semidwarf grain varieties some 20 years ago during the Green Revolution. Such an "asexual revolution" now appears to be within our scientific grasp as we approach the 21st century. The main determinant of its occurrence may be the funding and international cooperation needed to bring it about.

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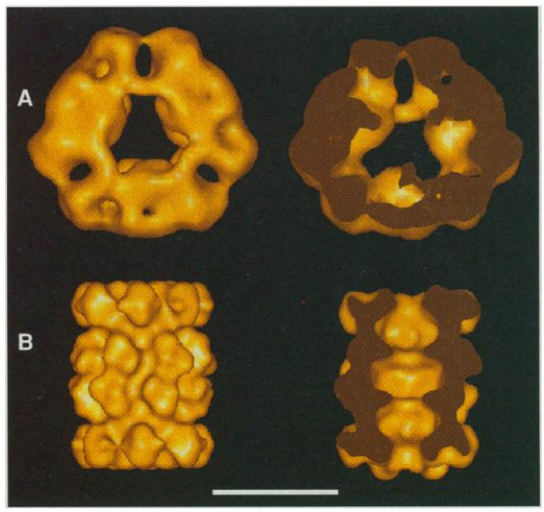
CELL BIOLOGY

Hats Off to the Tricorn Protease

Christine Schneider and F. Ulrich Hartl

Proteolysis, the last step in the life of a protein, is essential for the maintenance of cellular homeostasis (*1*). Proteases remove damaged or denatured proteins, recycle their amino acids, and are important for cell regulation. But how do cells tame the potentially destructive activities of these enzymes to ensure that they act only where needed? One solution—used by archaebacteria, eubacteria, and eukaryotes—has been to compartmentalize proteolytic enzymes within so-called molecular organelles (*2*), ring-shaped protein assemblies in which the proteolytically active center is sequestered from the rest of the cytosol. The paradigm is the proteasome, a cylindrical multimeric protein complex with a central proteolytic chamber, to which access is carefully controlled (see figure, part B) (*1*, *3*). This ancient organizational principle has been extremely successful. Indeed, most protein degradation in the cytosol of archaebacteria and of eukaryotic cells is thought to be proteasome-mediated. But now on page 1385 of this issue, Tamura *et al.* (*2*) report the surprising discovery of a new proteasome-like particle, a large tricorn complex (TRI) abundant in the thermophilic archaebacterium *Thermoplasma acidophilum* (see figure, part A). A comparison of TRI and the proteasome reveals both interesting parallels and differences.



Destructive siblings? Structures of (**A**) TRI and (**B**) the proteasome from *Thermoplasma acidophilum* at a resolution of 20 Å. Bar, 10 nm. [Figure courtesy of T. Tamura and W. Baumeister]

As beautifully documented by a three-dimensional reconstruction of the complex from electron microscopic images, the shape of the new protease is reminiscent of a tricorn, a hat with the brim turned up on three sides. Unlike the *Thermoplasma* proteasome, which is composed of four heptameric rings (two containing α and two containing β subunits), TRI consists of six identical subunits of 120 kD. The TRI complex is a trimer of dimers forming a toroid traversed by a channel along its threefold axis (part A of the figure shows the end-on view of the toroid on the left and a horizontal cut through the middle of the complex on the right). The quaternary structure of TRI is strikingly similar to that of the Gal6 or bleomycin hydrolase from *Saccharomyces cerevisiae* (*4*). Both proteins are hexamers with a central channel. In TRI the maximum width of the channel, thought to harbor the proteolytic sites, is 8 nm, whereas the proteolytic chamber of the yeast complex is only 4.5 nm, close to that of the *Thermoplasma* proteasome (5.3 nm). However, the sequence of the *tri* gene, also reported by Tamura *et al.* (*2*), is not related to Gal6 or bleomycin hydrolase, nor are any other TRI homologs found in the databases. The only detectable homology to other proteins occurs in a stretch of ~160 residues at the COOH-terminal of the TRI subunit. This segment is related to several COOH-terminal processing proteases of bacterial and eukaryotic origin and may form a domain that participates in substrate binding.

What is known about the function of TRI? Unlike the proteasome from the same organism, which has chymotrypsin-like cleavage specificity, TRI cleaves with a specificity more like trypsin, as established with model peptides. It is inhibited by tosyl-L-phenylalanine chloromethyl ketone, suggesting the involvement of a histidine residue in the active center [the proteasome has a threonine in the active site (*3*)]. Neither the natural substrate proteins of TRI

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nor the end products of its reaction have been defined.

Why does *Thermoplasma* need TRI in addition to the proteasome? Like the proteasome, TRI appears to cooperate with other factors (albeit in a different manner) to achieve its full proteolytic activity. These include two low molecular weight protein factors, F1 and F2 (*2*). Surprisingly F1, a 33-kD polypeptide (*5*), is itself a protease with homology to certain proline iminopeptidases and is capable of hydrolyzing a wide variety of substrate peptides. By means of a physical interaction with TRI, F1 may act on the proteolytic products generated by TRI. Thus, the picture of a new type of modular proteolytic system is emerging with TRI as its core; upon interacting with low molecular weight factors, intrinsic proteolytic activities of TRI may be enhanced and new activities generated (*2*, *5*).

The proteasome cooperates with an adenosine triphosphate (ATP)–dependent protein machine that is thought to unfold polypeptide substrates in preparation for subsequent cleavage (*3*). The proteolytic chamber of the proteasome, located in the center of the barrel, is flanked by two antechambers and, in the crystal structure (*3*), is accessible through narrow channels of no more than 1.3-nm width that can only be traversed by unfolded polypeptides (on the right in part B of the figure is a vertical cut through the proteasome cylinder). So far, there is no evidence for an ATP requirement for TRI. The access to the large central chamber in the TRI complex appears much wider, and less extensive unfolding of a substrate may be required for cleavage. TRI may cooperate with the proteasome by cutting substrates, such as thermally denatured polypeptides, into smaller pieces that unfold spontaneously to become competent for final degradation by the proteasome. No doubt future studies will provide answers to many of these questions and will clarify the significance of TRI as a novel proteolytic concept. Who knows what other secrets TRI keeps under its hat.

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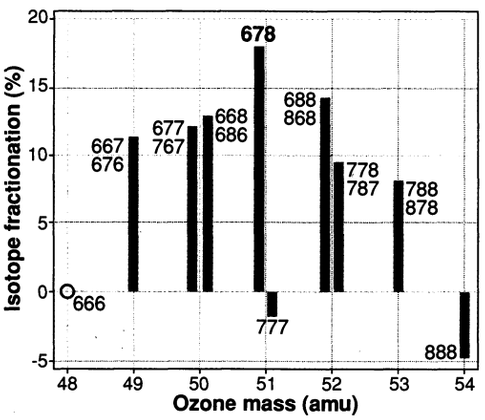
ATMOSPHERIC CHEMISTRY

Heavy Ozone—A Difficult Puzzle to Solve

Dieter Krankowsky and Konrad Mauersberger

Ozone makes up a small percentage of the atmosphere but is one of its most important constituents. It has a unique molecular composition consisting of three atoms of the same element that form an equilateral open triangle. Ozone is produced in a three-body reaction $O + O_2 + M = O_3 + M$, with M being a stabilizing molecule. Because there are three oxygen isotopes—^{16}O, ^{17}O, and ^{18}O—a variety of ozone isotopomers, usually called ozone isotopes, can be formed, ranging in mass from 48 to 54 atomic mass units (amu). Because ^{17}O and ^{18}O are minor species in atmospheric oxygen, only three ozone isotopes are present in measurable quantities in our atmosphere: the dominant molecule $^{48}O_3$ and, considerably lower in abundance, $^{49}O_3$ and $^{50}O_3$. Numerous laboratory studies and atmospheric measurements have shown that the heavy isotopes are enriched by 10% or more over values one would expect from a statistical distribution of oxygen in ozone. This imbalance is an unusually large isotope effect for such molecules. Theoretical analysis of the ozone formation process in terms of the standard kinetic isotope effect actually predicts a small mass-dependent depletion. Past attempts to find the physical processes leading to the large enrichments have not been successful. A report by Gellene (*1*) on page 1344 of this issue of *Science* provides a quantitative prediction of the symmetry-controlled ozone isotope fractionation.



Measured enrichment or depletion of all possible ozone isotopes [after Mauersberger *et al.* (*7*)]. The labels 6, 7, and 8 stand for ^{16}O, ^{17}O, and ^{18}O, respectively. Ozone was produced in two isotopically enriched oxygen mixtures of well-known composition at 70 torr and room temperature. The statistical abundance of each isotope was calculated and used to derive from the measured values the isotopic fractionation. The values in the graph are normalized so that $^{48}O_3$ has zero enrichment.

The first indication of an unusual isotope effect was found in stratospheric ozone, initially by mass spectrometric measurements on board balloons (*2*) and later through analysis of cryogenically collected ozone samples (*3*). Infrared emission and absorption spectroscopy from the ground and airborne vehicles (*4*) have confirmed the enrichments in heavy ozone. Tropospheric ozone repeatedly collected and analyzed has also shown enrichments of about 9 and 7% in $^{50}O_3$ and $^{49}O_3$, respectively (*5*).

Inspired by atmospheric measurements, investigators have studied in the laboratory the ozone formation process in great detail. Ozone has been produced both in oxygen of atmospheric composition and in isotopically enriched samples, extending the number of ozone isotopes to be studied. Results show that the isotope effect is large, typically 10 to 20%, and its magnitude depends on the pressure and temperature of the gas in which ozone is formed. This isotope effect cannot be explained by known physical processes such as diffusion or by kinetic effects, in which most isotope fractionations have their origin. The effect is mass independent, occurs in the gas-phase formation process, and exhibits a strong dependence on molecular symmetry, as first recognized by Heidenreich and Thiemens (*6*).

The symmetry dependence was clearly demonstrated when isotopically enriched oxygen mixtures were used to produce all possible isotopomers of ozone from 48 through 54 amu (*7*) (see figure). The asymmetrical molecule $^{16}O^{17}O^{18}O$ of mass 51 amu shows the largest enrichment, whereas six others composed of symmetric and asymmetric molecules are less enhanced independent of their mass. The two completely symmetrical molecules $^{17}O^{17}O^{17}O$ and $^{18}O^{18}O^{18}O$, however, are reduced in abundance in a mass-dependent

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