the peptide-MHC clusters that accumulate in the center of the mature synapse are directly proportional to the initial density of peptide-MHC complexes present in the lipid bilayer. Importantly, maximum proliferation of T cells is triggered as soon as a minimum threshold density of peptide-MHC complexes is reached in the mature synapse. The lowest initial density of peptide-MHC complexes in the bilayer that is capable of triggering synapse formation and T cell proliferation compares rather well with the minimum density of specific peptide-MHC complexes that must be displayed on antigen presenting cells to trigger T cell activation.

It is likely that the T cell receptor acts as a molecular processor, translating the different half-lives of its interactions with ligands into distinct biological outcomes (*5*). The Grakoui findings support this view: Short-lived T cell receptor–ligand interactions result in the formation of disorganized contact points that differ from those induced by longer lived interactions. These looser contacts, however, are still capable of triggering less demanding biological responses. Previous studies have shown that suboptimal peptides can weaken or even prevent a T cell response to an optimal peptide. It will be interesting to see whether this effect correlates with the ability of suboptimal peptide-MHC complexes to prevent the formation of immunological synapses.

Analyzing the lipid bilayers by fluorescent photobleaching recovery established that the peptide-MHC complexes in the center of the mature synapse are stable and do not exchange with peptide-MHC complexes localized outside the contact area. Examination of the removal by endocytosis of T cell receptors from the cell surface after stimulation with specific peptide-MHC complexes has prompted the suggestion that a single peptide-MHC complex can engage and sequentially trigger up to 200 specific T cell receptors in less than an hour (*6*). Because the experimental system of Grakoui *et al.* provides only indirect information about the dynamics of ligand-bound T cell receptors, it remains to be determined whether they undergo internalization and are replaced by new receptors. Combining the Grakoui imaging system with T cell receptors tagged with green fluorescent protein should settle this important issue.

It has been argued that ligand-occupied T cell receptors need to congregate into tight arrays to deliver effective intracellular signals (*7, 8*). Once the mature synapse has been formed, however, the density of signaling T cell receptors is expected to mirror the density of central peptide-MHC clusters, which the investigators calculated to be about 100 molecules/μm^2. This density is only slightly higher than that of unoccupied T cell receptors sitting on the surface of quiescent T cells, and so these results do not support the idea of densely packed arrays of signaling T cell receptors.

The findings of Grakoui and colleagues clearly link the biological potency of T cell receptor ligands to their capacity to orchestrate the active assembly of an immunological synapse. The distinctive contact points formed by the T cell surface may polarize the delivery of secondary messenger molecules to a confined region of the cell and protect the T cell receptor core from dephosphorylation by protein tyrosine phosphatases. We do not know whether the stereotyped behavior of immunological synapse formation documented by the elegant real-time imaging of T cells interacting with lipid bilayers applies to the meeting of naïve T cells and professional antigen presenting cells or to the sequential engagement of cytotoxic effector T cells with target cells. Resolution of these questions will have to await a sequel, which, if the current report is anything to judge by, should be well worth watching.

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PERSPECTIVES: ATMOSPHERIC SCIENCE

Summer in the Stratosphere

D. W. Fahey and A. R. Ravishankara

Since the discovery in 1985 that an ozone hole develops over the Antarctic in the late winter and early spring, intense research efforts have clarified the roles of atmospheric transport and chemistry in stratospheric ozone changes. The initial focus of the research was on the winertime conditions, but more recently the stratospheric polar summer has received increased scrutiny.

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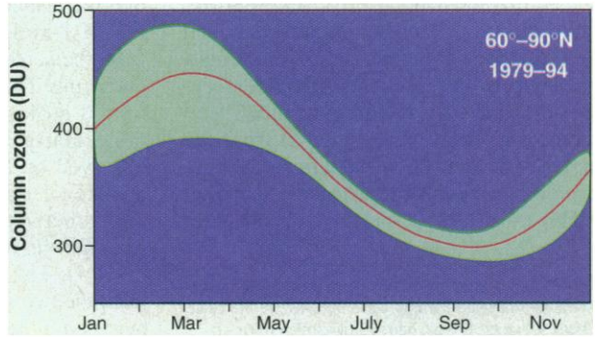
Average ozone concentrations in the polar stratosphere show a pronounced cyclical variation over the course of the year (see the figure at the right). In winter and early spring, ozone builds up at the poles as ozone-rich air is transported from lower latitudes toward the polar regions. But when transport to high latitudes slows and solar illumination increases in late spring and summer, catalytic ozone destruction leads to substantial ozone decreases (~30%). Here we discuss why ozone decreases in the summer polar stratosphere, how this decrease differs from the winter-spring ozone depletion, and how well we understand the underlying chemistry.

Ozone is produced via solar ultraviolet photolysis of oxygen and destroyed through catalytic cycles involving reactive nitrogen (NO_y), halogen (chlorine and bromine), and hydrogen species (HO_x) (*1–3*). Among the NO_y species, NO_x is the major catalyst (see the top panel in the figure on the next page). Stratospheric NO_y and HO_x are



A cycle of destruction and recovery. Annual cycle of column ozone abundances averaged over 60° to 90°N for 1979–94 (without years affected by volcanic eruptions). Data are from the TOMS satellite (W. Randel, National Center for Atmospheric Research). The mean value (red line) and range of values (green area) are shown. A similar seasonal cycle is revealed by data from high latitudes in the Antarctic region. Losses of ozone in the Arctic and Antarctic regions have lowered column values in both late winter–early spring periods during the last 10 to 15 years (*15*). Averaging the data for the Arctic over 60° to 90°N and not just inside the vortex masks the winter-spring decreases.

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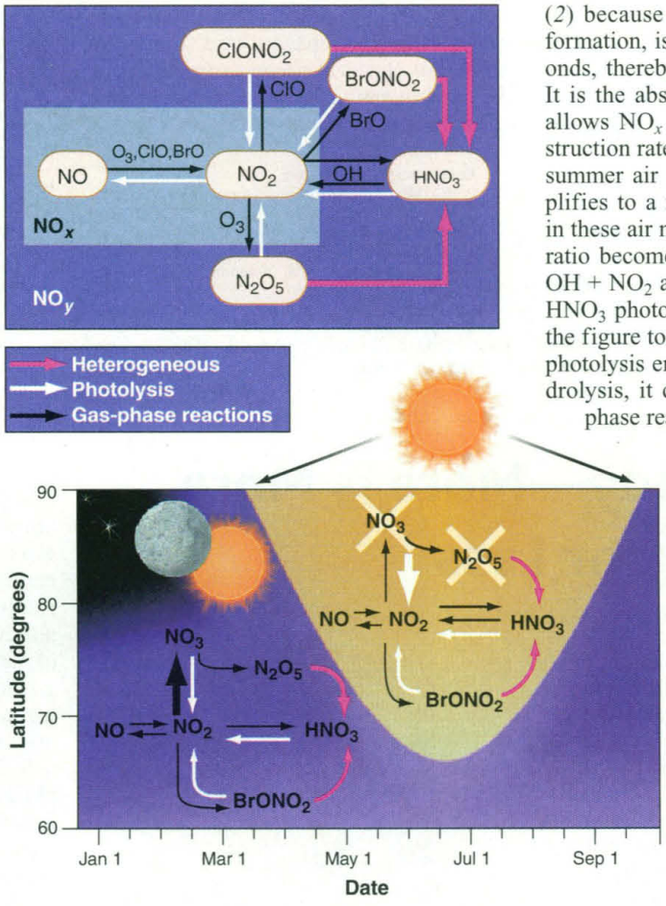
predominantly natural in origin, and their abundance has not been altered substantially by human activity (*1*). In contrast, the halogen content of the stratosphere has increased markedly over the past decades mainly because of human activity (*1*). This is the cause of the ozone hole. Atmospheric transport also affects stratospheric ozone concentrations; most ozone is produced in the tropical stratosphere and transported to higher latitudes.

The balance between photolytic production, transport, and chemical destruction determines the abundance of ozone at any particular stratospheric location. This balance is also strongly season dependent. In addition, the relative contribution of the three types of catalytic destruction of ozone differs between the summer and winter-spring (*2–4*). In winter and spring, the halogen cycle predominates over those of NO_x and HO_x. Throughout most of the year, chlorine, which originates from chlorofluorocarbons and related compounds, resides predominantly in molecules that do not react with ozone (chlorine nitrate and hydrochloric acid). But when stratospheric temperatures drop below 205 K over the winter-spring poles, heterogeneous reactions effectively convert these reservoir molecules to reactive chlorine species (such as chlorine monoxide). In concert with bromine, this reactive chlorine destroys ozone in catalytic reaction cycles at rates that locally can exceed 1% per day (*5*). When low temperatures cease near the end of winter, chlorine returns to its inactive forms and ozone levels begin to recover. The extent of this winter-spring depletion has increased with enhanced human-induced halogen emissions in the past decades. In contrast to winter and spring, polar stratospheric temperatures during the summer remain well above 205 K, precluding the intense heterogeneous activation of chlorine. Other loss mechanisms must then be responsible for the observed summertime ozone decrease.

During the summer, large regions of the polar stratosphere receive uninterrupted sunlight for many weeks (see the bottom panel in the figure above). Photolysis reactions, several of which are complete ozone destruction cycles, occur continuously under these conditions. As a result, the NO_x/NO_y ratio increases substantially, and NO_x becomes the predominant catalyst for ozone loss. Summer ozone production rates and the transport of ozone-rich air from lower latitudes and higher altitudes are too slow to offset this destruction (*3*), which can locally exceed 0.3% per day. Consequently, total ozone concentrations continuously decrease throughout high latitudes in late spring and summer.

The hydrolysis reactions of N_2O_5 and $BrNO_3$ on background sulfate aerosols are efficient with rates that are almost independent of temperature in the stratosphere (*1, 6, 7*). These reactions represent two important pathways that convert NO_x to HNO_3 within the NO_y family and thus reduce the NO_x available for ozone destruction. However, daily N_2O_5 production ceases abruptly with the onset of continuous photolysis in high-latitude air masses (*2*) because NO_3, the intermediate in its formation, is photolyzed within a few seconds, thereby preventing N_2O_5 formation. It is the absence of N_2O_5 hydrolysis that allows NO_x and its associated ozone destruction rate to increase so dramatically in summer air masses. The NO_y family simplifies to a near "gas-phase-only" system in these air masses because the NO_x/HNO_3 ratio becomes primarily controlled by the $OH + NO_2$ and $OH + HNO_3$ reactions and HNO_3 photolysis (see the bottom panel of the figure to the left). Although continuous photolysis enhances the role of $BrNO_3$ hydrolysis, it cannot compete with the gas-phase reactions.



Chemistry of summertime ozone destruction. (Top) Principal component species of stratospheric reactive nitrogen (NO_y) and their interconversion processes. Red arrow, heterogeneous process; white arrow, photolysis; and black arrow, gas-phase reactions. [Adapted from (*8*)] **(Bottom)** Important interconversion processes of reactive nitrogen in the polar stratosphere over the course of the year. The dark shading indicates regions that have interrupted solar illumination within a 24-hour period. The light shading indicates regions of uninterrupted solar illumination. The arrows roughly indicate conversion processes as in the top panel. The arrow thickness indicates the relative importance of the interconversion processes. The crosses indicate species that are at a negligible concentration.

Aircraft, balloon, and satellite measurements have confirmed the important role of NO_x in the summer stratosphere (*4, 8, 9*). Recent extensive in situ and remote measurements of NO_x initially could not be reproduced satisfactorily with highly constrained photochemical box models (*8, 9*). The availability of more accurate laboratory data for the $OH + NO_2$ (*10, 11*) and $OH + HNO_3$ (*12*) reactions dramatically improved the agreement (*8, 9*). These comparisons of observations and model simulations demonstrated a very accurate understanding of the fast photochemistry of stratospheric reactive nitrogen species over the summer poles.

However, ozone abundances calculated with various two- or three-dimensional models generally exceed observations at summer high latitudes (*13*). Including the new rate coefficients for the OH reactions with NO_2 and HNO_3 provides only modest improvements. The contribution to summer ozone destruction by chlorine and hydrogen species are much less than that of NO_x, and NO_x increases actually moderate the loss from the other catalytic cycles. Measurements of chlorine and hydrogen species in the summer stratosphere are generally sufficiently well represented by these models and are unlikely to be the source of the difference. Poleward stratospheric transport is weakest in the summer season. The discrepancies between measured and observed ozone amounts are most likely a result of inaccurate modeling of this transport. Thus, a more accurate representation of model transport is expected to improve these long-term simulations.

We now understand in some detail how the combined effects of transport, chemical ozone production, and catalytic ozone loss control ozone during the annual cycle of stratospheric conditions. The summer ozone decreases at high latitudes will persist in the future because natural (NO_x) rather than human-induced (halogen) species are primarily responsible for ozone destruction there. In contrast, the winter-spring ozone destruction will gradually lessen in the next decades as halogen emissions steadily decrease—barring other changes to the stratosphere such as major cooling of this region due to greenhouse gases (*14*). Although the summer and winter polar stratospheres have similar potential for chemical ozone destruction, differences in sunlight and temperature keep their ozone abundances poles apart.

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PERSPECTIVES: GENETICS

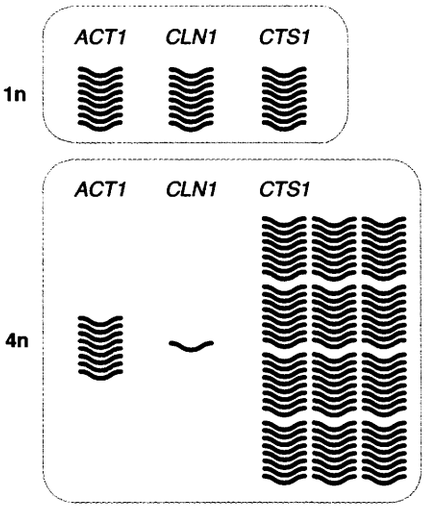
Polyploidy—More Is More or Less

Philip Hieter and Tony Griffiths

Biologists tend to think of the normal ploidy (number of complete chromosome sets) of cells as either diploid (2n) or haploid (n). Yet examples of polyploidy (more than two sets of chromosomes) abound among plants and animals (*1*, *2*). The bananas we eat are triploid (3n); wheat is hexaploid (6n). At least half of the natural species of flowering plants are polyploid and, although polyploid animal species are less common, some groups, such as salmonid fish and certain amphibians, have clearly evolved by doubling or tripling their ploidy.

Cells differing only by their ploidy are identical in terms of DNA sequence information and relative gene dosage, and yet are often quite different in terms of physiology, morphology, and behavior. How can this be so? A report by Galitski *et al.* on page 251 of this issue (*3*) provides a satisfying answer. In a convincing demonstration of the power of DNA chip technology, these authors found that yeast (*Saccharomyces cerevisiae*) with different ploidies had different patterns of gene expression. Their findings provide definitive evidence for a ploidy-driven mechanism of gene regulation that may be important in a variety of biological states.

Changes in ploidy during cell differentiation appear to be important in development. Almost all plants and animals generate specific sub-populations of polyploid cells by endoreduplication cycles (DNA replication in the absence of cell division) during tissue-specific differentiation (*4*). For example, the ploidy of megakaryocytes (the cells that produce blood platelets) ranges from 16n to 64n; that of cardiomyocytes (heart muscle cells) from 4n to 8n; and that of hepatocytes (liver cells) from 2n to 8n. A related phenomenon, polyteny (chromosomes consisting of multiple strands), is also found during development, the best-known example being the giant salivary gland chromosomes of insects. Many cancer cells are polyploid, raising the still unresolved issue of whether an increase in ploidy contributes to, or is a consequence of, tumor development (*5*).



Ploidy paradox. Yeast strains that differ only in their ploidy show different patterns of gene expression. The mRNA levels (wavy lines) for three genes are shown in haploid (1n) and tetraploid (4n) yeast strains. *ACT1*, like most genes, is not affected by an increase in ploidy. A small subset of genes is dramatically repressed (for example, *CLN1*) or induced (for example, *CTS1*) in response to increased ploidy. Changes in ploidy also affect the expression of many more genes, but not as dramatically.

Ploidy also varies by a factor of 2 during mitotic (G_1 versus G_2 phase) and meiotic (germ cell versus gamete) cycles of cell division. Mitotic cells double their ploidy during DNA synthesis, and ploidy is restored at cell division. Meiotic cells reduce their ploidy by half during gametogenesis, and ploidy is restored upon fertilization. Thus, changes in ploidy commonly occur both in normal states (during differentiation in multicellular organisms, in the evolution of species, and in the DNA replication and cell division of mitosis and meiosis) and under abnormal conditions such as disease.

The elegance and rigor of the experimental design in the Galitski *et al.* study could only be achieved in yeast at this point in time. First, using genetic trickery and a clever series of manipulations, a perfectly isogenic (genetically identical) set of yeast strains differing only in ploidy (1n, 2n, 3n, 4n) were constructed and compared. Second, yeast is the only eukaryotic organism for which whole-genome expression analysis (that is, the identity of each expressed gene and its level of expression) can be determined completely in a single experiment.

The investigators used DNA chip technology to analyze mRNA levels for all genes in yeast strains that varied only in their ploidy. They then searched the data for genes whose expression, relative to total gene expression, increased or decreased as the ploidy changed from haploid to tetraploid. Most genes showed no change in mRNA levels relative to total RNA. However, when the investigators introduced a stringent cutoff requiring a 10-fold difference in gene expression be-

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