estingly, RdRp is present at the chromatin regions comprising the outer *cen* repeats in *S. pombe* (*7*). Rather than inducing degradation or translational inhibition of mRNA, the shRNAs target chromatin modifications, presumably through a pairing mechanism (*24*), and interaction with chromatin-associated proteins. Although this recognition step is currently unclear, good candidates for linking shRNAs to chromatin are chromo domain proteins, such as Chp1 (*17*), Pdd1 (*18*), and the Clr4 HMTase itself. Intriguingly, the *Drosophila* Clr4 homolog, Su(var)3-9, contains an amino-terminal extension that resembles the elongation initiation factor eIF2γ (*25*). Following targeted H3-K9 methylation, the chromatin region can either be stably silenced by Swi6/HP1 incorporation (*8*), which may entail further repression by DNA methylation (*12*, *13*) or, in its most dramatic form, result in DNA elimination (*9*, *18*) (see the figure).

With the exception of the budding yeast *Saccharomyces cerevisiae*, which does not appear to contain components of the RNAi machinery or of H3-K9 methylation, the new pathway is predicted to trigger heterochromatin formation in complex organisms including mammals. For example, bidirectional transcription across mouse major satellite repeats has been described (*26*), suggesting that there are shRNAs in pericentric heterochromatin. In agreement with this interpretation, the definition of pericentric H3-K9 methylation is abrogated after ribonuclease treatment of permeabilized mouse cells (*27*). To extend these parallels further, it is conceivable that the initiating mechanisms for X-chromosome inactivation and imprinting, or even for transcriptional silencing mediated by Polycomb-group (Pc-G) proteins, may also use short RNAs. X-chromosome inactivation requires the *Xist* RNA as part of a large RNA scaffold; in addition, several Pc-G proteins are associated with the inactive X chromosome (*28*). Pc-G gene mutations impair RNAi in the worm *Caenorhabditis elegans* (*29*), and small RNAs direct dosage compensation in *Drosophila* (*30*). Thus, the new discoveries in *S. pombe* (*6*–*8*) and *Tetrahymena* (*9*, *18*) may reveal a key unifying signal for inducing chromatin alterations in most of the epigenetic transitions that occur during cellular differentiation.

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31. I thank M. Matzke (Salzburg), A. Bird (Edinburgh), and K. Nasmyth (Vienna) for helpful comments.

PERSPECTIVES: BIOMEDICINE

Gluten and the Gut—Lessons for Immune Regulation

Detlef Schuppan and Eckhart G. Hahn

Celiac sprue is an inflammatory disease that leads to destruction of the microscopic fingerlike projections of the small intestine called villi. The disease is triggered by ingestion of the gluten proteins contained in wheat, barley, and rye, and symptoms range from minor complaints to severe nutrient malabsorption (*1*). The report by Shan *et al.* on page 2275 of this issue (*2*) significantly enhances our understanding of celiac sprue pathogenesis and hints at potential intervention strategies to treat this common disease.

There are three remarkable features of celiac sprue: (i) It usually remits upon strict dietary avoidance of gluten; (ii) it requires a unique genetic background for antigen presentation—expression of the human leukocyte antigen (HLA) class II molecules DQ2 or DQ8; and (iii) patients have characteristic circulating mucosal (immunoglobulin A) autoantibodies to the ubiquitous enzyme tissue transglutaminase (tTGase) (*3*, *4*). What is intriguing is the connection between these three features. Gluten peptides presented in the context of HLA-DQ2 or HLA-DQ8 molecules elicit proliferation of intestinal T cells from celiac sprue patients and induce these cells to release inflammatory cytokines (*5*). The autoantigen, tTGase, catalyzes transamidation between a glutamine residue of peptide 1 (glutamine donor) and a lysine residue of peptide 2 (glutamine acceptor), creating stable covalent complexes among a limited set of mostly extracellular matrix proteins (*6*). This enzyme is highly expressed in the subepithelial cells of the gut, where it is stored in an intracellular inactive form. It is released in response to mechanical or inflammatory stress and is activated by high extracellular calcium levels. The strong affinity of tTGase for gluten reflects the fact that 30 to 50% of the amino acids in gluten are glutamine. This enzyme induces formation of aggregates of gluten and other antigens, which seems to be important for efficient antigen uptake by antigen-presenting cells of the immune system (*7*). At low pH or in the absence of glutamine acceptors, tTGase deamidates certain glutamine residues of gluten to glutamic acid. This posttranslational modification enhances binding of gluten epitopes to HLA-DQ2 or HLA-DQ8 and potentiates their ability to stimulate T cells (*8*). Several immunodominant gluten peptides (all substrates of tTGase) have been identified, but it is unclear to what extent these peptides reach the small intestinal mucosa after exposure to gastric and duodenal proteases.

In the new work, Shan and colleagues (*2*) isolated a unique 33–amino acid peptide from the 266–amino acid α2-gliadin (the homologous gliadins represent the major storage proteins of wheat and harbor most of gluten's antigenic epitopes). They tested the resulting peptide fractions against different gliadin-reactive T cell lines in the context of HLA-DQ2. Currently, more than

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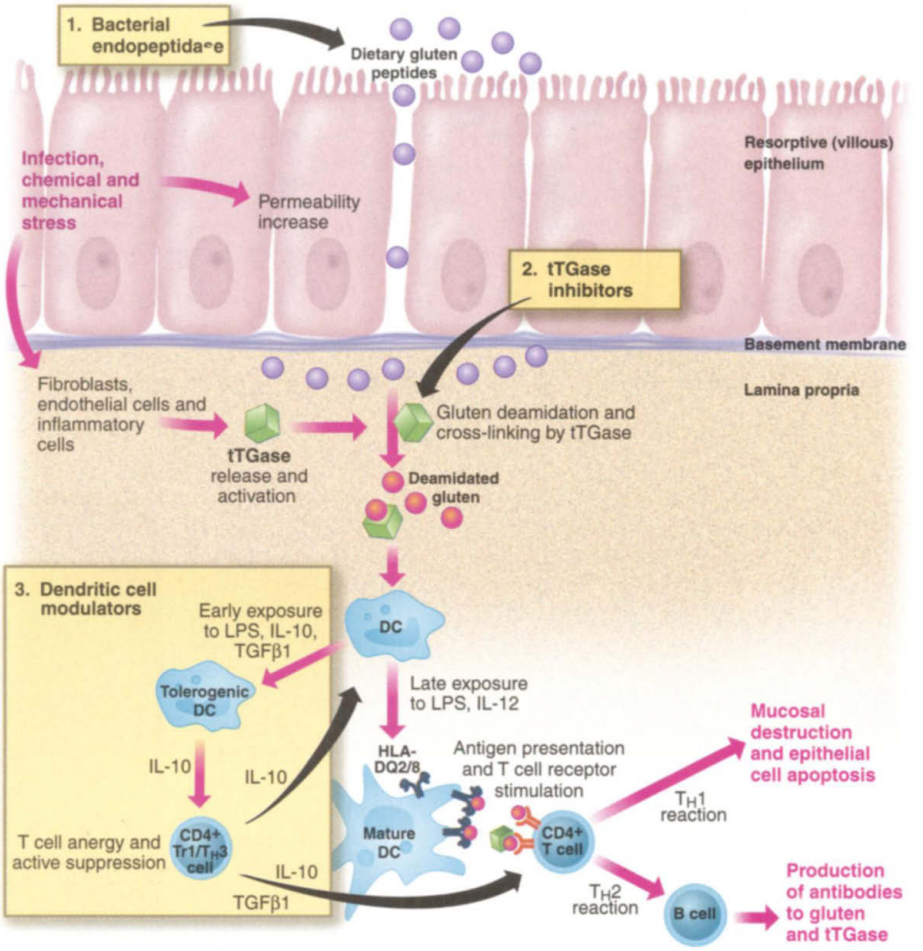
10 antigenic epitopes among the more than 50 gluten proteins have been described, and as many as 50 additional epitopes are thought to exist (*9*). Remarkably, the 33–amino acid peptide (which contained six partially overlapping antigenic hot spots) stimulated all of the T cell lines that the authors tested. Moreover, deamidation by tTGase potentiated this T cell stimulation. Addition of a bacterial prolyl endopeptidase caused degradation and loss of the antigenicity of this peptide, which is normally resistant to breakdown by endogenous proteases. This finding opens up the possibility that bacterial endopeptidases could be used to "detoxify" this and other immunodominant peptides of gluten.

Celiac sprue is perhaps the most common human genetic disorder, with a prevalence of 0.5 to 1% in Western, Arabian, and Indian populations. Patients with celiac sprue have to maintain a life-long, strictly gluten-free diet. Thus, there is a strong need to find ways to detoxify wheat, so that it can be eaten by these individuals. Current approaches include creation of a genetically modified wheat that is devoid of antigenic gliadin sequences. This assumes that the glutenins, which are structurally unrelated to gliadins and which are needed for their baking properties, do not harbor T cell–stimulatory sequences. However, two "toxic" glutenin epitopes have been described, and many more are thought to exist (*10*). This would necessitate the "knockout" of essentially all genes encoding wheat-storage proteins. Apart from general reservations about genetically modified foods, the product would probably have lost all of the properties of wheat. As Shan *et al.* suggest, supplementing the diet with a bacterial endopeptidase that destroys major immunodominant gliadin epitopes—and probably glutenin peptides as well (*10*)—is attractive and appears practical, because the protease could be ingested along with a diet containing gluten.

Research into celiac sprue pathogenesis has focused on gluten-reactive T cell lines or clones and the destructive T helper cell 1 (T_H1) reaction induced by immunodominant gluten peptides. However, intestinal immune regulation is complex, and we poorly understand mechanisms of T cell anergy or active suppression that are central to distinguishing between beneficial nutritional (or microbial) antigens and detrimental antigens in the gut. HLA-DQ2 or HLA-DQ8 is found in roughly 30% of Western populations, but celiac sprue is encountered in only 1 out of 50 carriers. Thus, most carriers must harbor some form of immune protection. Key immune regulators are dendritic cells—potent antigen-presenting cells that can prime T cells not only for destruction, but also for tolerance or anergy (*11*, *12*). In addition, the route and sequence of antigen supply determine whether the T cell response will be destructive or suppressive, as illustrated by experimental autoimmune neuritis. This autoimmune disease can be induced in mice by *subcutaneous* vaccination with an oligomeric, immunodominant myelin P2 peptide; in contrast, the same peptide after *intravenous* or *oral* application prevents or even reverses established disease (*13*).



New ways to treat celiac sprue. Molecular pathogenesis of celiac sprue and possible therapeutic interventions. Dietary gluten peptides reach the subepithelial connective tissue (lamina propria), particularly once intestinal mucosal integrity is impaired during infection or during mechanical or chemical injury. In genetically susceptible (HLA-DQ2/HLA-DQ8–positive) individuals, certain gluten peptides are displayed on professional antigen-presenting cells, in particular on dendritic cells, but also on B lymphocytes and activated intestinal epithelia. This results in driving of the $CD4^+$ T cell response toward either inflammation and tissue remodeling (T_H1 response) or antibody production (T_H2 response). The enzyme tTGase is released from endothelia, fibroblasts, and inflammatory cells residing in the lamina propria, where it encounters its prime external substrate, gluten. Cross-linking of gluten by tTGase potentiates its uptake and presentation by antigen-presenting cells, and its deamidation enhances its binding to HLA-DQ2/HLA-DQ8, resulting in the triggering of a vigorous T cell response. Possible intervention strategies (numbered boxes) include (1) addition of bacterial endopeptidase to the diet to destroy antigenic gluten peptides, such as the 33–amino acid peptide, that are resistant to gastrointestinal proteases; (2) treatment with tTGase inhibitors that block potentiation of gluten antigenicity; (3) directing dendritic cell differentiation toward promotion of $CD4^+$ T cell anergy or induction of tolerogenic (Tr1 and T_H3) T cells by, for example, early exposure to lipopolysaccharide (LPS) or the cytokines IL-10 and transforming growth factor–β1 (TGFβ1). (Not shown is the fact that dendritic cells and T cells circulate to mesenterial lymph nodes where important encounters with antigen take place, before returning to the lamina propria.)

But dendritic cell and suppressor T cell biology is ill-defined in celiac sprue. In addition, numerous external factors are involved in gastrointestinal nutrient and gluten processing. These include (i) gastric acid, which regulates pepsin activity and can be modified by nutrient supply or antiacid medications like proton-pump inhibitors; (ii) the activity of pancreatic and small intestinal proteases, which relies on pancreatic function; and (iii) the small in-

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testinal microbial milieu, which may alter epithelial permeability, admitting an influx of gluten and other antigens.

The multiple levels of immune regulation explain the observed broad spectrum of gluten sensitivity in patients with celiac sprue, and will allow us to test several intervention strategies. With the ease of obtaining duodenal biopsies, immunomodulatory therapies can be verified in celiac sprue patients, an approach that should benefit all individuals who suffer from immune diseases of the intestinal system.

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PERSPECTIVES: PLANETARY SCIENCE

Active Volcanism on Io

Alfred S. McEwen

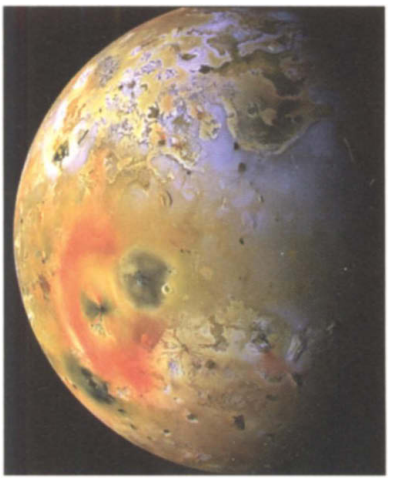
Jupiter's moon Io (see the first figure) is a bizarre world with hundreds of active volcanoes, high rugged mountains, a colorful surface rich in sulfurous materials, and giant (up to 500 km high) plumes of gas and dust that drive a thin atmosphere. High-resolution data returned by the Galileo spacecraft resolve some longstanding questions regarding Io's volcanism and pose new questions about its internal structure.

The four Galilean moons of Jupiter are planet-sized worlds. The three innermost moons have resonant orbits: Each time Ganymede orbits once, Europa orbits twice and Io four times, leading to substantial tidal heating of Europa and intense tidal heating of Io. In Greek mythology, Io was a nymph who was changed into a heifer by her lover Zeus but later regained her form. Likewise, the massive Jupiter (Zeus) periodically deforms Io, which has an eccentric orbit forced by the periodic tugs of Europa and Ganymede. The resulting world may provide clues to understanding very ancient volcanism on Earth.

The Voyager 1 flyby in 1979 first revealed the exotic surface and active volcanism of Io (*1*). The nature of the volcanism was hotly debated. Some scientists, such as Carl Sagan (*2*), argued that sulfur volcanism was burying a silicate subcrust. Others, such as Michael Carr (*3*), argued for silicate volcanism and crust (similar to that on Earth) with thin sulfurous coatings.

Sulfurous volcanism could not produce temperatures above ~700 K, whereas basaltic lavas on Earth range from 1300 to 1450 K. Voyager instruments and Voyager-era telescopic observations revealed hot spots with temperatures of up to 650 K, consistent with sulfur volcanism. But these measurements lacked the sensitivity and wavelength coverage needed to detect small areas with higher temperatures.

The first convincing evidence for surfaces too hot for sulfur volcanism was acquired in 1986 (*4*). During the next 10 years, ground-based measurements yielded several other detections of such high temperatures, and it became apparent that there were at least a few sporadically active silicate eruptions. But a second key test—the spectral reflectance of the dark regions suspected of being silicate lavas—could not be accomplished from Earth-based telescopes. In recent years, NASA's Galileo mission has provided some of these missing data after overcoming some formidable obstacles.



Global view of Jupiter's moon Io. Enhanced color composite image from data obtained in September 1997 by Galileo. One of the most dramatic changes from previous images was the appearance of a new dark spot (upper right edge of the big red ring of Pele), 400 km in diameter, which surrounds the volcanic center Pillan Patera.

When the Galileo spacecraft was launched in 1989 after delays such as the shuttle Challenger explosion, it had to take a longer, less direct path to Jupiter than originally planned. After launch, the umbrella-like high-gain antenna failed to open properly. The mission team was forced to drastically revise the science planning, returning just a small fraction of the expected data via a small, low-gain antenna.

Galileo included much more sensitive instruments than Voyager, such as the Solid State Imaging (SSI) system (sensitive from 400 to 1000 nm) and the Near-Infrared Mapping Spectrometer (NIMS) (sensitive from 700 to 5200 nm). SSI could detect only areas hotter than ~700 K and usually when Io was in eclipse (in Jupiter's shadow) to eliminate reflected and scattered light. NIMS had the ideal spectral coverage for detecting both the temperatures and spectral reflectances expected from silicate lavas, but had limited spatial resolution (120 km or more), except during the one close Io flyby in December 1995 expected when Galileo first arrived at Jupiter.

Unfortunately, Galileo's troubles were not over. Because of a tape recorder anomaly, no remote-sensing data of Io's surface were collected during the Io flyby. Galileo subsequently proceeded to be a great success, with several extensions beyond the 2-year nominal mission, including six close Io flybys. But by the time of the first return to Io in 1999, the spectral grating of NIMS had became stuck and several detectors were dead or degraded. NIMS could therefore acquire useful data at only 13 rather than hundreds of wavelengths. The greatest scientific toll of the early tape recorder anomaly was the loss of NIMS spectra that could spatially resolve the dark patches to determine their compositions.

Despite these early troubles, the Galileo mission has provided unprecedented insights into Io's volcanism. The first distant Galileo observations of Io were made in the summer of 1996. The first SSI image of Io in eclipse revealed eight definite hot spots and several other likely sites of active silicate eruptions. The first NIMS images revealed 14 hot spots with single-temperature fits ranging from ~400 to 600 K; more sophisticated models revealed hot spots with temperatures above ~1000 K in most or all of these images. By 1998, Galileo had identified a total of 41 hot spots (*5*), all in the dark patches that cover only about 1.4% of Io's otherwise bright sur-

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