"inverter" built from chemically doped nanotubes on a silicon substrate. Schön *et al.* also demonstrated an inverter assembled from field-effect transistors based on a monolayer of small organic molecules (*11*) and even on single molecules (*12*), each only 2 nm long.

As impressive as these four very recent demonstrations of molecular circuits are, they build on a long series of important steps by a number of researchers striving to make molecular-scale computers (*2–4*). The concept for molecular electronic devices and circuits dates back to the seminal 1974 work of Aviram and Ratner (*13*). Only in the past few years have scientists realized key experimental demonstrations of molecules that serve as wires and switches, which may be divided into four broad categories, according to the type of molecule or molecular-scale structure used to make the devices. The categories (see the figure) are semiconductor and metal nanowires (*7*, *9*), carbon nanotubes and fullerenes (*6*, *8*, *14*), small organic molecules (*11*, *12*, *15–19*), and biomolecules (*20*, *21*). Structures from three of these categories are used in the recent advances described above.

Despite the broadly based and encouraging recent progress, a set of technical challenges still must be overcome to make a robust, commercially viable computer integrated on the molecular scale. Circuits must be produced that are molecular scale in their entirety, not just incorporating molecular-scale components. Developing intrinsic metrics (see above) or other means for readily establishing molecular spacing between components and devices would be an important step in that direction. Advances in the chemistry of nanotubes may make it faster and easier to manipulate them and to produce or select nanotubes with specific structures and electrical properties. The nanotube-based circuits discussed above still require selection and placement through time-consuming, arduous nanomanipulation.

The very small sizes of molecules make it possible, in principle, to fit a trillion molecular devices in a square centimeter. What does one do with a trillion devices? Even if the problem of heat dissipation can be overcome for so many densely spaced electrical devices, how can they be harnessed for useful computation? At this level of integration, geometric and dynamic bottlenecks from the proliferation of interconnects, as well as intrinsic latencies, which have been observed even in less densely integrated highly parallel processors, will present new challenges unless innovative architectural approaches can be found. Finally, how does one assemble a trillion devices per square centimeter quickly, inexpensively, and with molecular precision? This facility seems necessary to fulfill the promise of molecular electronics and would have revolutionary implications for nanotechnology.

Until now, though, one of the major challenges facing molecular electronics investigators was the assembly of individual molecules or molecular-scale structures into functioning logic circuits. The fact that four groups have achieved this goal almost simultaneously is an indicator of how far molecular electronics and nanotechnology have come and is very encouraging concerning their robust promise for the future.

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PERSPECTIVES: IMMUNOLOGY

# Antigen Presentation—Losing Its Shine in the Absence of GILT

**Colin Watts**

Before $CD4^+$ T cells of the immune system can be activated, they must engage specific class II major histocompatibility (MHC)/peptide complexes on the surface of antigen presenting cells (APCs) such as dendritic cells and B lymphocytes. The T cell then decides whether to proliferate based on the amount and quality of these complexes and the presence on the APCs of other so-called costimulatory signals. Class II MHC/peptide complexes are assembled within the endosomes and lysosomes of APCs prior to their expression on the cell surface. Both the secretory and endocytic pathways furnish these endosomes and lysosomes with newly synthesized class II MHC molecules, antigen captured by endocytosis, proteolytic enzymes, and other dedicated chaperone proteins. Add the acidic environment required for everything to work optimally, and you have the complete specification for a "reaction vessel" designed to load class II MHC molecules with diverse antigenic peptide species.

Well, not quite. On page 1361 of this issue, Maric *et al.* reveal a new element of the antigen presentation machinery in the guise of an enzyme called GILT (*1*). As its name implies, GILT (γ interferon–inducible lysosomal thiol reductase) catalyzes the reduction of disulfide (S-S) bonds in protein substrates endocytosed by APCs. To assess GILT's involvement in class II MHC-restricted presentation of antigenic proteins containing disulfide bonds, Maric *et al.* generated a GILT-deficient mouse. So far, they have tested only a limited set of antigens in their mouse, but for the principal test case, hen egg lysozyme (also called HEL), the results are clear-cut: In the absence of GILT, presentation of two major lysozyme antigenic epitopes to T cells is partially or completely abrogated. These data provide the first demonstration that the battery of proteolytic enzymes found in the endocytic pathway is not always sufficient to release the full spectrum of peptides for T cells to scrutinize.

A requirement for disulfide bond reduction in antigen processing was demonstrated some years ago. This disulfide bond reduction allows improved access of proteases to the antigen substrate (*2*) and is an evident requirement for those T cell epitopes that contain cysteine residues, which participate in S-S bridges in the native protein (*3*, *4*). But how is this reduction achieved? Although reductants such as free cysteine are known to be transported into lysosomes (*5*), the acidic conditions found there, although optimal for proteolysis, are unfavorable for disulfide bond reduction and exchange (*6*). This finding has led to the suggestion that the reduction step may need to be catalyzed in a fashion analogous to, but the reverse of, protein

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disulfide isomerase (PDI)–catalyzed formation of disulfide bonds in the endoplasmic reticulum (*7*). However, no enzyme capable of mediating mixed disulfide exchange reactions at acidic pH had been described.

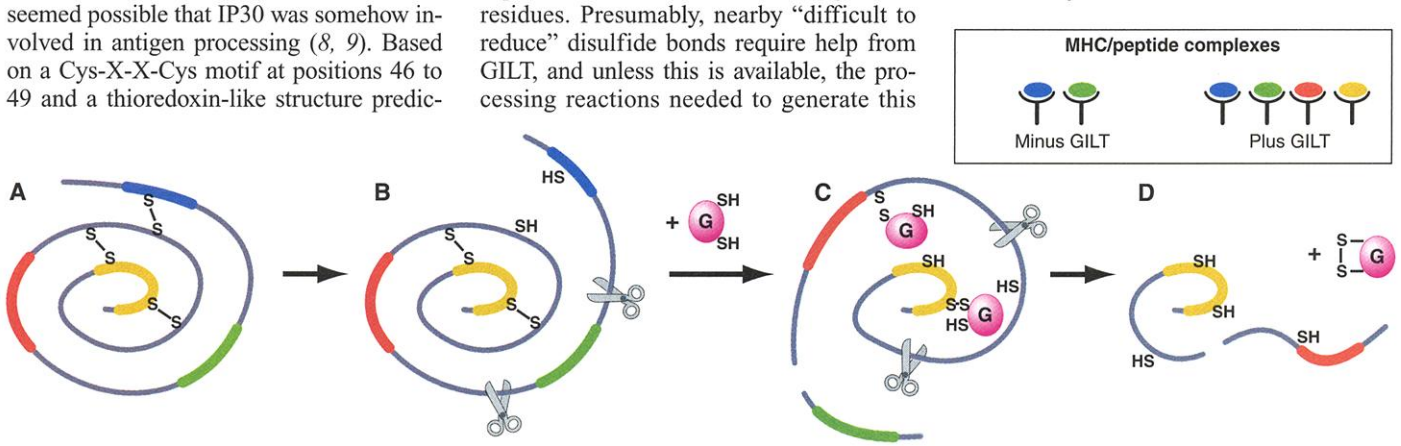
The trail that led to GILT started with its identification as a glycoprotein called IP30. This glycoprotein was found in endosomes and lysosomes, organelles known to host class II MHC peptide-loading events in B cells and other APCs. Because production of IP30 could be induced by interferon γ and because of its rather enigmatic association with certain class II MHC alleles, it seemed possible that IP30 was somehow involved in antigen processing (*8, 9*). Based on a Cys-X-X-Cys motif at positions 46 to 49 and a thioredoxin-like structure predicted, IP30 was postulated to have thiol reductase activity (*8*). This guess turned out to be correct. Mutation of either $Cys^{46}$ or $Cys^{49}$ abolished the reductase activity of IP30, which, unlike other thiol reductases such as thioredoxin, was maximally active at acidic pH (*9*). Moreover, production of IP30 (by this time renamed GILT) in GILT-deficient cells enhanced the reduction and proteolysis of endocytosed protein substrates containing disulfide bonds (*9*). The new work by Maric *et al.* (*1*) finally establishes the importance of GILT in class II MHC–restricted antigen presentation.

Using the well-studied model antigen hen egg lysozyme (*10*, *11*), which contains four disulfide bonds, Maric *et al.* (*1*) show that the display of certain lysozyme peptides to T cells is strikingly abrogated in APCs from GILT-deficient mice. These investigators analyzed the appearance on the APC surface of four different lysozyme peptides associated with $A^b$ class II MHC molecules. Three of the four "epitopes" contain cysteine residues that contribute to disulfide bonds. The results are interesting and not necessarily what one might have predicted. Two overlapping epitopes that share $Cys^{30}$ were presented normally by GILT-free APCs. Another, defined by the peptide 74–88, which contains two cysteine residues that participate in different S-S bonds, was not presented at all; the fourth, defined by amino acid residues 46 to 61, was presented poorly. Presentation of the 74–88 peptide was rescued by preincubating hen egg lysozyme with recombinant GILT.

The picture after immunization of GILT-free mice with lysozyme was similar, except that the response to 46–61 was now completely absent. Interestingly, the 46–61 epitope does not itself contain cysteine residues. Presumably, nearby "difficult to reduce" disulfide bonds require help from GILT, and unless this is available, the processing reactions needed to generate this epitope do not take place (see the figure). This fits with earlier work showing that this region of hen egg lysozyme requires more extensive processing and is probably loaded in later compartments of the class II MHC pathway compared with other regions (*12*). It will be interesting to see whether a pattern of GILT-dependent and GILT-independent T cell epitopes emerges for other antigens.

What next for GILT and the GILT-free mouse? The question of substrate choice arises. How is GILT able to target S-S bonds in hen egg lysozyme and other antigen substrates but not those of other proteins resident in the same cellular compartment or of itinerant proteins passing through, such as receptors being recycled by the cell? Related to this, does GILT act on native, partially unfolded, or even partially processed substrates? Maric *et al.* present evidence that even native hen egg lysozyme can be a substrate for GILT in vitro. However, the mechanism of action originally proposed (*13*) involves nucleophilic attack by GILT's $Cys^{46}$ on a susceptible disulfide bond to give a GILT-substrate intermediate that is then resolved by internal disulfide exchange with GILT's $Cys^{49}$ (see the figure). For at least some substrates, partial unfolding or processing might be necessary to allow the access that such a mechanism would seem to require.

Maric *et al.* rule out the possibility that the deficient hen egg lysozyme responses after in vivo immunization are simply due to lack of appropriate lysozyme-specific T cells. Nonetheless, GILT is likely to have an impact on the array of peptides constitutively presented on APCs in lymphoid organs. It will be interesting to see, for example, whether there are differences in the T cell repertoire that develops in the thymus of GILT-free mice. Finally, could GILT be involved in other endosomal/lysosomal events? We do not yet know, but it is noteworthy that some bacterial and plant toxins, as well as manufactured immunotoxins containing disulfide bonds, require reduction of S-S bridges after endocytosis in order to exert their toxic effects (*14*). Conceivably, cells expressing GILT may be more easily intoxicated than those lacking this enzyme, a possibility that may have therapeutic implications.




**GILT required for access to HEL.** (**A** and **B**) In the absence of the lysosomal thiol reductase GILT, some (green, blue) but not all (yellow, orange) T cell epitopes can be generated from a protein antigen (*1*, *13*). (**C**) GILT may attack disulfide (S-S) bridges via $Cys^{46}$ to form a GILT-substrate intermediate that is resolved by disulfide exchange to release reduced protein substrate and oxidized GILT (**D**). Active GILT may be regenerated by low molecular weight reductants such as free cysteine. Free cysteine may also reduce some GILT-independent protein disulfide bonds (A and B). Scissors denote the action of proteolytic enzymes.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