strands, containing short DNA words associated with each of the Boolean variables. A wide variety of biochemical methods have been developed to encode Boolean variable assignments and to separate or identify the strands encoding an assignment of variables satisfying the formula.

Corn, Smith, and co-workers (*4*) have used surface chemistry techniques to solve a 4-variable instance of SAT. They affixed the DNA stands encoding all possible Boolean variable assignments onto a two-dimensional surface, and then applied restriction enzymes to destroy those DNA strands that do not satisfy the Boolean formula. A fluorescent optical readout was used to identify the remaining DNA strands that encode satisfying assignments for the given formula.

Supercomputers in the test tube

Landweber and co-workers (*5*) used a combination of DNA and RNA techniques to solve a 9-variable instance of SAT related to the well-known Knight's Problem in chess. They employed evolutionary methods to determine a set of RNA sequences used to eliminate those strands that do not satisfy the Boolean formula.

The SAT experiments of Adleman and co-workers (*3*) use hybridization of short DNA strands called stickers to encode truth assignments of 20 Boolean variables. Their latest breakthrough was made possible by the use of automated gel electrophoresis to separate out the DNA strands that have stickers encoding satisfying assignments of the Boolean formula.

The above protocols require many laborious separation and detection steps, which will only increase as the scale increases. These problems may be overcome by using autonomous methods for DNA computation, which execute multiple steps of computation without outside intervention. Autonomous DNA computations were first experimentally demonstrated by Hagiya and co-workers (*6*) using techniques similar to the primer extension steps of PCR and by Reif, Seeman, and co-workers (*7*) using the self-assembly of DNA nanostructures (*8*). Recently, Shapiro and co-workers reported the use of restriction enzymes and ligase (*9*).

Known protocols for the solution of medium-scale SAT problems may have useful applications in the biomolecular domain. For example, they may be used to execute Boolean retrieval queries on synthetic DNA tags in a "wet" database consisting of a vast store of genomic DNA (obtained from many organisms, individuals, cell types, and at many distinct dates), each tagged with a synthetic DNA strand that provides binary data such as the sampled individual's identification number, cell type, and date of sampling.

The separation techniques developed by Braich *et al.* for their SAT experiments may be used for exquisitely sensitive and error-resistant separation of a small set of specified molecules from a large combinatorial set of molecules. Quite apart from its use for the SAT problem, the technique may also have many other diverse biotechnology and security applications, such as the detection and tracing of minute amounts of toxic or explosive materials.

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PERSPECTIVES: TRANSCRIPTION

Proteasome Parts at Gene Promoters

Søren Ottosen, Francisco J. Herrera, Steven J. Triezenberg

Proteasomes are the protein complexes in eukaryotic cells that direct the degradation of ubiquitin-tagged (ubiquitinated) proteins. The 26*S* proteasome is composed of a 19*S* regulatory subcomplex (comprising a base and a lid) that recognizes polyubiquitinated proteins, and a 20*S* proteolytic subcomplex where these tagged proteins are degraded (see the figure). The proteolysis of ubiquitinated proteins is a feature of many cellular processes including chromosome stabilization, cell division, apoptosis, cell differentiation, and the stress response (*1*). Ubiquitin-tagged proteins are also involved in cellular events that do not require proteolysis, such as endocytosis and the localization of certain proteins in the nucleus (*2*).

Ubiquitin tagging modulates the stability of many transcription factors and even of RNA polymerases, the enzymes that drive gene expression. Intriguingly, the transcriptional activation domains of some transcription factors serve as signals for ubiquitination and degradation (*3*, *4*), suggesting that the proteasome itself is involved in transcription (*4*). In contrast, histone proteins, especially H2A and H2B—which form the nucleosomes around which chromatin is wrapped—are ubiquitinated, but apparently not for purposes of degradation. The participation of ubiquitin in nonproteolytic processes suggests that different components of the proteasome may be involved in proteolytic and nonproteolytic events. This hypothesis gains support from the work of Gonzalez *et al.* (*5*) published on page 548 of this issue. They show that adenosine triphosphatase (ATPase) enzymes of the 19*S* subcomplex (but not the proteases of the 20*S* subcomplex) become associated with genes that are being transcribed.

The roots of this story go back nearly a decade, when Johnston and colleagues discovered that specific alleles of the yeast *SUG1* and *SUG2* genes suppress the phenotype of a defective allele of *GAL4*, which

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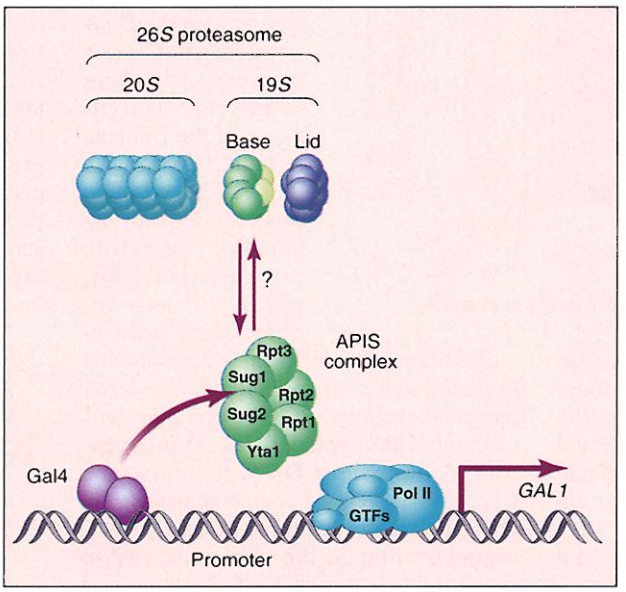
encodes a transcription factor that activates certain genes in response to galactose (*6, 7*). Moreover, the Sug1 and Sug2 proteins bind directly to the activation domains of Gal4 and other transcription factors (*8–10*), suggesting that they may be acting as coactivators. However, Sug1 and Sug2 were then found to be components of the proteasome (Rpt6 and Rpt4, respectively) (*11*). What could the proteasome possibly be doing in the midst of transcription? Protein degradation did not seem to be a likely answer. The suppressing *SUG* alleles do not increase the stability of Gal4; conversely, *SUG* alleles that do allow accumulation of Gal4 do not suppress the *gal4* mutant phenotype (*12*). Moreover, *SUG1* and *SUG2* alleles also rescue the phenotype of yeast with mutations in the cdc68 transcription elongation factor (*13*), implicating the Sug proteins in elongation of mRNA transcripts (*14*).

In the new work, Gonzalez *et al.* (*5*) show that the ATPases located in the 19*S* base compartment of the yeast proteasome associate with the *GAL1* and *GAL10* genes when the expression of these genes is switched on. They discovered this using the chromatin immunoprecipitation assay in which proteins and nucleic acids are covalently cross-linked by formaldehyde before precipitation by an antibody that recognizes the protein of interest. Specific DNA fragments associated with that protein can then be detected by the polymerase chain reaction. Gonzalez and co-workers found that the regulatory DNA region between the *GAL1* and *GAL10* genes, and sequences throughout the *GAL1* gene itself, were precipitated by antibodies to three proteasomal ATPases: Sug1/Rpt6, Sug2/Rpt4, and Yta1/Rpt5. They observed this association only when the yeast were grown under inducing conditions (in medium containing galactose) and when Gal4 was present. Strikingly, other proteasomal proteins from either the 20*S* proteolytic subcomplex or the 19*S* lid did not associate with *GAL1* DNA, regardless of whether this gene was transcribed. Thus, when attached to DNA, these ATPases do not associate with the rest of the proteasome.

The Sug ATPases do not associate with the promoter of the *GAL1* and *GAL10* genes in yeast strains bearing a truncated Gal4 activation domain. However, the *sug2* allele that suppresses the growth defect of this *gal4* mutant also restores the association of Sug1 with the *GAL1-10* promoter. Remarkably, all six of the proteasomal ATPases—but not proteins either in the 19*S* lid or the 20*S* subcomplex—bound to the Gal4 activation domain in protein interaction assays in vitro (although direct protein interactions with Gal4 were observed only for Sug1 and Sug2). These results suggest that a complex containing the six ATPases of the 19*S* base, which the authors call APIS (AAA proteins independent of 20*S*), interacts with the Gal4 activation domain and is recruited to the *GAL1* gene in the presence of galactose (see the figure).



Proteasomal parts at sites of gene activation. The 26*S* proteasome comprises a 20*S* proteolytic subcomplex and a 19*S* regulatory subcomplex. The 19*S* particle can be subdivided into a lid and a base that contains six ATPases. Expression of the yeast *GAL1* gene, induced by addition of galactose to the growth medium, results in association of the 19*S* ATPases with the *GAL1* promoter. This association requires the transcriptional activator Gal4, which directly interacts with two of the ATPases, Sug1 and Sug2. Whether the ATPases are a prerequisite for transcriptional activation, or whether they serve to recruit the proteasome to switch off gene activation, remains to be determined.

This report is noteworthy for its observation that proteasomal components are intimately engaged with the transcriptional machinery, but with no apparent link to proteolysis carried out by the proteasome. These results were foreshadowed by work showing that a similar complex of 19*S* ATPases interacts with the TATA-box binding protein in vitro (*15*). This complex, like that in the Gonzalez *et al.* work, does not contain proteins of the 20*S* particle nor of the 19*S* lid, but does include certain general transcription factors and related proteins. Together, these reports imply that the 19*S* base particle (or APIS) can exist as a separate biological entity with distinct transcriptional activities that are independent of the proteasome. It is still not clear what the APIS complex does during transcription. One exciting possibility is that the ATPases generate energy to drive the assembly, initiation, or elongation activities of the transcription machinery, which may involve remodeling of the massive protein complexes assembled at gene promoters. A precedent for this possibility comes from a related family of ATPases in bacteria, mitochondria, and chloroplasts, the Clp/Hsp100 chaperone proteins. These chaperones help to degrade proteins, but they are also needed to remodel protein complexes involved in transcription, replication, and transposition (*16*).

Another important question is how the APIS complex gets recruited to activated genes. Although the authors show a direct interaction between the Gal4 activation domain and two of the six proteasomal ATPases, they speculate that ATPase recruitment could be modulated by ubiquitination of Gal4. Transcriptional activation domains can serve as signals for ubiquitination (*3*), which may have either a positive or negative impact on gene expression (*4, 17*). One model consistent with these observations posits that attachment of a single ubiquitin moiety to Gal4 enables transcriptional activation to occur (activator "licensing") by promoting the interaction of the transcription factor with key protein targets, including perhaps the APIS complex. But, as monoubiquitination is insufficient to trigger proteasome-mediated degradation, subsequent ligation of additional ubiquitin groups may be required to target the transcription factor to the 26*S* proteasome for degradation. Thus, the APIS complex may recruit the remainder of the proteasome to transcriptionally active genes, thereby dampening or down-regulating gene expression. Gonzalez *et al.* (*5*) hint at this possibility by noting that components of the 20*S* subcomplex and 19*S* lid can be detected at the *GAL1* promoter much later during gene expression. In another example, ubiquitination can promote the replacement of coactivator proteins by corepressor proteins at genes regulated by the LIM homeodomain transcription factor family (*18*). Thus, APIS and the proteasome may provide an actively regulated endpoint for gene expression stimulated by a given transcription factor.

Exciting questions that are waiting to be explored include whether the APIS complex contributes enzymatically to transcriptional activation or is merely involved in transcription factor degradation, and

whether ubiquitination of transcription factors triggers recruitment of APIS to genes that are to be transcribed.

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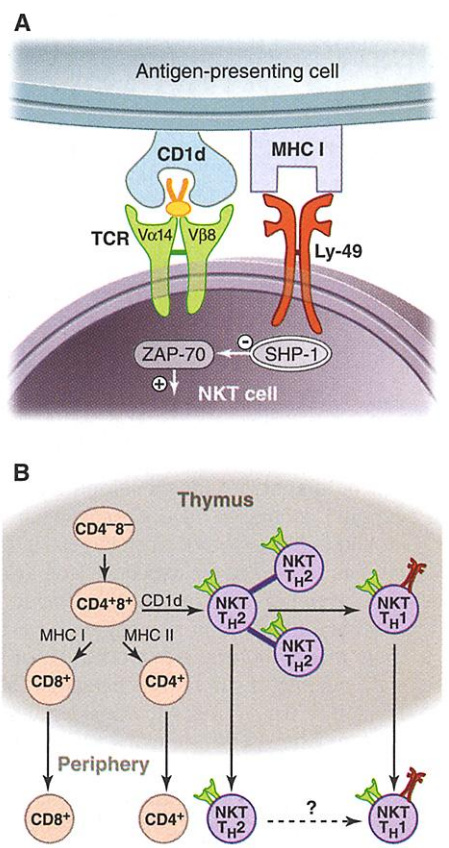
T Before NK

H. Robson MacDonald

Natural killer T (NKT) cells are a minor subset of mature lymphocytes that, as their name suggests, express receptors associated with both T cell and NK cell lineages. They have attracted a lot of attention because they regulate not only autoimmunity but also immune responses against microbes and tumors (*1*, *2*). NKT cells are able to carry out this wide array of tasks partly because, when activated, they secrete large amounts of cytokines, such as interferon-γ (IFN-γ) and interleukin-4 (IL-4). These hybrid lymphocytes express a heterodimeric αβ T cell receptor (TCR), as well as the NK cell receptors (NKRs) NK1.1 and members of the Ly-49 receptor family. When expressed by T cells, TCR is an activating receptor that transduces positive signals through mobilization of intracellular tyrosine kinases. In contrast, Ly-49 receptors expressed by NK cells are inhibitory receptors that recruit intracellular phosphatases, which dephosphorylate and hence inactivate kinases (see the figure, panel A). Thus, the coexpression of TCR and Ly-49 receptors by NKT cells raises the intriguing question of how these potentially opposing receptors are regulated during NKT cell development. Enter Benlagha *et al.* (*3*) on page 553 of this issue and Pellicci *et al.* (*4*) in the *Journal of Experimental Medicine* with a solution to this dilemma. Both groups identify a new intermediate cell in the NKT lineage that reveals how TCRs and NKRs operate during differentiation and maturation of NKT cells.

It is now generally accepted that most (if not all) NKT cells, like conventional T cells, originate in the thymus. However, the search for immature NKT lineage precursors in the thymus is complicated by the fact that mature NKT cells represent a very small fraction (0.3 to 0.5%) of total thymic T cells (thymocytes). To overcome this problem, Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) have exploited a recent technical advance that allows the direct identification of NKT lineage cells by virtue of their restricted TCR specificity. Whereas conventional T cells express diverse TCRs that recognize short peptides in association with highly polymorphic major histocompatibility complex (MHC) molecules, the highly conserved TCR on NKT cells exclusively recognizes glycolipids bound to the monomorphic CD1d molecule (see the figure, panel A). Although the endogenous and foreign glycolipids recognized by the TCR on NKT cells under physiological conditions are not known, a synthetic glycolipid (α-galactosyl ceramide) bound to CD1d mimics their effects. Hence, by preparing fluorescent tetramers of α-galactosyl ceramide bound to CD1d it is possible to track very small numbers of NKT lineage cells by flow cytometry (*5*, *6*).

With this approach, Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) identified a new subset of thymocytes that bind to CD1d tetramers but do not express NKRs, such as NK1.1 and members of the Ly-49 inhibitory receptor family. When purified and injected directly into the thymus of a genetically marked syngeneic recipient mouse, NKR^- $tetramer^+$ cells gave rise to NKR^+ $tetramer^+$ cells in both the thymus and peripheral lymphoid tissues. This observation provided direct evidence for a lineage relationship between these two populations. Surprisingly, quantitation of NKR^- and NKR^+ subsets of $tetramer^+$ cells among recent thymic emigrants (using in situ fluorescein isothio-



Balancing act. (**A**) NKT cells express a conserved αβ TCR (composed of Vα14 and Vβ8 chains) that recognizes glycolipids bound to CD1d, as well as inhibitory Ly-49 receptors that bind MHC class I molecules. Whereas TCR ligation leads to activating signals mediated by tyrosine kinases (such as ZAP-70), engagement of Ly-49 receptors recruits phosphatases (such as SHP-1) that can potentially neutralize kinase activity. (**B**) During T cell development in the thymus, $CD4^-8^-$ precursor cells give rise to immature $CD4^+8^+$ thymocytes that randomly express TCRs. $CD4^+8^+$ thymocytes expressing TCRs with appropriate affinity for MHC class I and II molecules, respectively, develop further into conventional $CD8^+$ and $CD4^+$ T cells. Rare $CD4^+8^+$ thymocytes expressing TCRs that bind to CD1d develop along the NKT cell lineage, first undergoing proliferation and subsequently expressing inhibitory NKRs such as Ly-49. During this maturation process the cytokine-producing potential of NKT cells evolves from a T_H2 to a T_H1 pattern. Both immature and mature NKT cells can be exported from the thymus to the periphery, but it is not known whether NKT cell maturation can take place outside of the thymus.

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