Genome-Wide Insertional Mutagenesis of *Arabidopsis thaliana*

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ilar to that seen in ChAT knockout mice (*17*). This may result from perturbed neuronal activity. The most unique aspect of the *reeler* phenotype is the failure to achieve input elimination that is normally completed by the end of the second postnatal week (*10*). NCAM deficiency (*18*) or the overexpression of trophic factors (*10*) caused a delay of synaptic editing due to continued remodeling and axonal sprouting. In *reeler*, however, sprouting was not observed, and multiple innervation was seen even in adult animals. Exogenous Reln promoted axonal withdrawal, strongly suggesting that Reln regulates synapse elimination. To our knowledge, Reln is the first identified molecule that is required for this process in vivo. Our pharmacological studies, demonstrating the mechanism of Reln activity, are consistent with previous studies that invoked the action of a serine protease in the removal of multiple terminals from the NMJ (*19*, *20*). Synapse elimination is a competitive phenomenon affected by electrical activity. We hypothesize that this activity may modulate Reln function, for example, by affecting its local concentrations in the synaptic cleft.

The absence of Reln in humans results in lissencephaly with cerebellar hypoplasia, a disease characterized by ataxia and, notably, abnormal neuromuscular connectivity (*21*). Genes disrupted in congenital forms of muscle dystrophy were recently implicated in neuronal migration (*22*). The requirement for Reln in both brain and muscle development further demonstrates that the extracellular environments of these tissues share essential molecules. Our findings suggest that Reln may affect synaptic maturation in the brain. Distinct alterations in cerebellar (*23*), hippocampal (*24*), and retinal circuitry (*25*) have been reported in *reeler* mice, and the number of dendritic spines is reduced in *reeler* heterozygous mice (*26*). Reln also affects long-term potentiation in the hippocampus (*27*). We speculate that Reln deficiency could lead to abnormal neuronal connectivity and that this could underlie the insurgence of cognitive disorders associated with Reln abnormalities, such as schizophrenia (*28*) and autism (*29*).

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Supporting Online Material

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Genome-Wide Insertional Mutagenesis of *Arabidopsis thaliana*

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Over 225,000 independent *Agrobacterium* transferred DNA (T-DNA) insertion events in the genome of the reference plant *Arabidopsis thaliana* have been created that represent near saturation of the gene space. The precise locations were determined for more than 88,000 T-DNA insertions, which resulted in the identification of mutations in more than 21,700 of the ~29,454 predicted *Arabidopsis* genes. Genome-wide analysis of the distribution of integration events revealed the existence of a large integration site bias at both the chromosome and gene levels. Insertion mutations were identified in genes that are regulated in response to the plant hormone ethylene.

One of the most significant findings revealed through analysis of genomes of multicellular organisms is the large number of genes for which no function is known or can be predicted (*1*). An essential tool for the functional analysis of these completely sequenced genomes is the ability to create loss-of-function mutations for all of the genes. Thus far, the creation of gene-indexed loss-of-function mutations on a whole-genome scale has been reported only for the unicellular budding yeast *Saccharomyces cerevisiae* (*2*–*4*). Although targeted gene replacement via homologous recombination is extremely facile in yeast, its efficiency in most multicellular eukaryotes does not yet allow for the creation of a set of genome-wide gene disruptions (*5*, *6*). Gene silencing has recently been used to study the role of ~86% of the predicted genes of the *Caenorhabditis elegans* genome in several developmental processes (*7*, *8*). The RNA interference (RNAi) method has, however, several drawbacks, including the lack of stable heritability of a phenotype, variable levels of residual gene activity (*9*–*11*), and the inability to simultaneously silence several unrelated genes (*12*).

By comparison, the creation of genome-wide collections of sequence-indexed insertion mutants has several advantages (*5*). We selected *Agrobacterium* T-DNA to generate a large collection of sequence-indexed *Arabidopsis* insertion mutants. About 150,000 transformed plants (T1 plants) expressing a T-DNA–located kanamycin-resistance gene (*NPTII*) were selected and individually propagated (*13*). To estimate the number of unlinked T-DNA insertions per plant line, the segregation of antibiotic resistance was scored in the progeny of 100 T1 plants. The average number of T-DNA insertions per line was found to be ~1.5 [a number similar to other T-DNA collections (*14*)], and therefore, the entire collection was estimated to contain 225,000 independent T-DNA integration events. Given its genome size of ~125,000 kb, average gene length (x) of ~2 kb, and a random distribution of insertion events, and disregarding that insertions in essential female gametophyte genes cannot be recovered, there is a 96.6% probability (P) of obtaining an insertion in an average *Arabidopsis* gene [where $P = 1 - (1 - [x/125{,}000]^n)$ and n = the total number of insertions in the genome (*15*)].

To determine the precise genomic location of each T-DNA integration event, we developed a high-throughput insertion-site recovery system (*13*). In total, 127,706 T1 plants were processed, resulting in 99,230 T-DNA/genome junction sequences (GenBank accession numbers). The integration site of each T-DNA was located by alignment of each junction sequence with the five *Arabidopsis* pseudochromosomes [GenBank release date 20 August 2002; (*13*)]. Insertions in low-complexity regions and tandem repetitive DNAs, including 180–base pair centromeric elements and ribosomal RNA gene repeats [~12 million base pairs (Mbp)], were excluded from this analysis. Also excluded were apparent polymerase chain reaction (PCR) plate cross-contaminants and T-DNA insertions in large, perfectly duplicated (100% sequence identify) chromosomal regions, which appeared to be artifacts of chromosome pseudomolecule assembly from individual bacterial artificial chromosome clones. In total, a conservative set of 88,122 high-quality T-DNA integration-site sequences were mapped onto the genome sequence, and a single genomic location was unambiguously determined. These sequences were used for all of the analyses presented below (Table 1, table S1).

Our analysis of these 88,122 T-DNA insertion site sequences revealed that mutations had been identified in 21,799 of the 29,454 annotated genes or ~74.0% of the *Arabidopsis* genes. In addition, two or more alleles have been identified for 15,265 genes. Analysis of multiple alleles is often crucial for gene function studies, since the mutations not linked to a T-DNA could be as high as ~60% (*14*). With the exception of transposons, no significant bias was detected for T-DNA insertions for any of the gene functional categories (*16*). A highly nonuniform chromosomal distribution of integration events was observed (Fig. 1A). We found preferred sites of T-DNA integration or "hot spots," as well as "cold spots" [Fig. 1A, fig.S1, and (*13*)]. As for whole chromosomes, fewer T-DNA integration events were consistently observed in regions surrounding each of the five centromeres (Fig. 1, B to F). The density of T-DNA insertion events was closely correlated with gene density along each chromosome: The number of T-DNA integration events decreased dramatically as our examination moved toward the centromeres from the gene-rich chromosome arms. These pericentromeric regions in *Arabidopsis* chromo-

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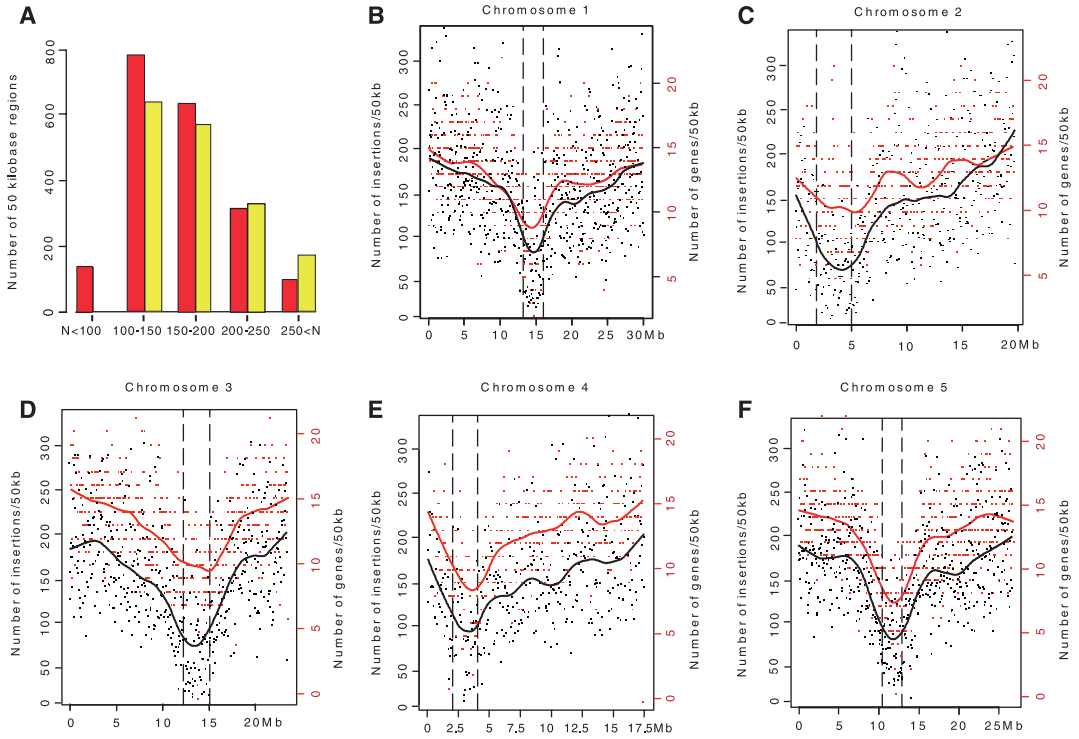

Fig. 1. Nonuniform distribution of T-DNAs in the *Arabidopsis* genome. (**A**) Comparison between the observed and random distribution of T-DNAs. The genome was divided into windows of 50 kb, and the windows were binned according to number of insertions. The expected number was calculated by independently permuting the insertions across windows on each chromosome. Twenty such permutations were used to estimate the expected values and variance-covariance matrix of the counts that was used in significance testing. The observed distribution is shown in yellow, and the expected random distribution is in red. There is an excess of fragments with either high or low amounts of actual T-DNA insertions (hot spots, right side; cold spots, left side). (**B** to **F**) T-DNA and gene distribution along the five chromosomes. The chromosomes were divided into windows of 50 kb. The number of T-DNA insertions and the number of predicted genes in each 50-kb window were plotted in black and red, respectively. The black and red lines represent the best fitting function for the T-DNA and gene distribution. The area between the discontinuous vertical lines corresponds to the pericentromeric regions deduced from references (*1*, *17*).

somes are known to have lower gene density and a higher concentration of transcriptionally silent transposons and pseudogenes (*1*, *17*).

Next, we examined the preference for T-DNA insertion events within particular genetic elements, including 5′ and 3′ untranslated regions (UTRs), coding exons, introns, and predicted promoter regions (*13*). The coordinates for each of these elements were deduced either from full-length cDNA sequences, which are available for 11,930 genes (table S2), or from gene predictions from the latest release of the *Arabidopsis* genome annotation (table S1). No significant differences were observed between the frequencies of insertion events in 5′UTRs versus 3′UTRs, nor were there differences between coding exons versus introns [Table 1, fig. S2, and (*13*)]. However, a significant bias was seen against integration events in introns and coding exons in favor of 5′UTR, 3′UTRs, and promoters. Moreover, when all intergenic regions were compared with all genes, we detected a small bias toward T-DNA insertions in the intergenic regions. Although there were no effects of G + C content on T-DNA integration sites observed at the genome scale (*13*), we found a positive correlation between the G + C content and the number of insertions in promoters, 5′UTRs, exons, and intergenic regions. Similarly, we detected a negative correlation and no correlation between the G + C content and insertion frequency in introns and 3′UTRs, respectively (fig. S2).

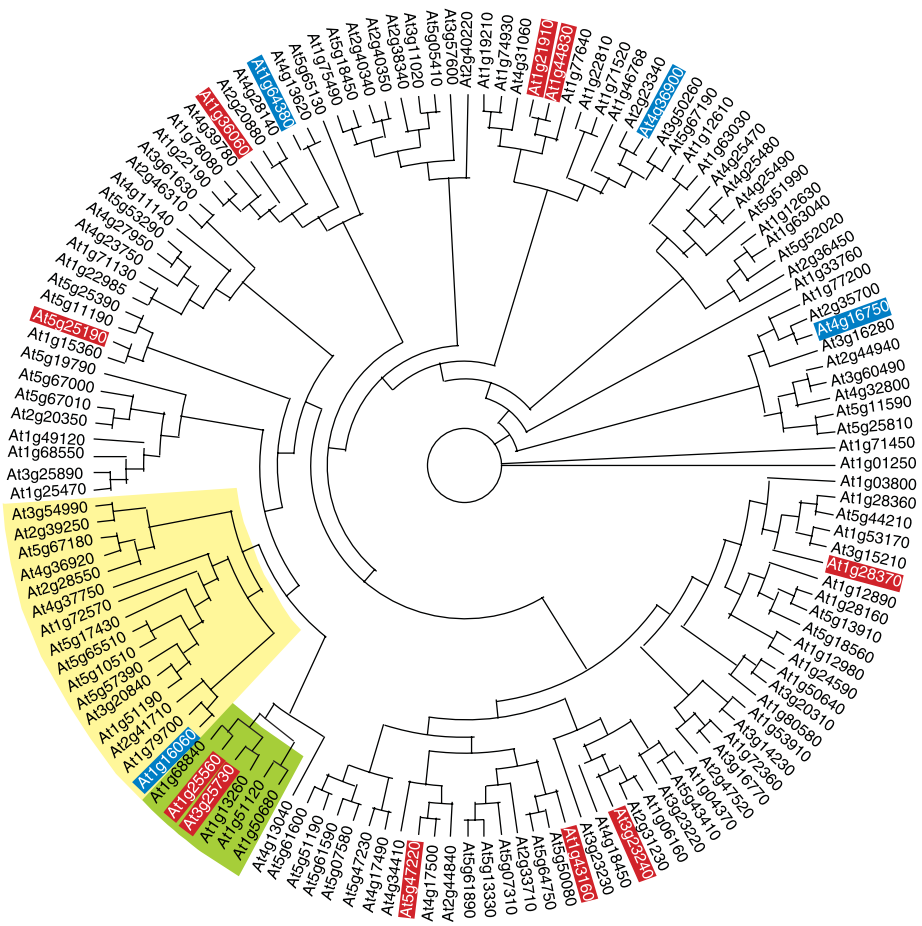

Fig. 2. Functional analysis of the AP2/EREBP multigenic family. Neighbor-joining tree of AP2 domain–containing proteins in *Arabidopsis* was constructed with ClustalWPPC and PAUP3.1.1 software (see supplementary data). The non-EREBP AP2-containing proteins are on the same branch of the tree and are highlighted in yellow. Six EREBP-like genes closely related to the *AINTEGUMENTA* family (*EDF*s) are highlighted in green. AP2 domain–encoding genes that were induced or repressed by ethylene by a multiple of at least two are highlighted in red and blue, respectively. Insertions in promoters or transcribed regions were found for 69 of the 141 AP2 domain–encoding genes and are marked with asterisks.

Table 1. Distribution of T-DNA insertions in genes and intergenic regions.

	Chr. 1	Chr. 2	Chr. 3	Chr. 4	Chr. 5	Total
Promoter	5,488	3,376	4,452	3,076	4,900	21,292
5′UTR	1,243	737	951	680	1,099	4,710
Coding exon	5,089	2,960	3,988	2,871	4,440	19,348
Intron	2,663	1,507	1,840	1,681	2,284	9,975
3′UTR	1,621	914	1,263	966	1,535	6,299
Intergenic regions	6,861	4,323	5,180	3,813	6,321	26,498
Total	22,965	13,817	17,674	13,087	20,579	88,122

Although the precise mechanism of T-DNA integration in the host genome is not fully understood, a variety of host proteins appear to play important roles not only in T-DNA transport but also in integration processes (*18*). For example, the plant VIP2 protein, which is thought to interact with the transcriptional machinery, directly interacts with *Agrobacterium* VirE2 (a bacterial protein associated with the T-strand) (*19*). It is conceivable that the bias toward promoters and UTRs is the result of preferential interaction of the Vir proteins with host proteins involved in initiation or termination of transcription.

As recently reported for HIV integration into the human genome, the process of DNA integration can be significantly affected by gene activity (*20*). Thus, another plausible model for integration site preference is that uncoiling of the DNA helix during transcription initiation and termination at 5′ and 3′UTRs may allow greater accessibility to the T-DNA integration machinery (*20*). To test this possibility, we assessed genome-wide gene expression levels using unique expressed sequence tags (ESTs) present in GenBank for each *Arabidopsis* gene, as well as microarray analysis to examine the expression levels for ~22,000 genes in plants grown under a variety of different conditions (table S3). We observed no significant correlation between the level of gene expression and the frequency of T-DNA integration. Caveats of this conclusion are that the exact cell-type infected by *Agrobacterium* is not known and that mixed-stage flowers may not be adequately representative of expression in the highly specialized female gametophyte cells that are the most likely target for transformation (*21*, *22*).

To test the utility of the sequence-indexed *Arabidopsis* insertion mutant collection for genome-wide functional analysis, we targeted genes in the response pathway of the plant hormone ethylene (*23*). This simple hydrocarbon is an essential regulator of plant disease resistance, fruit ripening, and a variety of other important developmental processes in plants. The transcriptional activation of genes in response to ethylene depends on the plant-specific EIN3 and EIN3-like (EIL) family of DNA binding proteins, and these

are among the most downstream signaling components in the ethylene pathway (*23*). Thus far, the only described direct target of this family of transcription factors is *ERF1* (*ETHYLENE RESPONSE FACTOR1*) (*24*), a member of a large family of AP2-like DNA binding transcription factors known as EREBPs. To identify new genes involved in responses to this important plant-growth regulator, we used Affymetrix gene expression arrays to examine the RNA levels of more than 22,000 genes in response to ethylene (*13*). We identified 628 genes whose levels of expression were significantly altered by treatment with exogenous ethylene; 244 genes were induced and 384 genes were repressed by hormone treatment (table S4). The distribution according to ontology of these genes indicated that ethylene affected genes involved in many types of biological processes, from metabolism to signal transduction (*16*). In total, by searching our sequence-indexed T-DNA insertion-site database (*25*), T-DNA insertion mutations for 179 inducible and 287 repressible genes were identified (i.e., for 74.2% of all ethylene-regulated genes) (*26*). This percentage is in agreement with the total proportion of genes disrupted in this collection (74%). Of particular interest, in addition to *ERF1*, we found that the expression levels of 14 of 141 AP2 domain–containing genes were affected by ethylene treatment of etiolated seedlings (Fig. 2). In particular, four of six genes that encode proteins with two plant-specific DNA binding domains, AP2 and B3, were found to be ethylene-inducible [(*16*), Fig. 3B]; these genes were named *ETHYLENE RESPONSE DNA BINDING FACTORS1* to *4*.

By searching the sequence-indexed insertion mutant database and using gene-specific PCR primers with a multidimensional DNA pooling approach, we were able to identify insertion mutant plants for each of the *EDF* family members (*13*). Although no detectable alterations in morphology were observed in the ethylene responses in any of the single mutants (Fig. 3, A and B), we found significant ethylene insensitivity in multiple-mutant plants (Fig. 3, C and D). These findings reveal an important role for the *EDF1* to *4* genes in the response to ethylene. Moreover, the lack of observed phenotypes in the individual *edf* mutants implies a significant degree of functional overlap among the *EDF* gene family members. The residual ethylene sensitivity observed in the quadruple-mutant plants is consistent with the fact that the *EDF* genes represent only one branch of the ethylene response.

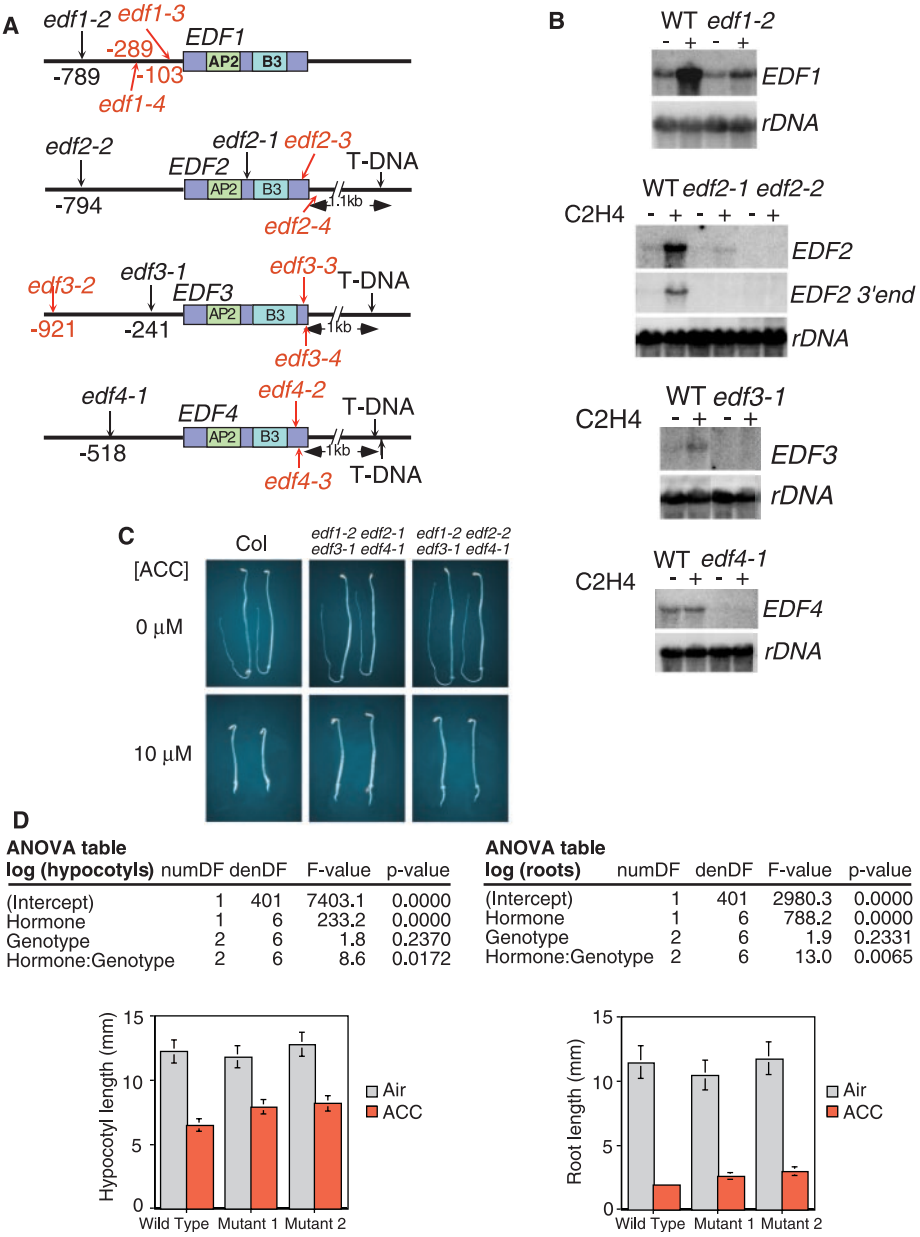

Fig. 3. *EDF* knockouts. (**A**) Schematic representation of the four *EDF* family members with the respective positions of the T-DNA insertions. AP2 and B3 domains are highlighted. The coordinates of the T-DNA insertions in the promoter regions are indicated with respect to the translation start site. Insertions marked in black and red were identified by PCR screening and database search, respectively. (**B**) Expression levels of the *EDF* genes in wild-type plants and the T-DNA mutants with or without 10 ppm ethylene. Total RNA was loaded at 30 µg per lane. (**C**) Ethylene-insensitive phenotypes of two different quadruple-mutant combinations. Wild-type and quadruple mutants were cold-treated and germinated as described in (*27*) for 5 days. (**D**) ANOVA tables for the logarithm of root and hypocotyl lengths. The hormone:genotype term indicates that both quadruple mutants respond to the hormone treatment significantly differently from the wild type. Error bars indicate 95% confidence interval.

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Screening for Nitric Oxide–Dependent Protein-Protein Interactions

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Because nitric oxide (NO) may be a ubiquitous regulator of cellular signaling, we have modified the yeast two-hybrid system to explore the possibility of NO-dependent protein-protein interactions. We screened for binding partners of procaspase-3, a protein implicated in apoptotic signaling pathways, and identified multiple NO-dependent interactions. Two such interactions, with acid sphingomyelinase and NO synthase, were shown to occur in mammalian cells dependent on endogenous NO. Nitrosylation may thus provide a broad-based mechanism for regulating interactions between proteins. If so, systematic proteomic analyses in which redox state and NO bioavailability are carefully controlled will reveal a large array of novel interactions.

Signal transduction is often coordinated within the confines of multiprotein complexes (*1*). Constitutive interactions among proteins subserve the initiation of signaling, and the emergence of higher order complexes that are required for signal processing and propagation relies on inducible, regulated protein-protein interactions (*1*, *2*). These dynamic interactions form the basis of complex regulatory circuits whose composition determines biological function. Identifying the makeup of these circuits is a major challenge, however, because they involve multiple low-affinity interactions that are controlled by dynamic posttranslational protein modifications in response to multiple stimuli (*2*).

Nitric oxide (NO) is a ubiquitous signaling molecule that operates through posttranslational modification of proteins (nitrosylation) (*3*). Specifically, NO targets cysteine thiol and transition metal centers to regulate a broad functional spectrum of substrates, including all major classes of signaling proteins. NO synthases (NOS) are localized within multiprotein signaling complexes (*3*, *4*), but whether NO can directly affect protein-protein interactions subserving signal transduction has not been considered, and prior biochemical and genetic analyses of protein-protein interactions have been carried out in the absence of NO.

To assess the possibility of NO-dependent regulation of protein-protein interactions in cells, we developed a modified yeast two-hybrid screening methodology (*5*). We first deleted the yeast flavohemoglobin (*6*), which consumes NO very efficiently and thus obfuscates NO signaling. We then established three complementary methods to identify NO-dependent protein-protein interactions. NO was delivered from a long-lived donor (Z)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate (DETA-NO; half-life ~18 hours in our assay) dispersed in solid agar, soft agar, or liquid medium, thus covering a range of nitrosylating conditions. We further established a concentration range of DETA-NO over which physiological amounts of NO could be generated in our assays without impairing yeast growth (fig. S1) (100 μM DETA-NO produced steady-state concentrations of ~300 nM NO in yeast culture medium, as determined with an NO electrode).

A well-characterized example of functional regulation by *S*-nitrosylation is the control of caspase-3–dependent death signaling. *S*-nitrosylation inhibits and denitrosylation facilitates the sequential activation of caspases within macromolecular complexes (*7*–*9*). However, the molecular mechanisms of action that enable *S*-nitrosylation to regulate signal transduction through these complexes remain poorly understood. We therefore conducted a two-hybrid screen of a cDNA library derived from cytokine-activated murine macrophages [which are frequently used to study the involvement of NO in apoptosis (*10*)] in the presence of NO. We initially screened ~4 million transformants for proteins that interact with procaspase-3 (bait). Thirty-five clones were isolated, from which prey plasmids retransfected into the bait strain caused at least threefold increases in growth in the presence but not the absence of NO. No growth was seen in yeast transformed with either bait or prey vector alone. Seventeen of these 35 clones showed NO-dependent growth a third time; 2 of 17 clones also showed at least threefold activation of *lacZ* transcription, an independent reporter of protein-protein interactions, in the presence versus absence of NO ($P < 0.001$). Thus, these clones both activated *lacZ* transcription and conferred histidine prototrophy in an NO-dependent manner. One of these clones contained a partial sequence (amino acids 158 to 927) of the apoptosis-related enzyme, acid sphingomyelinase (ASM) (Fig. 1A). These data establish the principle of NO-inducible protein-protein interactions.

To enable rapid large-scale screening, we developed an alternative method of genomic two-hybrid analysis in which *Escherichia coli* were transformed with plasmids pooled from yeast transformants that were previously grown for several days in histidine-deficient medium, supplemented continuously with NO (*5*). Growth in liquid medium circumvented the requirement for large numbers of plates, and thus for upfront processing of individual clones, and provided better control over NO delivery. The prey plasmids were isolated and retransfected into caspase-3 bait strains, whose NO-dependent growth was then assessed individually. About 1800 *E. coli* colonies were derived from a screen of ~4 million transformants; 499 of these contained prey plasmids, of which 50 appeared at least twice as determined by Hae III diges-

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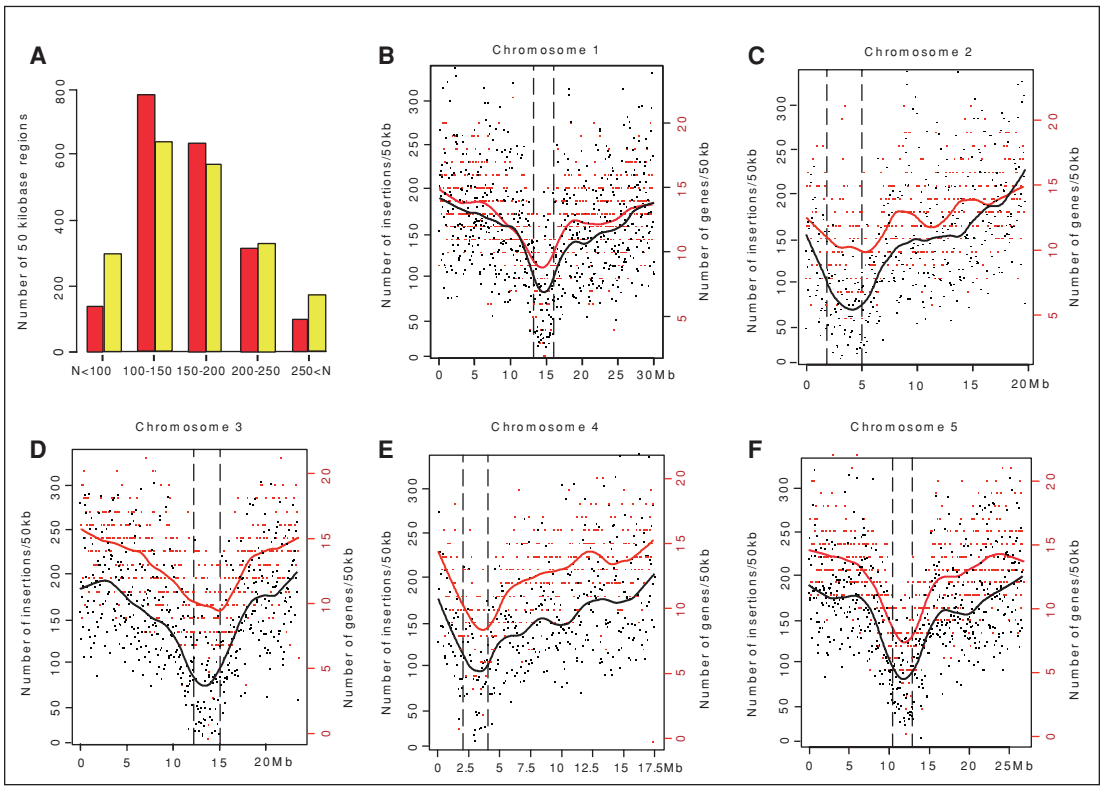
ERRATUM

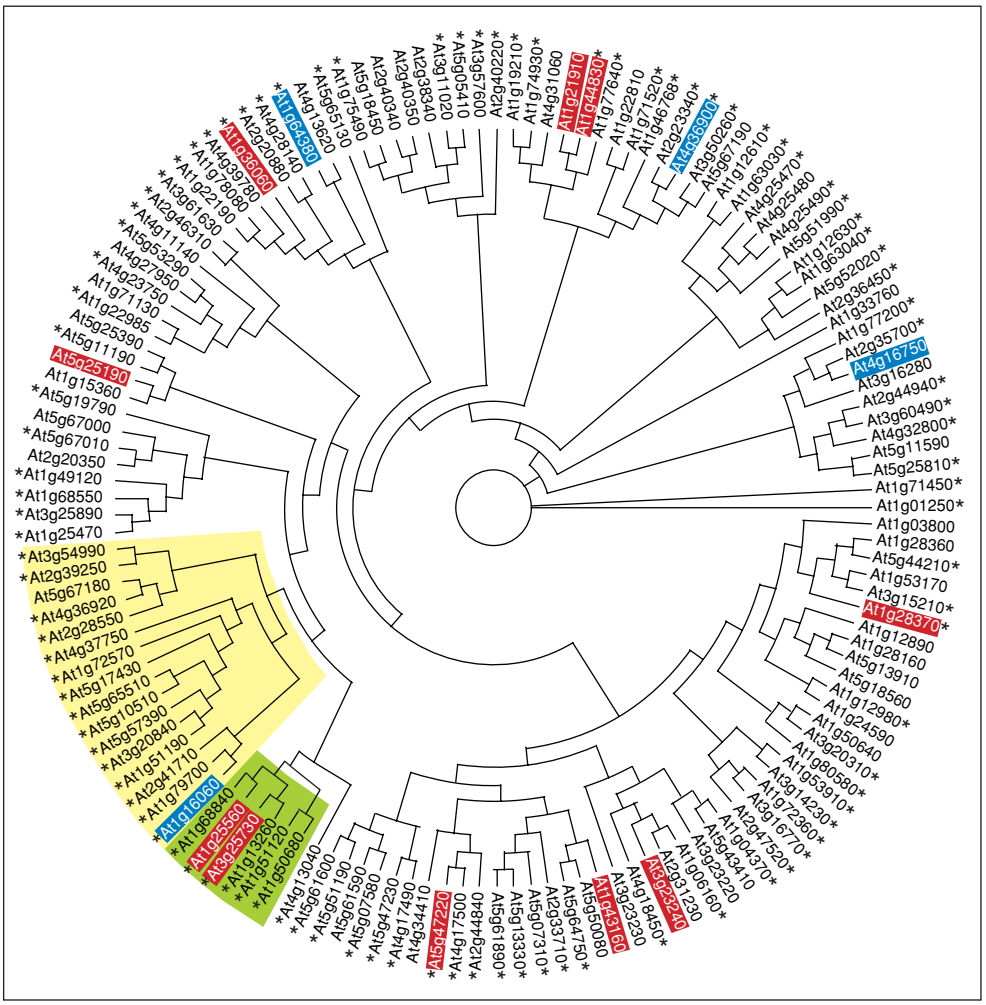
post date 26 September 2003



REPORTS: "Genome-wide insertional mutagenesis of *Arabidopsis thaliana*" by J. M. Alonso *et al.* (1 Aug. 2003, p. 653). There were errors in two of the figures. In Fig. 1A (above), there was a bar missing from the graph. In Fig. 2 (on the following page), genes in which insertions in promoters or transcribed regions were found should have been marked with asterisks. The corrected figures are shown here.

ERRATUM

post date 26 September 2003, continued



LETTERS

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted by e-mail (science_letters@aaas.org), the Web (www.letter2science.org), or regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Hooked on Hooke

RICHARD STONE'S COGENT REVIEW OF Robert Hooke's incredible achievements ("Championing a 17th century underdog," News of the Week, 11 July, p. 152) evokes fascinating facets of his incomparably productive life. He produced weekly Royal Society demonstrations without compensation, promised lecture honoraria were never paid despite polite reminders, and the Council of the society even voted to deduct the amount from his promised salary.

Isaac Newton's aloofness toward and disparaging belittling of "this miserable philosopher" was not without benefit for him: He awaited Hooke's death before publishing his dormant "Optics" without fully acknowledging Hooke's prior work. Newton was not his only enemy; Henry Oldenberg, Secretary of the Royal Society, often omitted Hooke's name from recorded comments and rightful priority credits.

An original composite rendering of Hooke, at about age 25 to 30, using databases on a compu-sketch instrument coordinated by Henry Lee, Nick Skebeta, and Martin E. Gordon.

Their intensifying disputes caused Hooke to call Oldenberg a "trafficer in intelligence." Little wonder that Hooke's digestive tract required "tailoring" of his "stomach and gutt" by his "one dish/meal," supplemented by potable metals such as licking powdered silver, syrup of poppy seed, and liberal use of the famous ancillary treatments of clysters and bleedings with cuppings. Despite these problems, Hooke was able to perceive and correlate projected applications of his nearly 1000 inventions.

While always dressed in his personally chosen long fabrics, sewn by himself, he gregariously interacted in coffee shops with many notables, including Samuel Pepys, but was never able to sustain wide recognition of his work. Hooke died a feeble, depressed, reclusive man, despite his wealth of legacies to science and his personal wealth, found dormant in an iron chest filled with several thousand pounds of earned silver and gold coins.

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CREDIT: H. LEE, N. SKEBETA, AND M. E. GORDON

Hooke and Generation of Molds

IN HIS ARTICLE "CHAMPIONING A 17TH century underdog" (News of the Week, 11 July, p. 152), Richard Stone reports on the current interest in the scientific achievements of Robert Hooke (1635–1703), including his remarkable insights in several areas of biology. One aspect of his many contributions that has gone largely unnoticed by historians of science is his proposal of a spontaneous generation of microbes based on purely mechanical forces, which was prompted by his observations of various molds. As summarized by Lechevalier and Solotorovsky (*1*), both the drawings and descriptions that Hooke included in his 1665 *Micrographia* suggest that he observed the teleutospores of a rust, which, together with a blue mold "and several kinds of hairy mouldy spots" (*2*), could be found in decaying organic matter and which reproduced without seeds, requiring only a convenient substratum and the proper amount of warmth (*1*). However, a detailed reading of the *Micrographia* demonstrates Hooke's adherence to a more sophisticated scheme largely based on Cartesian mechanistic concepts. As he wrote, "I must conclude, that as far as I have been able to look into the nature of this Primary kind of life and vegetation, I cannot find the least probable argument to perswade me there is any other concurrent cause then such as purely Mechanical, and that the effects or productions are as necessary upon the concurrence of those causes as that a Ship, when the Sails are hoist up, and the Rudder is set to such a position, should, when the Wind blows, be mov'd in such a way or course to that or t other place; Or, as that the brused Watch, which I mention in the description of Moss, should, when those parts which hindred is motion were fallen away, begin to move, but after quite another manner then it did before" (*2*).

Not surprisingly, his explanation of the appearance of molds and other microorganisms lacks an evolutionary perspective. Nonetheless, the delightful analogy used by Hooke demonstrates that he accepted a continuity between the nonliving and the living without invoking any vital force of supernatural character. To substantiate his claims, he compared the emergence of molds with that of the "silver tree," a dendritic structure with plantlike morphologies formed from an amalgam of silver and mercury dissolved in nitric acid, which had been studied, among others, by his major foe Isaac Newton (*3*).

The temptation to compare biological structures with artifacts of purely inorganic nature may have begun with Newton and Hooke, but it did not end with them. In an attempt to understand the origin and nature of life, 19th century scientists like Leduc and Herrera devoted themselves for several decades to the production of lifelike structures from various combinations of crystals and inorganic fluids, as part of the now largely forgotten fields of "synthetic biology" and "plasmogeny" (*4*). Advocates of complexity theory, which likens the emergence of complex patterns in dynamical systems with biological phenomena (*5*), do not shy away from such comparisons, which also have a bearing on the ongoing discussions of the significance of complex morphologies as biological signatures in early Archean sediments and Martian meteorites (*6*). Sometimes our current debates have a long genealogy.

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Risks of Genetically Engineered Crops

STEVEN H. STRAUSS'S GENERAL CONCEPT that the inherent riskiness of a genetically engineered (GE) crop should determine the

extent of regulation ("Genomics, genetic engineering, and domestication of crops," Policy Forum, 4 Apr., p. 61) makes sense in principle. The bigger issue is that the Department of Agriculture (USDA) and the Environmental Protection Agency (EPA) have not adequately identified which transgenic crops should receive limited or heightened review based on relative risk to health or the environment. The issue applies to GE crops that have received inadequate scrutiny, as well as those that may receive more scrutiny than necessary.

Several cases of inadequate USDA oversight have been identified in recent National Academy of Sciences (NAS) reports. For example, NAS criticized USDA's assessment of cross-breeding between transgenic virus-resistant squash and wild sexually compatible relatives (*1*, *2*). NAS also criticized insufficient USDA oversight of transgenic avidin-containing maize (*2*). In these and other cases, more stringent oversight is needed, not less.

Additionally, contamination of soybeans and possibly corn, due in part to inadequate USDA monitoring policies for transgenic "pharma" crops, resulted in fines for the company ProdiGene and erosion of food industry confidence in the regulation of transgenic technology (*3*). More stringent confinement policies by the USDA, such as requiring redundant physical and biological isolation techniques, could minimize similar incidents. In response to public concern, USDA is revising its oversight of "pharma" and "industrial" crops, but concern remains that food contamination will not be prevented. The utilization of nonfood "pharma" crops would better prevent food contamination.

Although it may be difficult to delineate GE traits that could allow reduced regulation at the field trial level, Strauss suggests domesticating phenotypes coded by genes similar to genes from the crop genus as a starting point. However, predicting invasiveness of plants based on particular traits has not been reliable (*4*). In retrospect, traits from the crop sorghum transferred to a wild relative in the same genus have been implicated in the notorious weediness of johnsongrass (*5*). Therefore, it is premature to exempt "agronomic" GE traits from regulation.

The problems outlined here and by Strauss are symptomatic of inadequate effort by USDA and EPA to determine how to assess and rank environmental risks of GE crops. USDA's GE risk assessment grant program amounts to only about $3 million per year. EPA's Office of Research and Development eliminated GE risk-assessment research in the mid 1990s and now has only a shoestring program. The public confidence in GE crops that Strauss desires—especially in the era of Enron—will only occur when sufficient resources are devoted to developing testing requirements for GE crops based on input from independent scientists and the public. Confidence would be further enhanced if Congress gave the FDA the authority to conduct safety approvals of new GE foods.

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Response

GURIAN-SHERMAN SUGGESTS THAT IT IS premature to reduce regulations for genetically engineered organisms (GEOs). However, my Policy Forum and another recent essay (*1*) did not suggest a general reduction of regulations, but instead called for greater discrimination in how regulations are implemented, based on the trait and the degree of evolutionary novelty of the genes employed. Others have made similar suggestions (*2*).

I agree that more stringent and tailored rules for pharma-crops, as the USDA Animal and Plant Health Inspection Service has already put into place, are warranted. What I am suggesting is that regulatory agencies could also be doing the same customizing of regulatory scrutiny at the other end of the novelty spectrum—by relaxing regulatory oversight for traits where there is a strong a priori case to be made that they will be neutral or domesticating (i.e., will not improve invasiveness or weediness). The intention is to make GEO regulation more congruent with that of conventional crop improvement. Breeding, as a result of its large benefits for agricultural productivity and human health, continues to have overwhelming social support in the absence of any government regulation, despite tangible levels of ecological and toxicological risk.

It is unclear to me why the ability to predict invasiveness of exotic organisms placed into novel ecosystems, usually without their native assembly of parasites and predators, is viewed by Gurian-Sherman as relevant to assignment of homologous genes to risk classes. In terms of information novelty, the product of new genomes × new biotas × new abiotic environments is astronomically more complex than that of individual, carefully selected homologous genes and putative domestication traits in familiar

species. In neither case is the risk absolute zero, but the ecological and evolutionary uncertainties are many orders of magnitude apart. In addition, nowhere did I propose that all "agronomic" traits be exempt from regulation, as Gurian-Sherman implies.

My Policy Forum focused on regulations that pertain to exploratory (small-scale) field testing. It did not discuss exemption from oversight at the point of commercialization, nor even suggest exemptions for large-scale (precommercial) field trials. It was a modest attempt to begin to identify a class of GEOs that are very safe and thus might not be encumbered by the stigmas, costs, and complexities of crops with ecologically novel genes. Without such a class, and thus a less encumbered breeding pathway, small companies and public-sector researchers will continue to find it difficult to use GEO methods to take full advantage of genomic knowledge in crop breeding. Indiscriminate regulation of GEOs also confuses the public about risk and novelty, inflaming rather than helping to resolve the GEO debate. The net result is likely to be large opportunity costs in the form of lower food quality, higher food prices, poorer health, and greater environmental impacts from agriculture.

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Good and Bad Amyloid Antibodies

ANTIBODIES THAT RECOGNIZE AN OLIGOMERIC state common to different amyloidogenic proteins described by R. Kayed *et al.* ("Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis," Reports, 18 Apr., p. 486) advance our understanding of Alzheimer's disease (AD) and other amyloid diseases, and provide a tool for probing such amyloid conformations in patients and in animal and cell culture models. Kayed *et al.* establish that the antibody they generated recognizes only oligomers of amyloid beta-peptides 1-40 and 1-42 (Aβ40 and Aβ42) that contain a minimum of eight peptide copies (octamers) and that it does not recognize amyloid fibrils. Previous studies had suggested that Aβ40 and Aβ42 are particularly toxic to cells when they are in an early stage of the peptide aggregation process (*1*, *2*). The findings of Kayed *et al.* confirm and extend this notion by showing that the state-specific amyloid antibody inhibits the cytotoxicities of a range of amyloidogenic peptides, including those

involved in prion disorders, Parkinson's and Huntington's diseases, and type II diabetes.

Efforts to develop a vaccine for AD based on immunization with Aβ42 or administration of Aβ antibodies (passive immunization) have encountered mixed results. Several laboratories have documented the clearance of Aβ aggregates from the brains of transgenic mice expressing mutant amyloid precursor protein, a mouse model of AD (*3*, *4*). However, the mouse studies did not establish whether the Aβ antibodies produced by or given to the mice modified the neurotoxicity of the Aβ in the brains of the mice. In an initial clinical trial in which AD patients were administered Aβ to elicit an immune response, some of the patients appeared to be benefiting from the vaccine (*5*), but several patients developed encephalitis (*6*). Although Kayed *et al.* found that their antibody against oligomeric Aβ was capable of protecting cultured cells against the toxicity of such forms of Aβ, we have found that several other Aβ antibodies potentiate the neurotoxicity of Aβ (*7*). Previous studies showed that Aβ generates reactive oxygen species, including hydrogen peroxide, only when the peptide is in an aggregating oligomeric form (*8*, *9*). When the latter process occurs when Aβ is in contact with cell membranes, lipid peroxidation occurs, resulting in perturbed membrane transporter and ion channel functions that can lead to cell death (*10*).

Aβ antibodies might facilitate the formation of a toxic peptide conformation (*7*). However, the possibility that the interaction of the antibodies with Aβ catalyzes or enhances the generation of reactive oxygen species should also be considered. Lerner and colleagues (*11*, *12*) have shown that many antibodies can convert molecular oxygen into hydrogen peroxide and short-lived hydroxylating radical species such as hydrotrioxy radical. When we tested several different Aβ42 antibodies to determine their ability to modify the amount of oxidative damage to cells induced by Aβ42, some of the antibodies increased the damage, whereas others decreased the damage. Because many different Aβ antibodies are produced in response to immunization with Aβ, our findings suggest that some of the antibodies may exacerbate the neurodegenerative process. Passive immunization with Aβ antibodies with predetermined effects on Aβ clearance and toxicity might reduce or eliminate potentially serious side effects resulting from vaccination with Aβ.

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A Clarification on Data Availability

JOHN R. LOTT JR. RECENTLY RESPONDED ("Research fraud, public policy, and gun control," Letters, 6 June, p. 1505) to an earlier Editorial ("Research fraud and public policy," D. Kennedy, 18 Apr., p. 393) that stressed the need for integrity in research and alluded to serious allegations of academic misconduct by Lott in his efforts to advance the thesis that more guns will lead to less crime. In the course of his reply, Lott seems to deflect attention from the charges that have been leveled against him by making an untrue allegation that Ian Ayres and I have failed to give him the data related to our work showing that adoptions of concealed carry laws are not associated with drops in crime. As I assume Lott knows (since he responded to our paper), we state in footnote 33 of our paper "Shooting down the more guns, less crime hypothesis" (*1*) that the data set and computer programs we used are available on the Web, and indeed they are. In fact, I have always made my data available to any researcher for this work and every other research project I have worked on (and Lott has asked for and received from me data on other research projects of mine).

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Reference
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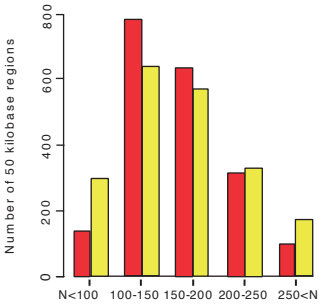
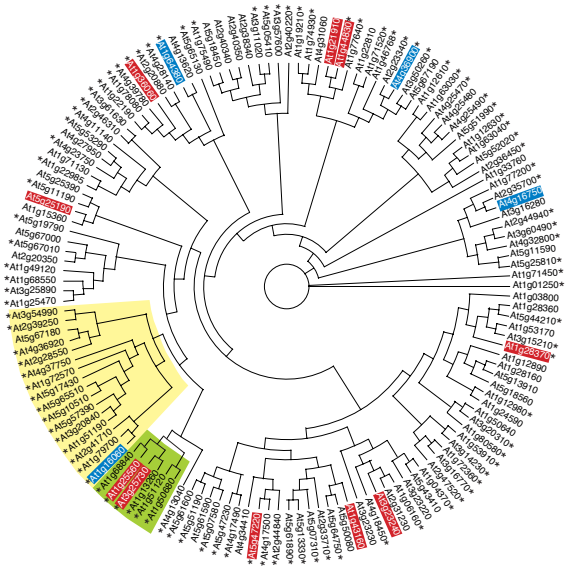
CORRECTIONS AND CLARIFICATIONS



REPORTS: "Genome-wide insertional mutagenesis of *Arabidopsis thaliana*" by J. M. Alonso *et al*. (1 Aug., p. 653). There were errors in two of the figures. In Fig. 1A (left), there was a bar missing from the graph. In Fig. 2 (below), genes in which insertions in promoters or transcribed regions were found should have been marked with asterisks. The corrected figures are shown here.



REPORTS: "Collection, mapping, and annotation of over 28,000 cDNA clones from *japonica* rice" by the Rice Full-Length cDNA Consortium (18 July, p. 376). Under the RIKEN part of the author list, three of the authors' names were spelled incorrectly: Wataru Hashizume, not Wataru Hashidume; Yoshiyuki Ishii, not Yoshiki Ishii; and Hideaki Konno, not Hedeaki Konno. Also, one author name was missing: Ayako Yasunishi. In the acknowledgments in reference 17, the following names should not have appeared: W. Hashizume, K. Imotani, A. Miyazaki, and A. Yasunishi.

REPORTS: "Reelin promotes peripheral synapse elimination and maturation" by C. C. Quattrocchi *et al*. (1 Aug., p. 649). The affiliation listed for the fifth author, David Benhayon, is incorrect. He is at St. Jude Children's Research Hospital, Memphis, TN 38105, USA, and Health Science Center, University of Tennessee, Memphis, TN 38163, USA. There was also information missing from the acknowledgments in the final reference. The work was supported by a grant from NIH/National Institute of Neurological Disorders and Stroke grant NS36558, and the work was also supported in part by NIH Cancer Center Support CORE grant P30 CA21765 and the American Lebanese Syrian Associated Charities.

VIEWPOINT: "Special section: Building signaling connections: Regulators of cerebellar granule cell development act through specific signaling pathways" by D. Vaudry *et al.* (6 June, p. 1532). The final sentence of the legend of Fig. 1 on p. 1533 should read: "Symbols: ⊥, inhibition; ↓, activation, or in the case of PARP and actin, degradation, which leads to cell death."