creased appetite and changes in food preference to hyperorality and consumption of nonfoods. Such phenomena may arise out of a disabled network involving OFC and amygdala, whereby routine (learned) food cues no longer recruit motivationally appropriate representations of food-based reward value.

Theoretical and computational models of reward learning postulate the concept of motivational "gates" that traffic information flow between internal representations of the CS+ and the UCS (*2*, *30*). These gates are the targets of motivational signals (likely a combination of sensory, visceral, autonomic, and interoceptive factors) and determine the likelihood that stimulus-reward associations activate appetitive systems. The neural mechanisms that support these processes are not well characterized. Our data show that neural responses evoked by a CS+ in amygdala, OFC, ventral striatum, insula, cingulate, and hypothalamus are directly modulated by hunger states, indicating that this structural network underpins Pavlovian incentive behavior in a manner that meets the requirements of a motivational gate.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/301/5636/1104/DC1
Materials and Methods
Tables S1 to S3
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SIR1, an Upstream Component in Auxin Signaling Identified by Chemical Genetics

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Auxin is a plant hormone that regulates many aspects of plant growth and development. We used a chemical genetics approach to identify *SIR1*, a regulator of many auxin-inducible genes. The *sir1* mutant was resistant to sirtinol, a small molecule that activates many auxin-inducible genes and promotes auxin-related developmental phenotypes. *SIR1* is predicted to encode a protein composed of a ubiquitin-activating enzyme E1–like domain and a Rhodanese-like domain homologous to that of prolyl isomerase. We suggest a molecular context for how the auxin signal is propagated to exert its biological effects.

Auxin has been implicated in almost every aspect of plant growth and development. Molecular genetics studies on auxin-resistant *Arabidopsis* mutants (*1*, *2*) and biochemical analyses of early auxin-inducible genes (*3*, *4*) have elucidated many aspects of auxin signaling. The current model of auxin signaling suggests that negative regulators such as the auxin/indole-3-acetic acid (Aux/IAA) proteins are targeted for degradation in an auxin-dependent manner through the ubiquitin-related protein degradation machinery, thereby derepressing a network of genes to guide proper growth and development (*5*).

Analysis of auxin signaling by classical genetics is complicated by auxin polar transport, a process whereby an auxin-concentration gradient is maintained among neighboring cells (*6*). This process limits the accessibility of certain cells and tissues to exogenous auxin, and therefore certain auxin signaling components might well be missed from genetic screens for mutants that are resistant to exogenous auxin. Moreover, auxin polar transport and auxin signaling are well-coordinated, interdependent processes, making it difficult to isolate and specifically analyze either process. Here we identify a tool to modulate auxin signaling exclusively, without affecting auxin polar transport, in order to analyze the molecular mechanisms of auxin signaling.

We used a chemical genetics (*7*) approach to identify an auxin signal transduction component in *Arabidopsis*. Sirtinol (Fig. 1A), an inhibitor of the Sirtuin family of nicotinamide adenine dinucleotide (NAD)–dependent deacetylases in *Saccharomyces cerevisiae*, affects root and vascular tissue development in *Arabidopsis* (*8*). Sirtinol specifically activates many auxin-inducible genes, thereby promoting several auxin-related developmental phenotypes. With this approach, we identified the gene *SIR1* as a key regulator of many auxin-inducible genes.

We screened for compounds that could alter the expression pattern and/or levels of an auxin reporter line, DR5-GUS (*9*). Sirtinol caused up-regulation and ectopic expression of the auxin reporter gene (*10*) that was induced throughout the plant, with the highest expression levels along the vascular tissues (Fig. 1B). We next performed a microarray analysis using the *Arabidopsis* "whole genome" chip (Affymetrix) to determine whether sirtinol activated the expression of auxin-inducible genes at the transcriptional level (*10*). Of the 23,000 genes on the chip, about 16,000 genes gave signals that were significantly above background levels in all samples. Sirtinol treatment

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induced 138 genes that were expressed at least 2.5 times as high in the sirtinol-treated samples as in the water-treated seedlings. Many of the known auxin-inducible genes, including Aux/IAA genes, were represented among the 138 sirtinol-induced genes (Table 1). The gene expression profile of sirtinol-treated plants was similar to that of auxin-treated plants: More than 65% of the genes induced by sirtinol were also induced by auxin (table S1).

The rapid degradation of negative regulators, such as Aux/IAA proteins induced by auxin to activate the expression of many auxin-inducible genes, is a hallmark of auxin signaling (*11*, *12*). Using IAA17/AXR3-GUS transgenic *Arabidopsis* plants (*1*), we found that sirtinol treatment, like auxin treatment, led to a rapid degradation of the AXR3-GUS fusion protein (Fig. 1C), indicating that sirtinol-induced gene expression could also arise from regulated-protein degradation.

We next asked whether sirtinol promoted auxin-related developmental changes. Sirtinol inhibits primary root elongation and hypocotyl development in light-grown seedlings (*8*) (Fig. 2A). Seedlings grown in total darkness on sirtinol-containing media had short hypocotyls and lacked an apical hook, which is characteristic of auxin-treated or auxin-overproducing dark-grown seedlings (Fig. 2B) (*13*, *14*). Sirtinol also stimulated adventitious root formation (Fig. 2C). Defoliation was often observed at the bottom of the hypocotyls, where adventitious root growth was initiated (Fig. 2C). Transferring sirtinol-treated plants to unsupplemented Murashige and Skoog (MS) medium accelerated the formation of adventitious roots (Fig. 2C). Defoliation and adventitious root formation are phenotypes commonly associated with auxin-overproducing mutants in *Arabidopsis* (*13*), and exogenous auxin treatment is known to lead to the inhibition of primary root elongation and promotion of adventitious root development (*15*). The similarities between the phenotypes of sirtinol-treated plants and auxin-treated or auxin-overproducing plants are consistent with our observation that sirtinol treatment activates auxin-inducible genes and thus amplifies auxin signal output.

Sirtinol also affected leaf development in *Arabidopsis*. Sirtinol-treated seedlings often developed cup-shaped organs (Fig. 2D). When sirtinol-treated seedlings were transferred to MS media, the bottom part of the cup turned green and the whole tube-like leaves continued to elongate to form a trumpet-like structure (Fig. 2D). The shoot meristem of the sirtinol-treated plants was buried inside the cup. When the cup-like plants were transferred to MS media or soil, normal true leaves developed (Fig. 2D). The ability to regulate plant development in such a timed and reversible manner suggests the utility of small molecules in dissecting a variety of developmental processes in any model organism.

Although sirtinol activates auxin-inducible genes and partially phenocopies auxin-treated plants, it caused some additional phenotypes. For example, cup-shaped true leaves were not observed in auxin-treated plants. It may be that sirtinol is more effectively transported to cells that exogenous auxin cannot reach because of auxin polar transport. This is consistent with the observation that auxin polar transport inhibitors affected leaf initiation and patterning when applied locally (*16*). We tested the responses of known auxin mutants to sirtinol treatment. All the tested auxin-signaling mutants [*axr1* (*12*), *axr2* (*17*), *tir1* (*18*), and *nph4* (*19*)] were less sensitive to sirtinol than the wild type (Fig. 2E), whereas the tested auxin-transport mutants [*aux1* (*20*), *pin2* (*21*), and *tir3* (*22*)] responded similarly to the wild type (Fig. 2E), indicating that sirtinol affects only auxin signaling and probably is not transported via the auxin polar transport system.

We then undertook a genetic screen for mutants that were resistant to the effects of sirtinol, in an attempt to isolate sirtinol targets or downstream components. After screening 60,000 ethylmethane sulfonate–mutagenized M2 *Arabidopsis* seeds, we identified a single mutant, *sir1* (for sirtinol resistant 1), which displayed elongated primary roots, elongated hypocotyls, and normal cotyledons in the presence of 25 μM sirtinol (Figs. 2E and 3A). The *sir1* mutation also suppressed the ectopic expression of the auxin reporter genes that were induced by sirtinol treatment (Fig. 3B), suggesting that *SIR1* regulates auxin-inducible genes.

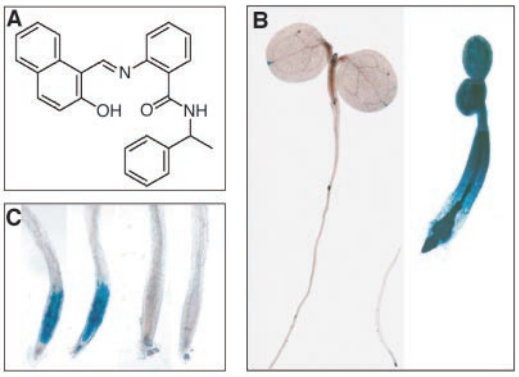

Fig. 1. Regulation of auxin-inducible genes by sirtinol. (**A**) The chemical structure of sirtinol. (**B**) Activation of the auxin-reporter gene DR5-GUS. Seedlings grown on half-strength (0.5×) MS (left) and on 25 μM sirtinol (right) for 5 days were visualized by staining with X-glucaronide for β-glucaronidase activity. (**C**) Degradation of the AXR3-NT-GUS fusion induced by sirtinol treatment. Expression of AXR3-NT-GUS was induced by heat shock at 37°C for 2 hours. Twenty min after the heat-shock, seedlings were further treated for 20 min with either water (the left two seedlings) or 25 μM sirtinol (the right two seedlings).

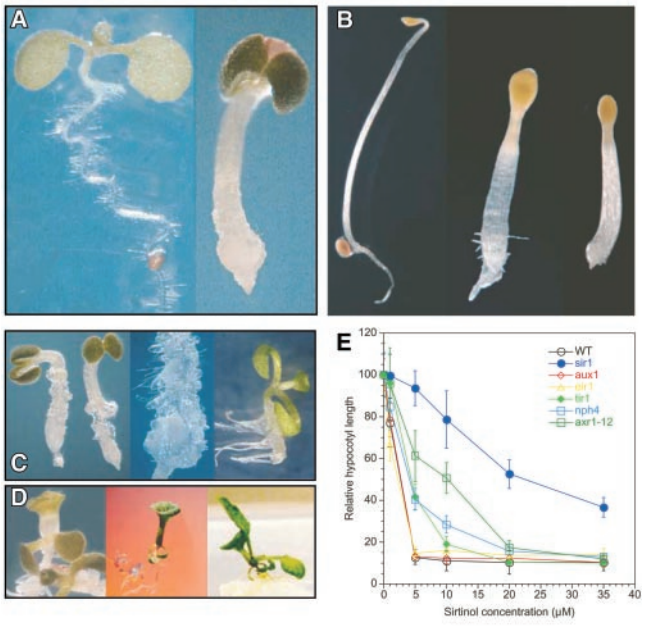

Fig. 2. The effects of sirtinol treatment on *Arabidopsis* growth and development. (**A**) Wild-type (WT) *Arabidopsis* grown on 0.5× MS media (left) and 25 μM sirtinol (right) for 5 days. (**B**) *Arabidopsis* seedlings grown in total darkness on MS media (left), 10 μM sirtinol (middle), and 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D) (right) for 3 days. (**C**) Stimulation of adventitious root growth by sirtinol. The left two seedlings are wild-type, grown on 25 μM sirtinol for 8 days. The third plant from the left is a wild-type seedling grown on 25 μM sirtinol for 5 days and then transferred to 0.5× MS media for 1 day (only the hypocotyl structure is shown). The right seedling is wild-type, grown on 25 μM sirtinol for 5 days and then grown on 0.5× MS for 3 days. (**D**) Development of a tubelike true leaf in a sirtinol-treated *Arabidopsis* seedling (left). The tube-like true leaf can elongate and develop into a trumpet-like structure when transferred to MS media (middle), and normal true leaves developed when the sirtinol-treated plants continued to grow on MS media (right). (**E**) Effects of sirtinol on known auxin mutants. Seedlings were grown in the dark for 3 days and hypocotyl lengths were measured. For clarity, the *axr2* and *tir3* data are not shown in the graph.

When grown on unsupplemented MS media, *sir1* resulted in additional phenotypes, including smaller cotyledons and shorter primary roots than wild-type seedlings. In addition, *sir1* plants were a paler green than the wild type and had far fewer lateral roots (Fig. 3C). When *sir1* plants were grown in soil in a greenhouse, their development was delayed (Fig. 3D). Finally, *sir1* was hypersensitive to exogenous auxin in a root elongation assay (Fig. 3E), indicating that SIR1 may negatively regulate auxin signaling.

sir1 was recessive and it segregated from a back-cross F2 population at a frequency of 25%, indicating that the associated phenotypes arise from a mutation in a single gene. The *sir1* gene was mapped to the bottom of chromosome V within an interval of 65 kb (Fig. 4). We sequenced all the open reading frames (ORFs) in the 65-kb interval and found a point mutation that resulted in a substitution of a highly conserved serine with a phenylalanine residue in ORF At5g55130 (GenBank accession no. NP 200324) (fig. S1A). Transformation of *sir1* with a 5-kb genomic fragment that contained only the ORF of At5g55130 and its regulatory sequences restored the sirtinol sensitivity and reversed other *sir1* phenotypes, providing proof that At5g55130 is in fact SIR1 (Fig. 4). *SIR1* was expressed throughout all stages of *Arabidopsis* development (fig. S2).

At5g55130 was originally annotated as a molybdopterin synthase sulfurylase, because of its homology to *Escherichia coli* MoeB protein (*23*). However, PSI-BLAST searches (fig. S1A) indicate that SIR1 is the *Arabidopsis* homolog of Uba4 from *S. cerevisiae*, a ubiquitin-activating enzyme E1–like protein (*24*) (Fig. 4 and fig. S1A). *S. cerevisiae* does not use molybdopterin as a cofactor and lacks all other molybdopterin biosynthesis machinery, and this indicates that the Uba4 homolog in *Arabidopsis* could participate in processes other than just molybdopterin biosynthesis. Pfam searches find that *SIR1* has two major functional domains (Fig. 4 and fig. S1A): an N-terminal domain that is conserved among molybdopterin synthases, vitamin B_1 synthases, and ubiquitin-activating enzymes (E1), and a C-terminal Rhodanese-like domain that shares homology with the C-terminal domain of *Arabidopsis* prolyl *cis-trans* isomerase. Each member of the E1/MoeB/ThiF superfamily catalyzes the adenylation of a C-terminal carboxyl group of a small protein, with adenosine triphosphate (ATP) as a substrate. The nucleotide-binding site in MoeB is very similar to that of the NAD-binding Rossmann folds (*25*). Given that sirtinol inhibits the Sirtuin family of NAD-dependent deacetylases in *S. cerevisiae* (*8*), our results suggest that the putative ATP-binding site in SIR1 may be a binding site for sirtinol, which is also consistent with our findings that the *sir1* mutation occurred in the vicinity of the putative ATP-binding site and that SIR1 was a negative regulator of auxin signaling (fig. S1A). The Rhodanese domain is an alpha/beta fold that was originally found in Rhodanese proteins and later in a variety of other proteins (*26*). Extensive BLAST searches indicate that Rhodanese domains are uncommon in *Arabidopsis* but do occur in SIR1, in one of three prolyl isomerases, and in a small family of proteins involved in senescence (fig. S1, B and C).

Given that *sir1* is hypersensitive to auxin and that SIR1 appears to be a direct target for sirtinol, we propose that SIR1 normally functions as a negative regulator in auxin signal-

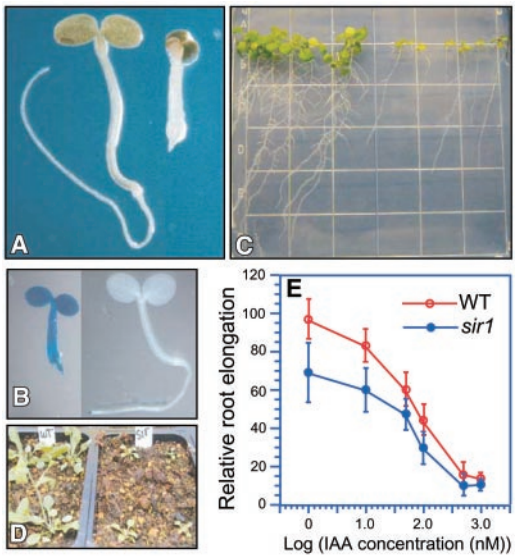

Fig. 3. *sir1* was resistant to sirtinol treatment. (**A**) *sir1* seedlings developed normally in the presence of 25 μM sirtinol. (**B**) Suppression of sirtinol-induced ectopic expression of DR5-GUS by the *sir1* mutation. Left, DR5-GUS grown on 25 μM sirtinol; right, *sir1*/DR5-GUS grown on 25 μM sirtinol. (**C**) In the absence of sirtinol, *sir1* plants are smaller in stature and have shorter primary roots and fewer lateral roots than wild-type plants. (**D**) Mature *sir1* plants were pale green and had delayed development. (**E**) *sir1* is hypersensitive to auxin. Root elongation was measured with 5-day-old seedlings that were transferred for 2.5 days to plates that contained various concentrations of auxin.

Table 1. Microarray analysis of gene expression induced by sirtinol and auxin treatment. *a*, absent, indicating that the microarray signal is below background levels; *nc*, no change in expression level. The Gene Access no. is the GenBank accession number. The numbers in the sirtinol and auxin columns refer to the fold-change from untreated samples.

Gene access no.	Gene product	Sirtinol	Auxin
At2g23170	GH3-like protein	200.0	1096.0
At3g03840	Putative auxin-induced protein similar to SAUR	36.6	2697.0
At1g59500	Auxin-regulated protein GH3	33.1	99.5
At4g32280	Similar to Aux/IAA proteins	27.1	60.3
At3g03830	Putative auxin-induced protein similar to SAUR	27.1	66.7
At3g58190	Putative protein	24.5	99.5
At4g38850	Small auxin up RNA (SAUR-AC1) protein	20.0	44.7
At5g03350	Putative protein	18.2	*a*
At1g29440	Auxin-induced protein	16.4	33.1
At2g15490	Putative glucosyltransferase	16.4	2.7
At3g56710	SigA binding protein	14.9	1.6
At1g29490	Unknown protein	14.9	493.0
At1g29460	Auxin-induced protein	13.5	27.1
At2g36770	Putative glucosyl transferase	13.5	2.0
At1g29500	Auxin-induced protein	12.2	44.7
At1g29450	Auxin-induced protein	12.2	60.0
At4g14560	Auxin-responsive protein IAA1	11.0	74.0
At5g47370	Homeobox–leucine zipper protein–like	11.0	30.0
At4g36110	Putative auxin-induced protein	11.0	270.0
At1g17170	Putative glutathione transferase	11.0	2.7
At5g18060	Auxin-induced protein–like	10.0	40.5
At3g56400	DNA-binding protein–like	10.0	*nc*
At3g50340	Putative protein	10.0	55.0
At1g29510	Auxin-induced protein	10.0	16.4
At2g21200	Putative auxin-regulated protein	10.0	7.4
At4g16515	Expressed protein	9.0	41.0
At1g05680	Putative indole-3-acetate beta-glucosyltransferase	9.0	*a*
At3g15540	Early auxin-induced protein IAA19	7.4	67.0
At1g29430	Auxin-induced protein	7.4	30.0
At1g26770	Expansin 10	7.4	2.0
At2g29490	Putative glutathione *S*-transferase	7.4	2.2
At5g54510	Auxin-responsive–like protein	6.7	33.0
At5g52900	Unknown protein	6.0	20.0
At5g02760	Protein phosphatase	6.0	37.0

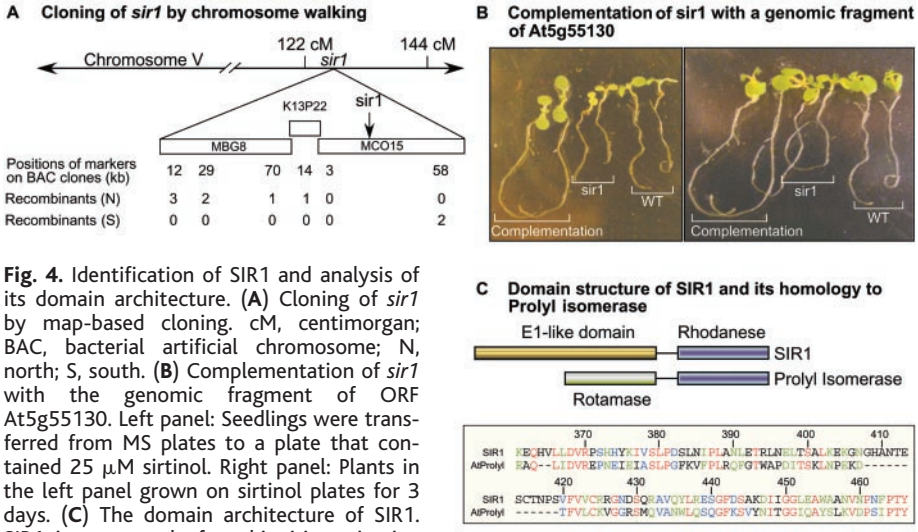

Fig. 4. Identification of SIR1 and analysis of its domain architecture. (**A**) Cloning of *sir1* by map-based cloning. cM, centimorgan; BAC, bacterial artificial chromosome; N, north; S, south. (**B**) Complementation of *sir1* with the genomic fragment of ORF At5g55130. Left panel: Seedlings were transferred from MS plates to a plate that contained 25 μM sirtinol. Right panel: Plants in the left panel grown on sirtinol plates for 3 days. (**C**) The domain architecture of SIR1. SIR1 is composed of a ubiquitin-activating enzyme E1–like domain and a Rhodanese-like domain that shares significant homology with that of *Arabidopsis* prolyl *cis-trans* isomerase.

ing by dampening the positive auxin signals. The negative role of SIR1 is inhibited when sirtinol binds to SIR1, and therefore, the positive auxin signal runs unchecked and gives rise to the high auxin phenotypes we observed (Fig. 2, B to D). SIR1 functions upstream of the Aux/IAA genes and the corresponding protein degradation machinery, and thus, auxin-resistant mutants of these components are also resistant to sirtinol (Fig. 2E). Therefore, by carrying out a sirtinol-resistant instead of an auxin-resistant mutant screen, we can not only identify components in the negative regulatory loop that should also be auxin-hypersensitive, but also identify all of the positive auxin-signaling components downstream of SIR1.

Although the detailed molecular mechanisms of how SIR1 may negatively regulate auxin signaling are not fully understood at present, the domain architecture of SIR1 offers important clues. Auxin is believed to regulate the degradation of Aux/IAA proteins through a ubiquitin-related system (*11*, *12*). Phenotypes resulting from dominant mutations in Aux/IAA proteins arise from alterations in the stability of those proteins, but exactly how this happens is not yet clear [as reviewed in (*27*)]. All such mutations occur at or in the vicinity of two apparently essential proline residues (*27*). Given that SIR1 and one of the *Arabidopsis* prolyl isomerases each contain similar single-copy Rhodanese domains, it is conceivable that these molecules associate via these domains. Formation of such a complex may provide both a mechanism to regulate the conformation of the critical proline residues in Aux/IAA proteins in an auxin-dependent manner and a route to translate such a conformational change to a signal for protein degradation, presumably through the E1-like enzyme activity of the N-terminal domain of SIR1. Although prolyl isomerases have been found in many organisms and have been shown to regulate the stability of important cellular proteins (*28*), BLAST results find that the active-site rotamase domains of prolyl isomerases occur in conjunction with Rhodanese domains only in *Arabidopsis* and the rice *Oryza sativa*, suggesting that these particular prolyl isomerases may regulate a plant-specific process.

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Materials and Methods
Figs. S1 and S2
Table S1

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