was 100% bootstrap support for the separation of the mouse species in both tissues.


10. All RNA pools were hybridized 4 to 6 times to the same set of filters in order to estimate interexperimental errors and to minimize their effects through the combined analysis of several experiments. Signals that were at least 5 times above background and not influenced to more than 25% by neighboring spots were further analyzed. A gene was regarded as differently expressed if it fulfilled two criteria: (i) The difference in signal between two species was at least two-fold; and (ii) the signal between the two species was significantly different as determined by a paired t test. Sixteen differently expressed genes were analyzed by Northern blots, and 1 of 12 that were detected by the Northern analyses yielded results contradictory to the arrays, whereas the remaining 11 showed that it was a valid probe. Sufficient data were obtained to quantitatively and qualitatively similar in all three species to that detected by the arrays. Details of experimental procedures are available on Science Online at www.sciencemag.org/cgi/content/full/296/5656/340/DC1 on http://email.eva.mpg.de/~khaitov/supplement1.html.

11. The distance between two expression profiles of two species in a given tissue was determined by summing up the absolute ratios of the included genes given by the formula: \( \sum_{i=1}^{n} \log \frac{x_{i}}{x_{i}^{*}} \), where \( n \) is the number of included genes, and is the normalized intensity of gene \( j \) as measured in species \( j \). In order to avoid the contribution of genomic differences, only those differently expressed genes were considered that did not show the same expression difference in two or more tissues. The resulting distance matrix was used to build neighbor joining trees (19) as implemented in the PHYLIP package (20). The data are available at http://email.eva.mpg.de/~khaitov/supplement1.html

12. We retrieved nonmitochondrial nucleotide sequences from M. spretus (10 sequences) and M. caroli (11 sequences) from GenBank and compared them with the corresponding M. musculus sequence. The average number of substitutions at silent sites was estimated to be 0.025 \pm 0.006 for M. spretus and 0.045 \pm 0.006 for M. caroli.


16. Fractionation, 2D gel electrophoresis, and matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry were done as described in (21–23).


23. Tomato plants harboring the ripening-inhibitor (rin) mutation yield fruits that fail to ripen. Additionally, rin plants display enlarged sepals and loss of inflorescence determinacy. Positional cloning of the rin locus revealed two tandem MADS-box genes (LeMADS-RIN and LeMADS-MC), whose expression patterns suggested roles in fruit ripening and sepal development, respectively. The rin mutation alters expression of both genes. Gene repression and mutant complementation demonstrate that LeMADS-RIN regulates ripening, whereas LeMADS-MC affects sepal development and inflorescence deter

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R E P O R T S

A MADS-Box Gene Necessary for Fruit Ripening at the Tomato Ripening-Inhibitor (Rin) Locus

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The maturation and ripening of fleshy fruits is a developmental process unique to plants and affects the quality and nutritional content of a significant portion of the human diet. Although specific fruit-ripening characteristics vary among species, ripening can be generally described as the coordinated manifestation of changes in color, texture, flavor, aroma, and nutritional characteristics that render fruit attractive to organisms receiving sustenance in exchange for assisting in seed dispersal (1, 2).

Fruit species are classically defined as one of two ripening types, climacteric and nonclimacteric, where the former display a burst in respiration at the onset of ripening, in contrast to the latter. Climacteric fruit typically increase biosynthesis of the gaseous hormone ethylene, which is required for the ripening of fruit such as tomatoes, bananas, apples, pears, and most stone fruit. Nonclimacteric fruit, including strawberries, grapes, and citrus fruits, do not require climacteric respiration or increased ethylene for maturation. Molecular ripening research has focused primarily on ethylene, but little is known of control before ethylene induction, nor of common regulatory mechanisms shared by climacteric and nonclimacteric species (3).

The tomato is a model for analysis of ripening due originally to its significance as a food source and diverse germplasm, and more recently, the availability of molecular tools (4) and efficient transformation (5). A number of tomato-ripening mutants have been useful for research and breeding (3). Especially interesting is the recessive ripening-inhibitor (rin) mutation that inhibits all measured ripening phenomena, including the respiratory climacteric and associated ethylene evolution, pro-vitamin A carotenoid accumulation, softening, and production of flavor compounds (5). The rin mutant exhibits ethylene sensitivity, including the seedling triple response (7), floral abcission, and petal and leaf senescence. Nevertheless, rin fruit do not ripen in response to exogenous ethylene, yet they display induction of at least some ethylene-responsive genes, indicating retention of fruit ethylene sensitivity (8). We interpret these results to mean that the RIN gene encodes a genetic regulatory component necessary to trigger climacteric respiration and ripening-related ethylene biosynthesis in addition to requisite factors whose regulation is
outside the sphere of ethylene influence. As such, *RIN* acts upstream of both ethylene and nonethylene-mediated ripening control. We previously reported mapping of the *rin* locus to tomato chromosome 5 (9). Here, we report cloning and characterization of two MADS-box genes at the *rin* locus. MADS-box genes encode transcription factors that, in plants, primarily regulate floral development (10, 11), yet have never before been demonstrated to regulate ripening.

The only reported mutation at the *rin* locus arose spontaneously in a breeding line developed by H. Munger (at Cornell University) (12). In addition to ripening inhibition, the mutant exhibits large sepals and a loss of inflorescence determinacy (Fig. 1, A to C). Genetic complementation of only the ripening phenotype of the original *rin/rin* mutant, with a recessive large sepal mutant (*macrocalyx*, mc), suggested that the lesion at *rin* affected two adjacent loci controlling ripening and sepal development, respectively (12).

Previous mapping indicated that *rin* resides on a tomato 365-kb yeast artificial chromosome (YAC) clone, [Yrin9 (9)], and the analysis summarized in Fig. 2 confirmed this hypothesis (13). Yrin9 was used as a hybridization probe to identify putative *rin* cDNAs (13). Gene-expression analysis revealed two Yrin9-derived cDNA clones with altered transcripts in mutant fruit (Fig. 3A). C34 showed constitutive expression during maturation of normal fruits, whereas C43 was induced coincident with ripening. Both sequences hybridized to mRNA that was identical to *rin* fruit. This result, in combination with increased transcript size for both probes in *rin*, suggested that the *rin* lesion resulted in the fusion of gene sequences represented by C34 and C43. Reverse transcription–polymerase chain reaction (RT-PCR) of *Rin/Rin* and *rin/rin* fruit RNA (14) confirmed this hypothesis (Fig. 3B). Sequencing of both cDNAs, in addition to the *rin* RT-PCR product (15), verified that a deletion occurred in the mutant, yielding a chimeric RNA (Fig. 3C). PCR of *Rin/Rin* genomic DNA (16) indicates that 2.6 kb separates the C43 and C34 coding regions (Fig. 3C).

C34 and C43 sequencing revealed that both are members of the MADS-box family of transcription factors (15). MADS-box genes encode proteins characterized by the conserved MADS-box DNA binding domain and have been isolated from numerous eukaryotic organisms (17). Plant MADS-box genes are primarily associated with the regulation of floral development (10, 11, 18). The best described plant MADS-box family is from *Arabidopsis*, with at least 47 genes identified, although many remain to be defined functionally (19).

We named the genes corresponding to C43 and C34, *LeMADS-RIN* and *LeMADS-MC*, respectively, based on analyses described herein.
MADS-box genes as regulators of fruit ripening. LeMADS-RIN is required to initiate climacteric respiration and associated ethylene biosynthesis in addition to ripening factors that cannot be complemented by supplemental ethylene. Consequently, LeMADS-RIN is upstream of ethylene in the regulatory cascade and may represent a global developmental regulator of ripening potentially shared among climacteric and nonclimacteric species.

In support of this hypothesis, we have isolated a cDNA (FvMADS-9) from strawberries (a nonclimacteric fruit), using LeMADS-RIN as a probe (25). FvMADS-9 displays fruit-specific expression (25) and clusters close to LeMADS-RIN in phylogenetic analysis (Fig. 4).

LeMADS-RIN closely related to sequences derived from petunia and peppers (Fig. 4) but for which no functional characterization has been reported. The near identity of these sequences from additional members of the Solanaceae suggests potential involvement in ripening. Interestingly, unlike tomatoes and peppers, petunia fruit are not fleshy and undergo a maturation process ending in senescence, dehydration, and dehiscence similar to that of Arabidopsis silique. Functional analysis of FPB4 could lead to additional insights regarding maturation of different fruit types.

Ripe fruits serve as a significant portion of the human diet, directly affecting human
nutrition and health. LeMADS-RIN represents a molecular bridge between the extensive- 
ly studied phenomena of floral development and fruit ripening/ethylene response with re-
gard to the cascade of ethylene-regulated events associated with climacteric ripening being de-
pendent on a member of the floral developmen-
t–associated MADS-box family. This discovery 
opens a new research frontier in fruit ripening.

For example, because MADS-box genes are 
known to act as multimers (26), one could 
logically predict that additional MADS-box 
genomes might affect ripening.

From a practical perspective, the rin 
mutation is widely used in tomato hybrid 
cultivars to yield fruit with a long shelf life 
and acceptable quality. Tomatoes heterozy-
gous for the rin allele remain firm and ripen 
over a protracted period (presumably due to 
reduced levels of functional RIN protein) 
permitting industrial-scale handling and ex-
panded delivery and storage opportunities. 
LeMADS-RIN is a rare example of a gene 
whose effects are documented a priori, sug-
gestng excellent potential for practical ge-
netic modification of fruit ripening and 
quality characteristics.

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13. Details of Yr99 mapping, cDNA isolation, plant 
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org/cgi/content/full/296/5566/343/DC1.
14. C34 (LeMADS-MC) primers are C34F 5′-GAAGATCG-
GAACGAAATAAGTGAATTAAAC′ and C34R 5′-
GCGTCTTATACAGTGCTTCTTCTGCAAA3′. 
C43 (LeMADS-RIN) primers are C43F 5′-GACCG-
GAAGAGTATGGCAATATTGATAAC3′ and C43R 5′-
GCGTCTTATACAGTGCTTCTTCTGCAAA3′.
15. GenBank accession numbers are C34 (LeMADS-MC), 
AF448521; C43 (LeMADS-RIN), AF448522; and C43/
34 (in mutant), AF448523.
16. Primers were designed to amplify the region separat-
ing the C34 and C43 transcribed regions, as shown in 
Fig. 3C. The primers used were designated as C34R 5′-
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Alteration of Lymphocyte 
Trafficking by Sphingosine-
1-Phosphate Receptor Agonists

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Blood lymphocyte numbers, essential for the development of efficient immune 
responses, are maintained by recirculation through secondary lymphoid organs. We 
show that lymphocyte trafficking is altered by the lysophospholipid sphingosine-
1-phosphate (S1P) and by a phosphoryl metabolite of the immunosuppressive 
agent FTY720. Both species were high-affinity agonists of at least four of the five 
S1P receptors. These agonists produce lymphopenia in blood and thoracic duct 
lymph by sequestration of lymphocytes in lymph nodes, but not spleen. S1P 
receptor agonists induced emptying of lymphoid sinuses by retention of lympho-
cytes on the abluminal side of sinus-lining endothelium and inhibition of egress 
in lymph. Inhibition of lymphocyte recirculation by activation of S1P receptors may 
result in therapeutically useful immunosuppression.

Fig. 1. The structures of FTY720, S1P and related synthetic compounds.