The Nru I site is in the first *ptc* exon. The resulting plasmid, KO1, was linearized with Xho I and electroporated into RI embryonic stem cells that were subjected to double selection and analyzed by Southern (DNA) blot [A. L. Joyner, *Gene Targeting: A Practical Approach* (Oxford Univ. Press, New York, 1993), pp. 33–61]. Targeted clones were expanded and used for injection into C57Bl/6 blastocysts [B. Hogan, R. Beddington, F. Costantini, E. Lacy, *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994), pp. 196–204].
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Epidermal Cell Differentiation in *Arabidopsis* Determined by a *Myb* Homolog, *CPC*

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The roots of plants normally carry small hairs arranged in a regular pattern. Transfer DNA–tagged lines of *Arabidopsis thaliana* included a mutant with few, randomly distributed root hairs. The mutated gene *CAPRICE* (*CPC*) encoded a protein with a Myb-like DNA binding domain typical of transcription factors involved in animal and plant development. Analysis in combination with other root hair mutations showed that *CPC* may work together with the *TTG* gene and upstream of the *GL2* gene. Transgenic plants overexpressing *CPC* had more root hairs and fewer trichomes than normal. Thus, the *CPC* gene determines the fate of epidermal cell differentiation in *Arabidopsis*.

The cellular organization of the primary root of *Arabidopsis thaliana* is relatively simple and invariant (*1*). During the maturation of the root epidermis in *A. thaliana*, each cell ultimately becomes either a root hair (trichoblast; which we shall hereafter term a root hair cell) or a hairless cell (atrichoblast) (*2*, *3*). This choice may be determined by the position of the cell relative to the underlying cortical cell layer. Epidermal cell files that make contact with two cortical cell files by lying over the junction between the two cortical cell files are root hair cells. Epidermal cells that contact only one cortical cell file are hairless cells. Primary roots in wild-type *Arabidopsis* normally have eight files of cortical cells (Fig. 1F). Root hairs are tip-growing, tubular-shaped outgrowths that help to anchor roots, interact with soil microorganisms, and assist in the uptake of water and nutrients. *TRANSPARENT TESTA GLABRA* (*TTG*) and *GLABRA2* (*GL2*) are genes that determine whether epidermal cells differentiate into root hair cells or hairless cells (*3*, *4*). In *ttg* and *gl2* mutants, all of the epidermal cell files differentiate into root hair cells independent of their position relative to the underlying cortical cells. The *GL2* gene encodes a homeodomain protein that is expressed preferentially in the differentiating hairless epidermal cells (*4*, *5*). Although the *TTG* gene has not been cloned yet, it is believed to encode a protein with a Myc-like domain or a protein positively regulating a Myc-like gene, because the phenotype of the *ttg* mutant can be complemented by introducing a maize Myc gene, *R*, into the mutant. When the *R* gene is overexpressed in a wild-type plant, all of the root epidermal cells differentiate into hairless cells (*3*, *6*). Thus, *TTG* and *GL2* may inhibit the differentiation of root epidermal cells into root hair cells.

From a T3 population of transfer DNA (T-DNA)–tagged lines (*7*), we isolated a mutant with fewer than normal root hairs, which we named *caprice* (*cpc*) for the irregular distribution of root hairs (Fig. 1B). *cpc* is a nuclear mutation, not allelic to other known mutations. Heterozygous plants show the wild-type phenotype. From a cross between heterozygotes, about one-fourth (67/324) of the offspring had few root hairs, which indicated that *cpc* is a single, recessive mutation. The number of root hairs in the primary root of the *cpc* mutant was about one-fourth of that of the wild type (Table 1). The morphology and size of the root hairs produced by the *cpc* mutant were indistinguishable from those of wild-type hairs. The addition of 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, at 5×10^{-6} M induced root hair production in *cpc* seedlings; however, the number of hairs was about 30% of that of the ACC-treated wild type, indicating that ethylene cannot rescue the phenotypic deficiency of the *cpc* mutant (*8*).

To examine how the CPC gene works in combination with the *GL2* and *TTG* genes, we analyzed the phenotype of double mutants (Table 1). The *cpc gl2* double mutant had about the same number of root hairs as the *gl2* mutant, showing the *gl2* mutation to

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be epistatic to the *cpc* mutation. The *cpc ttg* double mutant had more root hairs than the *cpc* mutant, but less than the *ttg* mutant, indicating either that the *CPC* and the *TTG* gene products work together or they work in two independent pathways that control the number of root hairs.

We obtained a 2-kb genome sequence adjacent to the left border of T-DNA by inverse polymerase chain reaction (PCR) and confirmed the close linkage between the *cpc* locus and the T-DNA insertion (9). The chromosomal location of *CPC* was mapped by restriction fragment length polymorphism (RFLP) to the lower arm of chromosome II, 3.1 centimorgans from the m336 marker (*10*). From a genomic library and a cDNA library made from the roots of 5-day-old seedlings (*11*), we obtained four genomic and five cDNA clones carrying *CPC*. The five cDNA clones were isolated by screening 3×10^6 clones. No *CPC* mRNA was detected by Northern hybridization. These two results indicated that transcription of *CPC* is very rare in the root tissues. The *CPC* gene has an open reading frame of 1170 base pairs (bp) composed of three exons of 233, 88, and 263 bp and two introns of 73 and 513 bp (Fig. 2A). The longest cDNA (Fig. 2B) is 584 bp long, and the predicted CPC protein contains 94 amino acid residues. The *CPC* gene has a potential TATA box about 30 bp upstream of the putative transcriptional initiation site. An additional open reading frame in the inverse orientation was found 3 kb upstream of the 5′ terminal of *CPC*. The corresponding cDNA clone, number 51, was isolated, but the function of the gene is unknown.

We used genomic fragments, designated A, B, and C (Fig. 2A), to complement the *cpc* mutation by transformation (*12*). All three fragments were able to complement the *cpc* mutation (Fig. 1C); root hair numbers were equivalent to the wild type (Table 1). Because fragment C contained 525 bp of sequence upstream of the 5′ terminal of the cDNA, and fragment B contained 309 bp of sequence downstream of the cDNA, these untranscribed regions may be sufficient for the correct expression of *CPC*.

The deduced amino acid sequence of the CPC protein showed homology to the DNA binding domain of the proto-oncogene Myb (Fig. 2C). Although the other known Myb proteins have two or three Myb domains, the *CPC* gene encodes only one Myb-like domain, which most resembles the second Myb-like repeat in other plant Myb proteins and the third repeat in the Myb proteins of vertebrates. The Myb DNA binding domains are sequences of about 50 amino acids that form helix-turn-helix folds (*13*). The two tryptophan residues are con-

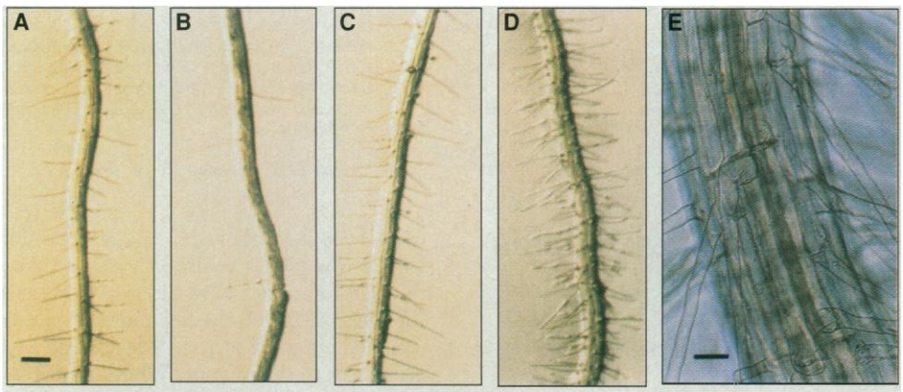
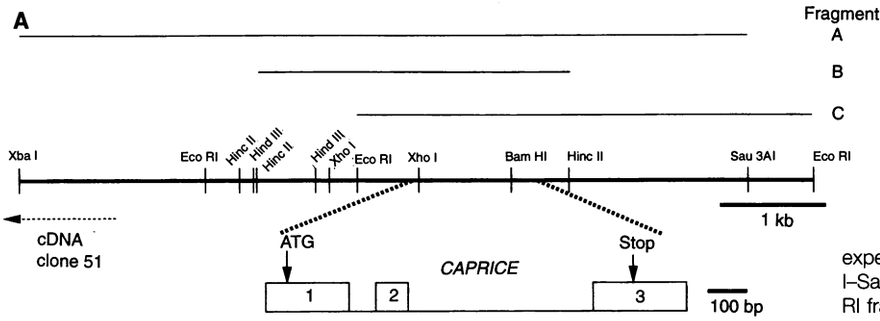
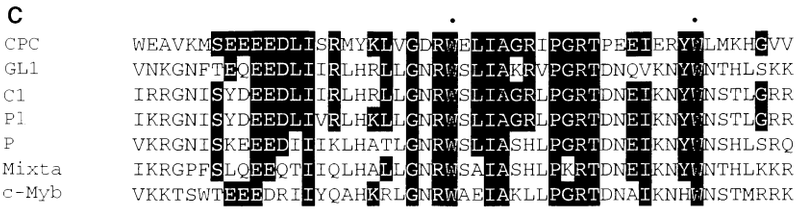


Fig. 1. Patterns of root hair formation in the major root of 5-day-old seedlings. Root surfaces were photographed with a binocular microscope (Olympus SZH). (**A**) Wild type, (**B**) *cpc* mutant, (**C**) *cpc* mutant complemented by the C fragment (see Fig. 2A), (**D**) transgenic plant harboring *35S::CPC* construct, and (**E**) enlarged picture of (D) taken with a camera attached to an Olympus PROVIS AX microscope. All epidermal cell files have root hairs. Scale bars: 200 μm in (A) to (D), and 30 μm in (E). (**F**) Drawing of a transverse section of the root. Eight epidermal cells that form root hair cells are indicated by arrowheads.





B

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 1 MFRSDKAEKMDKRRRRQSKAKASCSEEVSS 30
31 IEWEAVKMSEEEEDLISRMYKLVGDRWELI 60
61 AGRIPGRTPEEIERYWLMKHGVVFANRRRD 90
91 FFRK 94
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Fig. 2. Chromosome structure of *CPC* and the deduced amino acid sequence of its gene product. (**A**) Genomic structure of the *CPC* region. Genomic fragments used for complementation experiments are shown above the map: A, 7.3-kb Xba I–Sau 3AI fragment; B, 3-kb Hinc II fragment; C, 4.4-kb Eco RI fragment. Open boxes indicate the exons. (**B**) The deduced amino acid sequence of CPC. The region homologous to the Myb DNA binding domain is highlighted. The nucleotide sequence data has been submitted to the DNA Data Bank of Japan, European Molecular Biology Laboratory, and GenBank databases with accession number AB004871. (**C**) Sequences of the Myb domain of CPC compared with that of the C1, PI, and P proteins from maize (*14*); the GL1 protein from *Arabidopsis* (*15*); the Mixta protein from *Antirrhinum majus* (*16*); and the human c-Myb protein (*13*). Highlighted residues indicate amino acids that are identical to those of CPC. The W residues marked by dots indicate the position the tryptophan residues conserved in the Myb domain. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

served in the same position as in other Myb proteins. CPC has no proline-rich or acidic domain typical of a transcriptional activation domain. Insertion of a T-DNA into the CPC Myb homologous region converted the codon of the 75th amino acid residue, tyrosine (TAT), to a stop codon (TAA). Therefore, the CPC protein of the mutant lacks the COOH-terminal 19 amino acids that include part of the Myb domain. Several plant genes carrying Myb domains have been isolated. Mutation of maize genes *Pl*, *C1*, or *P* results in a defect of pigment synthesis (*14*). An *Arabidopsis* mutant carrying a mutation in the *GL1* gene lacks trichomes (*15*). Mutation of the *Mixta* gene in *A. majus* modifies the cell shape of the petal epidermis (*16*).

To examine the function of the CPC protein, we analyzed the phenotype of transgenic *Arabidopsis* plants in which the CPC gene was overexpressed under the control of the *35S* promoter of the cauliflower mosaic virus (*17*). Northern blot experiments showed that the expression of CPC was increased in both the roots and leaves of the transgenic plants (*18*). The *35S*::CPC transgenic plants had ectopic root hairs in the roots (Fig. 1, D and E). Almost all of the root epidermal cells formed a root hair in two independent transgenic lines. The phenotype was the same as that of the *gl2* and *ttg* mutants (Table 1 and Fig. 1E). In addition to the ectopic root hair formation, the *35S*::CPC transgenic plants lacked trichomes on the leaves, stems, and sepals (Fig. 3). Trichome formation is controlled by another Myb gene, *GL1* (*15*). It is supposed that in the wild type, *GL1* is a Myb gene expressed in shoots, whereas CPC is one expressed in roots. The phenotype of *35S*::CPC seedlings could be interpreted to indicate that the ectopic expression of CPC might interfere with the action of *GL1* on *TTG* or *GL2* (both of which block root hair formation) but induce trichome formation. *GL2* is the more likely relevant target, because the phenotype of *cpc gl2* and *cpc ttg* double mutants indicated that CPC may work together with *TTG* and upstream of *GL2* in the developmental pathway of root hair formation. Expression of *GL2* may be regulated negatively by CPC but positively by *GL1*, because the GL1 protein carries a transcriptional activation domain, whereas the CPC protein does not. If this is true, it is reasonable that transgenic plants overexpressing the CPC gene would show the same phenotype of the *gl2* mutant.

Both trichomes and root hairs are outgrowths of epidermal cells. Our results may indicate that the two cell differentiation systems are controlled by certain common pathways regulated by the Myb-like proteins.

Table 1. Root hair number and epidermal cell length of various plant lines. Values represent the mean ± SD. Hair number indicates the number of root hairs formed on a segment of a root with an average length of epidermal cells (=*ab*/1000). The control of the complementation experiment is of plants transformed by the vector pARK5 without genomic fragments. N.T., not tested.

Genotype	Number of root hairs per mm (*a*)	Length of epidermal cell (μm) (*b*)	Hair number
WT	43.2 ± 1.0	257.1 ± 10.9	11.1
cpc	10.6 ± 0.6	289.0 ± 12.0	3.1
gl2	111.8 ± 6.2	199.1 ± 5.4	22.3
ttg	98.2 ± 3.8	220.3 ± 10.4	21.6
gl2 cpc	100.4 ± 2.4	223.4 ± 6.7	22.4
ttg cpc	65.2 ± 2.6	249.3 ± 8.1	16.3
	Complementation experiment		
Control	11.8 ± 0.8	N.T.	
A	41.4 ± 1.8	291.3 ± 12.2	12.1
B	55.6 ± 1.4	254.9 ± 17.7	14.2
C	62.2 ± 2.8	N.T.	
	Overexpression experiment		
35S::CPC line 1	135.2 ± 4.8	172.2 ± 9.9	23.3
35S::CPC line 2	102.4 ± 3.2	226.3 ± 4.5	23.2

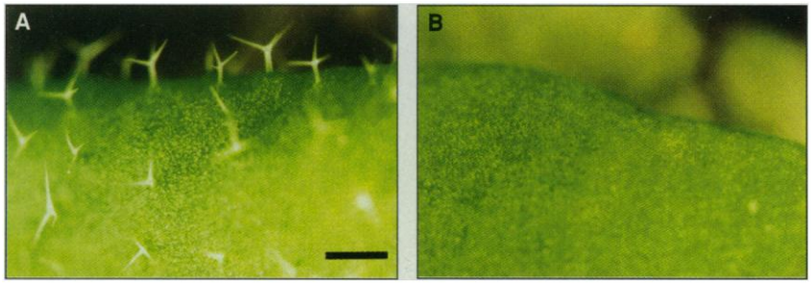

Fig. 3. Trichome pattern on the surface of the rosette. (**A**) Wild type. (**B**) Transgenic plant harboring *35S::CPC* construct. Scale bar, 500 μm. Photos were taken with a camera attached to a binocular microscope (Leica wild M420).

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9. From the T3 population we isolated a plant with only one Hind III band (4.9 kb) by Southern (DNA) blot analysis using the T-DNA left border as a probe. This fragment contained about 2.2 kb of *Arabidopsis* genomic DNA. We obtained the genomic sequence flanking the T-DNA using the inverse PCR method according to Deng *et al.* [X.-W. Deng *et al.*, *Cell* **71**, 791 (1992)]. Southern blot analysis revealed that all of the 24 F_2 plants with the mutant phenotype carried the 4.9-kb band homozygously. This result indicated that the location of the T-DNA insertion and the *cpc* locus were tightly linked.
10. Mapping experiments with recombinant inbred lines were conducted by use of RFLP of Bam HI–digested genomic DNA [C. Lister and C. Dean, *Plant J.* **4**, 745 (1993)]. The recombination frequency was calculated by the Map Maker program [E. S. Lander *et al.*, *Genomics* **1**, 174 (1987)].
11. A λ-Dash II genomic library was constructed with DNA from an aerial portion of 4-week-old wild-type *Arabidopsis* (ecotype WS). The amplified library (total 1.5×10^5 recombinants) was screened with the inverse PCR fragment used as a probe. We isolated four independent phage clones. A λ-ZAPII cDNA library was constructed with polyadenylated RNA isolated from primary roots from wild-type *Arabidopsis* seedlings (ecotype WS). The synthesis of root cDNA was performed with a ZAP-cDNA synthesis kit (Stratagene). The unamplified library (total 3×10^6 recombinants) was screened with the genomic 5-kb Xba I–Bam HI fragment used as a probe.
12. Three genomic fragments were ligated to a binary vector, pARK5, and introduced into an agrobacterium strain, C58C1 [H. Anzai, K. Yoneyama, I. Yamaguchi, *Nucleic Acids Res.* **18**, 1890 (1990)]. We then introduced them into *Arabidopsis* (ecotype WS) by the vacuum-infiltration method [N. Bechtold, J. Ellis, G. Pelletier, *C. R. Acad. Sci. Paris Life Sci.* **316**, 1194 (1993)].
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19. We thank the Nottingham Arabidopsis Stock Centre (University of Nottingham, UK) for providing the seeds of *gl2-1* and *ttg-1* mutants; S. Ishiguro and other members of our laboratory for suggestions; D. M. Marks and J. W. Schiefelbein for discussions; and A. Kawai for making the transgenic *Arabidopsis* lines. Supported by a Grant-in-Aid for Scientific Research (A) number 08408031 and a Grant-in-Aid for Scientific Research on Priority Areas number 06278103 from the Japanese Ministry of Education, Science, Culture and Sports and by funds from the Joint Studies Program for Advanced Studies from the Science and Technology Agency of Japan.

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TECHNICAL COMMENTS

Distribution and Causation of Species Endangerment in the United States

A. Dobson *et al.* (*1*) provide a description of the geographic distribution of endangered species in the United States. They also examine the associations between the density of endangered species and the intensity of human economic activities, with the use of the annual statistical survey of the United States (*2*). Their effort (*1*) was too abbreviated for prudent policy implications. The statistical survey of the United States does not provide data on all economic activity, and it says nothing of endangerment causation. Extrapolating correlation to causation is fraught with assumption (*3*).

With the use of the only encyclopedic account of endangered species available (*4*–*6*), we compiled a database of the 877 American threatened and endangered species listed by the U.S. Fish and Wildlife Service up until 1995 and the causes of their endangerment that have been operational since passage of the Endangered Species Act. We identified 18 causes of endangerment (Table 1).

Most endangered species are endangered by several causes, and it is rarely possible to determine the relative importance of each cause. By the time a species is endangered, however, any loss of individuals is critical, so that the "relativity" of importance loses relevance for any given species. We suggest, therefore, that the importance of a cause to overall species endangerment generally corresponds to the frequency with which it is found to endanger species.

Dobson *et al.*(*1*, p. 552) found that "the overall density of endangered species is correlated with one anthropogenic and one climatic variable (correlation coefficient $r^2 = 0.80$, $P < 0.01$): the value of agricultural output and either average temperature or rainfall." Agriculture is a major cause of endangerment, but it is less important than nonnative species and urbanization (*7*). Furthermore, there is a host of economic activities that greatly exceeds agriculture in importance, in a cumulative sense (Table 1).

The emphasis of Dobson *et al.* (*1*) on the concentration of endangered species in "hot spots" tends to discount the fact that species are endangered in all 50 states (*8*). (Agriculture alone endangers species in 35 states and Puerto Rico.) Many people might welcome the new study without a concomitant care for the species diversity. It offers policymakers living in 47 states an opportunity to skirt the issue by pointing to Hawaii, California, and Florida and claiming that sanctuaries in those states are sufficient. If population size and per capita consumption are not addressed in the policy arena, then accelerated extinctions will clearly proliferate, and human economy will be severely and forcefully adjusted to fit within the limits of its natural capital stocks. Other efforts (for example, assessments of species distribution) may delay economic adjustment from an administrative time perspective, but can only prolong extinction for a blink of evolutionary time.

Table 1. Causes of endangerment for species classified as threatened or endangered by the U.S. Fish and Wildlife Service.

Cause	Number of species endangered by cause and rank of frequency*	Number of species endangered and rank of frequency†
Interactions with nonnative species	305 - 1	115 - 8
Urbanization	275 - 2	247 - 1
Agriculture	224 - 3	205 - 2
Outdoor recreation and tourism development	186 - 4	148 - 4
Domestic livestock and ranching activities	182 - 5	136 - 6
Reservoirs and other running water diversions	161 - 6	160 - 3
Modified fire regimes and silviculture	144 - 7	83 - 10
Pollution of water, air, or soil	144 - 8	143 - 5
Mineral, gas, oil, and geothermal extraction or exploration	140 - 9	134 - 7
Industrial, institutional, and military activities	131 - 10	81 - 12
Harvest, intentional and incidental	120 - 11	101 - 9
Logging	109 - 12	79 - 13
Road presence, construction, and maintenance	94 - 13	83 - 11
Loss of genetic variability, inbreeding depression, or hybridization	92 - 14	33 - 16
Aquifer depletion, wetland draining or filling	77 - 15	73 - 15
Native species interactions, plant succession	77 - 16	74 - 14
Disease	19 - 17	7 - 18
Vandalism (destruction without harvest)	12 - 18	11 - 17

*Including Hawaiian and Puerto Rican species. †Not including Hawaiian and Puerto Rican species.

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