Serratia. In extracts, however, the inhibitors were active against the *Pseudomonas* and *Serratia* deacetylases. Because the lipid A content was not reduced in living cells of *Pseudomonas* and *Serratia* treated with inhibitors (*12*, *20*), it may be that our compounds did not penetrate these bacteria or were actively extruded by them.

Our compounds were capable of curing mice infected with live *E. coli* (Table 3). The mouse model used for this purpose (Table 3) is relevant to the clinical situation of bowel surgery or bowel injury. The model can also be used to eliminate antibacterial compounds that are ineffective because they bind to animal tissues and proteins. Two intraperitoneal doses of L-159,692 or L-161,240 rescued mice [calculated median effective doses (ED_{50}'s) of 36 and 15 mg per kilogram of body weight, respectively] from an *E. coli* infection that was 100% fatal without treatment. This suggests that deacetylase inhibitors could be developed for treatment of Gram-negative infections. Deacetylase inhibitors might also lower the complications of Gram-negative sepsis by reducing the amount of endotoxin released from bacteria when other antibiotics are used as the primary therapy (*21*, *22*).

Table 2. Minimal inhibitory concentrations of deacetylase inhibitors against a panel of Gram-negative bacteria. Bacterial strains were wild-type mouse-virulent isolates. Minimal inhibitory concentrations were determined as in Table 1. In most experiments, concentrations over 100 μg/ml were not examined. The inhibitors did not kill or slow the growth of Gram-positive bacteria, yeast, or Chinese hamster ovary cells at 100 μg/ml; only bacteria that make lipid A were killed.

Bacterial strain	MIC (μg/ml)		
	L-573, 655*	L-159, 692	L-161, 240
E. coli (MB2884)	200 to 400	50	1
Enterobacter cloacae (MB2646)	>100	100	6
Klebsiella pneumoniae (MB4005)	>100	50	13
Serratia marcescens (MB3548)	>100	100	>100
Proteus mirabilis (MB3125)	>100	>100	50
Pseudomonas aeruginosa (MB3286)	>100	>100	>100

Table 3. Deacetylase inhibitors L-159,692 and L-161,240 protect mice from lethal septicemia with *E. coli* strain MB2884. A systemic infection was established by intraperitoneal (i.p.) injection of *E. coli* MB2884 diluted in brain-heart broth. Challenge doses contained 9 to 33 median lethal doses. Antibiotics were administered i.p. immediately after the infecting dose (0 hours) and again 6 hours later. Five mice per group were tested at each of several doses of inhibitor. The test was terminated 7 days after infection, and the survival records of that day were used to calculate the amount of antibiotic that should protect 50% (ED_{50}) of the infected, treated animals (*25*). Before the actual experiment, two mice were treated for 7 days with 100 mg of each compound per kilogram of body weight (a sevenfold excess over the maximal dose used in the infection model) without adverse side effects (such as seizures or lethargy) or reduced food intake.

Dose (mg/kg)	Survivors/infected mice (*n*) after 7 days	
	L-159,692 treatment	L-161,240 treatment
3.125	0/5	0/5
12.5	1/5	1/5
50	3/5	5/5

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CKI1, a Histidine Kinase Homolog Implicated in Cytokinin Signal Transduction

Tatsuo Kakimoto

Although cytokinin plays a central role in plant development, little is known about cytokinin signal transduction. Five *Arabidopsis thaliana* mutants that exhibit typical cytokinin responses, including rapid cell division and shoot formation in tissue culture in the absence of exogenous cytokinin, were isolated by activation transferred DNA tagging. A gene, *CKI1*, which was tagged in four of the five mutants and induced typical cytokinin responses after introduction and overexpression in plants, was cloned. *CKI1* encodes a protein similar to the two-component regulators. These results suggest that CKI1 is involved in cytokinin signal transduction, possibly as a cytokinin receptor.

Cytokinin regulates many physiological events such as nutrient metabolism, expansion and senescence of leaves, and lateral branching (*1*). Moreover, it induces cell division, chloroplast development, and shoot production in cells grown in culture (*2*). However, little is known about the biosynthesis and mechanism of action of cytokinin (*3*). This report describes the isolation of cytokinin-independent mutants of *Arabidopsis*, generated by activation transferred DNA (T-DNA) tagging (*4*), and the use of these mutants to identify a gene involved in cytokinin signal transduction.

Calli derived from hypocotyl segments of 50,000 seedlings of *A. thaliana* were transformed (*5*) with *Agrobacterium* containing pPCVICEn4HPT, a transformation vector with a T-DNA containing a tetramer of the enhancer of the cauliflower mosaic virus (CaMV) 35S RNA promoter (*4*). After integration of such a T-DNA into the genome, expression of genes adjacent to the integra-

Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560, Japan.

tion site should become deregulated, thus creating a dominant mutation. If a gene involved in cytokinin biosynthesis, recognition, or signal transduction is tagged, then the normal response of the callus to cytokinin might occur in the absence of exogenous cytokinin. Under normal culture conditions, *Arabidopsis* callus requires cytokinin for proliferation, greening, and shoot formation. Thus, to isolate cytokinin-independent mutant calli, transformed calli were screened for growth in the absence of exogenous cytokinin (6). Five calli were found that turned green, proliferated rapidly, and produced shoots in the absence of cytokinin (Fig. 1). These lines were named for the mutation *cytokinin-independent* (*cki1-1* through *cki1-4* and *cki2*). The lines *cki1-1*, *-2*, *-3*, and *-4* were unable to produce roots and normal flowers and all were sterile, but *cki2* produced roots and normal flowers and set seeds, and the ability of the calli to grow and form shoots in the absence of cytokinin was inheritable. In the same screen, another fertile mutant was obtained that formed many shoots in the absence of cytokinin, but no rapid proliferation occurred. Plants raised from the seeds sometimes produced many adventitious shoots on cotyledons and petioles when grown on hormone-free MS medium [reference in (5)], and this mutant line was named *many shoots* (*msh*).

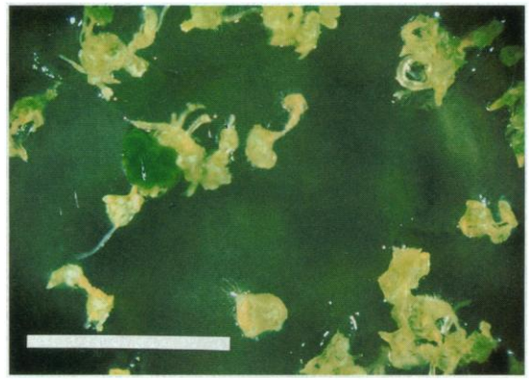
Southern (DNA) blot analysis with the enhancer fragment of the CaMV 35S RNA promoter as a probe revealed that *cki1-1* contained a single T-DNA insert (7), and this insert plus 10 kb of flanking plant-derived DNA was rescued in a plasmid, pC1S1 (Fig. 2) (8).

To examine whether the rescued DNA fragment could confer cytokinin-independent development, I converted it to Ti plasmid pC1S1Ti (9) and introduced it into wild-type calli by *Agrobacterium*-mediated transformation (Fig. 3, A to D). Calli transformed with pPCVICEn4HPT, the control vector, produced roots but no shoots in the presence of auxin as the sole plant hormone (Fig. 3B), but in the presence of cytokinin, either alone (7) or together with auxin, the transformed calli proliferated rapidly, turned green, and produced shoots (Fig. 3A). In contrast, calli transformed with pC1S1Ti exhibited all the phenotypic characteristics of the cytokinin responses, including rapid proliferation, greening, shoot production, and failure to produce roots in the presence of auxin as the sole plant hormone (Fig. 3D). Experiments conducted in the absence of auxin gave essentially the same results. Deletion analysis showed that the region covering both the T-DNA and the plant-derived DNA that flanked the right T-DNA border sequence confers cytokinin-independent development (7).

Cognate cDNAs of this plant-derived sequence were cloned, and one of them, cCKI1-16 (10), was studied in detail. Calli transformed with p35ScCKI1, a Ti plasmid that contained cCKI1-16 downstream of the CaMV 35S RNA promoter (11), showed all normal responses to cytokinin in the absence of exogenous cytokinin (Fig. 3, E to H).

RNA blot analysis revealed that a large amount of the *CKI1* message of about 3.7 kb was present in the *cki1-1* line, whereas the message was not detected in wild-type tissues, such as whole seedlings, undifferentiated calli, shooting calli that had been treated with a strong cytokinin (thidiazuron), or rooting calli that had been treated with the auxin indolebutyric acid (7, 12).

When the 5′ half of cCKI1-16 was hybridized at high stringency (13) with Pst I– or Spe I–digested genomic DNA from wild-



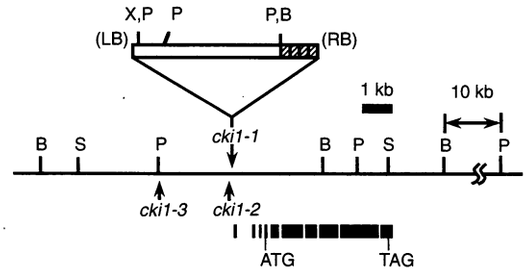


Fig. 1 (top). Screen for cytokinin-independent mutants. Wild-type *Arabidopsis* calli were transformed with pPCVICEn4HPT and cultured in the absence of phytohormones for 3 weeks. Note that a portion of one callus is green, whereas most of the transformed calli are a yellowish color. This green region proliferated rapidly and regenerated shoots in the absence of exogenous cytokinin and auxin. Bar, 1 cm. **Fig. 2 (bottom).** Genomic structure of the *CKI1* region. Sites of the T-DNA insert in *cki1-1*, *-2*, and *-3* are indicated by arrows. Structure of the T-DNA insert in *cki1-1* is indicated, with hatched boxes representing the tetramer of CaMV 35S RNA enhancers. Orientation of the T-DNA inserts in *cki1-1*, *-2*, and *-3* is the same. The position of the insert in *cki1-4* could not be defined because, in this strain, there appeared to have been a rearrangement in the upstream region of the *CKI1* gene. pC1S1 corresponds to the region between two Spe I sites with the T-DNA insert. Sequences that are common between cCKI1-16 and pC1S1 are shown below the diagram of the genomic structure as solid boxes, together with the presumed ATG initiation codon and TAG stop codon for the longest open reading frame. A region of 143 bp of the 3′ noncoding part of cCKI1-16, which was not carried by pC1S1, is not shown. The stop codon is at the Spe I recognition site. B, Bam HI; P, Pst I; S, Spe I; X, Xho I; LB, left border of T-DNA; RB, right border of T-DNA.

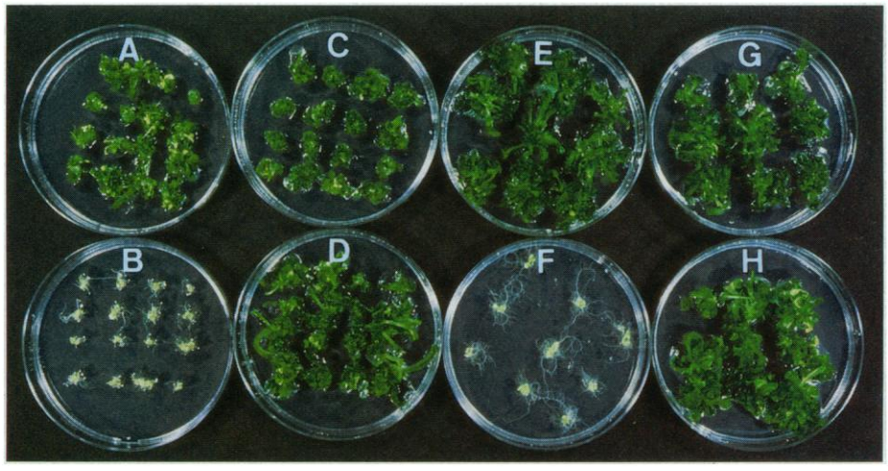


Fig. 3. Effects of transformation with the rescued DNA or with the cDNA of the *CKI1* gene fused under the CaMV 35S RNA promoter. Calli were transformed with pPCVICEn4HPT (**A** and **B**), pC1S1Ti (**C** and **D**), pMON530 (**E** and **F**), or p35ScCKI1 (**G** and **H**) and cultured for 18 days in the presence [(A), (C), (E), and (G)] or absence [(B), (D), (F), and (H)] of cytokinin [*trans*-zeatin (2 mg/liter)]. All plates contained indolebutyric acid (0.5 mg/liter) and cefotaxime (150 mg/liter). Plates in (A) through (D) contained hygromycin (20 mg/liter) and plates in (E) through (H) contained kanamycin (50 mg/liter).

type *Arabidopsis*, only fragments predicted by plant-derived sequences in pC1S1 were detected. A restriction fragment length polymorphism was detected between the genomes of the wild type and *cki1-1* (Fig. 4), and the sizes of the fragments detected in the *cki1-1* genome were consistent with the restriction map of pC1S1. This result indicated that the T-DNA and the *CKI1* gene are physically linked in the *cki1-1* genome. Southern blot analysis of the independently isolated *cki1-2*, *cki1-3*, and *cki1-4* lines indicated that the T-DNA also was inserted upstream of the *CKI1* gene in these lines (Fig. 2). Considering the genome size of *Arabidopsis* [10^8 base pairs (bp)] (*14*) and the number of calli screened (50,000) and assuming that, on average, one callus yielded two transformation events, as a lowest estimate of the frequency, an average of one tagging event every 1 kb was calculated. The result of this calculation is consistent with four tagging events of the gene *CKI1* in this experiment. Southern blot analysis revealed that *cki2* and *msh* lines did not have a T-DNA integration in the region between the Bam HI site (Fig. 2) about 7 kb upstream from the inferred start codon of the *CKI1* gene and the Pst I (Fig. 2) site about 12 kb downstream from the inferred stop codon of the gene. These observations, together with the results of Northern blot analysis, which could not detect the *CKI1* message in RNA from *cki5* and *msh* (*7*, *12*), suggest that, in both *cki2* and *msh*, the mutant phenotype might have been the result of activation of some gene or genes different from *cki1*.

Sequencing of cCKI1-16 revealed an open reading frame encoding 1122 amino acids corresponding to a predicted protein of 125 kD (Fig. 5A). The predicted CKI1 product has regions homologous to histidine kinase domains and receiver domains, respectively, of the two-component systems (Fig. 5, B to D). All of the five conserved motifs found in histidine kinases (*15*, *16*), including the putative autophosphorylation site at His^{405}, and the highly conserved amino acid residues of the regulators (*16*), including the putative phosphorylation site at Asp^{1050}, are present in CKI1. The deduced product of the *CKI1* gene has greatest similarity to the products of *LemA* (*17*) and *BarA* (*18*) in bacteria. The predicted CKI1 protein does not exhibit any homology to the proteins of two-component systems in regions outside the predicted histidine kinase domain and the receiver domain. The two-component systems are prevalent in bacterial signal transduction systems (*15*). Recently, such a system has also been found in yeast (*19*, *20*), and several genes with sequence similarities to these components have been identified in other eukaryotes (*21*–*24*). Like many bacterial histidine kinases, CKI1 has two potential transmembrane segments that flank the inferred extracellular domain in the NH_2-terminal region (Fig. 5, A and D). There are 11 potential signal sequences for N-glycosylation in the inferred extracellular domain.

The role of *CKI1* in cytokinin signal transduction is not clear. Overexpression of *CKI1* results in the characteristic effects of cytokinin action, suggesting that the product acts in regulation of cytokinin levels, in cytokinin recognition, or at an early stage of signal transduction. This being said, unexpected effects related to ectopic *CKI1* expression cannot be formally excluded. *ETR1*, which encodes a two-component histidine kinase homolog, was initially identified as a component of the ethylene signal transduction pathway (*21*). Recently, ETR1 was demonstrated to be an ethylene receptor through its ability to bind ethylene at its membrane-spanning region (*25*). By analogy, CKI1 could be a cytokinin receptor. It is noteworthy that, although

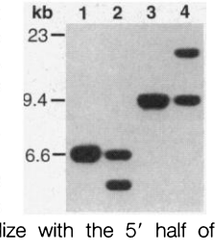


Fig. 4. Southern blot analysis of the wild-type and *cki1-1* lines. DNA from the wild type (lanes 1 and 3) and from *cki1-1* (lanes 2 and 4) was digested with Pst I (lanes 1 and 2) or Spe I (lanes 3 and 4), and fragments were allowed to hybridize with the 5′ half of cCKI1-16 (nucleotides 1 to 2652).

A

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MMVKVTKLVASRPIVVFCVLAFLVVVFECIWISNWRTTTENLVKEVASFTEDLRTSLVSEIENIGKFTYAKTNLSTIGLARVID
SYITNNDTGFTEIQTQIAPLLFVAYSTILQVSQVSYISRDGLMFSYIAESNTSVAVFANSSSNSSRGDYTWYTQTVDQLTGRLN
GNSTKSQSLDVTHTDWFQAAQSNNYTTAFVGTSLGGEDNETLIQSVVSLYSKKGLVSLGFPVKTLTEVLNSLNLHGEELYMWTK
DGTVLVREGSLNDSFFISNGSICFGRESNSLWSQCIPENCSSSGYEVEIKRLRYQAFCSVIEVSGVPLRYTLMFPNKGGATRIK
HQAEKAKYQLIVVMIFLGFGWPVWFVWFMMQATRREMHMRATLINQMEATQQAERKSMNKSQAFANASHDIRGALAGMKGLIDI
CRDGVKPGSDVDTTLNQVNVCAKDLVALLNSVLDMSKIESGKMQLVEEDFNLSKLLEDVIDFYHPVAMKKGVDVVLDPHDGSVF
KFSNVRGDSGRLKQILNNLVSNAVKFTVDGHIAVRAWAQRPGSNSSVVLASYPKGVSKFVKSMFCKNKEESSTYETEISNSIRN
NANTMEFVFEVDDTGKGIPMEMRKSVFENYVQVRETAQGHQGTGLGLGIVQSLVRLMGGEIRITDKAMGEKGTCFQFNVLLTTL
ESPPVSDMKVRQEIEAGGDYVSTPNLGLTINTSLGGSMNIRNLSPRFNNCLSSSPKQEGSRVVLLLKNEERRRVTEKYIKNLGI
KVTVVEKWEHLSYALERLFGFSPQSSMGRAECSLSCPSSRELPFIGMDGIDSRSQLPKRRSISFSAVVLLVIDAKTGPFFELCD
IVKQFRRGLPHGISCKVVWLNESSTRVSERGDISCSRPLHGSRLMEVLKMLPEFGGTVLKEPPTELQRESLLRHSFVAERSPKH
KVQEEGPSSMFNKKLGKRIMASTDSESETRVKSVRTGRKPIGNPEDEQETSKPSDDEFLRGKRVLVVDDNFISRKVATGKLKKM
GVSEVEQCDSGKEALRLVTEGLTQREEQGSVDKLPFDYIFMDCQMPEMDGYEATREIRKVEKSYGVRTPIIAVSGHDPGSEEAR
ETIQAGMDAFLDKSLNQLANVIREIESKRH
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B

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                                    ★
CKI1   385 ATQQAERKSMNKSQAFANASHDIRGALAGMKGLIDICRDGVKPGSDVDTTLNQVNVCAKDLVALLNSVLDMSKIE
LemA   286 ARKEALEASRIKSEFLANMSHEIRTPLNGILGFTHLLQK-SELTPRQFDYLGTIEKSADNLLSIINEILDFSKIE
BarA   282 AKKRAQEAARIKSEFLANMSHELRTPLNGVIGFTRLTLK-TELTPTQRDHLNTIERSANNLLAIINDVLDFSKLE
DhkA  1376 EKEAAEEANKAKSAFVATVSHEVRTPLSGVIGVSDLLLE-TNLSEEQRDYVQTIQKSSQALLTIINDILDYSKLE
ETR1   333 ARREAETAIRARNDFLAVMNHEMRTPMHAIIALSSLLQE-TELTPEQRLMVETILKSSNLLATLMNDVLDLSRLE
Slnl   556 AKIEAEAANEAKTVFIANISHELRTPLNGILGMTAISME-ETDVNKIRNSLKLIFRSGELLLHILTELLTFSKNV

CKI1   SGKMQLVEEDFNLSKLLEDVIDFYHPVAMKKGVDVVLDPHDGSVFKFSNVRGDSGRLKQILNNLVSNAVKFT-VDG  534
LemA   AGKLVLDNIPFNLRDLLQDTLTILAPAAHAKQLELVSLVYR-DTPLALS--GDPLRLRQILTNLVSNAIKFT-REG  431
BarA   AGKLILESIPFPLRSTLDEVVTLLAHSSHDKGLELTLNIKS-DVPDNVI--GDPLRLQQIITNLVGNAIKFT-ENG  427
DhkA   SRQLKMETLPFSIIETCQAVIHMLS-VAANDDVDILLRVPP-NVPRIIF--GDAMRMRQVLLNRLSNAIKFT-SRG 1520
ETR1   DGSLQLELGTFNLHTLFREVLNLIKPIAVVKKLPITLNLAP-DLPEFVV--GDEKRLMQIILNIVGNAVKFS-KQG  478
Slnl   LQRTKLEKRDFCITDVATQIKSIFGKVAKDQRVRLSISLFP-NLIRTMVLWGDSNRIIQIVMNLVSNALKFTPVDG  704

CKI1   589 VDDTGKGIPMEMRKSVFENYVQVRETAQG-HQGTGLGLGIVQSLVRL-MGGEI  649
LemA   453 VQDTGIGLSSQDVRALFQAFSQADNSLSRQPGGTGLGLVISKRLIEQ-MGGEI  504
BarA   449 IRDTGIGIPERDQSRLFQAFRQADASISRRHGGTGLGLVITQKLVNE-MGGDI  500
DhkA  1544 IEDTGIGIPQSLFDSIFEPFSQADNSTTRKYGGTGLGLSITKRLIEEVMGGTI 1600
ETR1   511 VKDSGAGINPQDIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNL-MEGNI  562
Slnl   857 VEDTGPGIDPSLQESVFHPFVQGDQTLSRQYGGTGLGLSICRQLANM-MHGTM  908
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C

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                                    ★
CKI1   986 KRVLVVDDN (49) FDYIFMDCQMPEMDGYEATREIR (10) PIIAVSGHDPGSEEARETIQAGMDAFLDKSL 1107
LemA   679 PRVLCVDDN (35) FDLVLMDVQMPGMDGRQATEAIR (11) PIVALTAHAMANE-KRSLLQSGMDDYLTKPI  786
BarA   668 MTVMAVDDN (35) FDLILMDIQMPDMDGIRACELIH (8)  PVIAVTAHAMAGQ-KEKLLGAGMSDYLAKPI  772
DhkA  2025 KKALIVEDN (36) FDIVFMDCQMPVLDGFQTTKIIR (11) NIVALSAGSSSSF-VQDCLDSGMDSFMGKPI 2133
ETR1   609 GLKVLVMDE (35) HKVVFMDVCMPGVENYQIALRIH (11) LLVALSGNTDKST-KEKCMSFGLDGVLLKPV  716
Slnl  1088 VKILVVEDN (41) YNMIFMDVQMPKVDGLLSTKMIR (7)  PIVALTAFADDSN-IKECLESGMNGFLSKPI 1197
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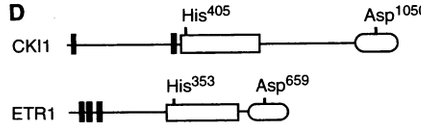

Fig. 5. (**A**) Deduced amino acid sequence of the *CKI1* gene product (*26*). Potential transmembrane regions are indicated with double underlining. Eleven potential sites of N-glycosylation between the putative transmembrane regions are indicated by single underlining. (**B** and **C**) Deduced amino acid sequence of CKI1, aligned with conserved regions of several histidine kinase and receiver domains of LemA of *Pseudomonas syringae* (*17*), BarA of *E. coli* (*18*), DhkA of *Dictyostelium* (*22*), Sln1 of *Saccharomyces cerevisiae* (*19*), and ETR1 of *A. thaliana* (*21*). Alignment was achieved with the ClustalW program (Human Genome Center, Baylor College of Medicine). Amino acids shown in boldface indicate residues that are identical to those found at the same positions in at least three of the other sequences. (B) shows the deduced amino acid sequence of CKI1 aligned with the histidine kinase domains. Boxes surround the five consensus motifs that are characteristic of histidine kinase domains, as compiled by Perkinson and Kofoid (*15*). Asterisk at His^{405} indicates a putative phosphoryl group acceptor. (C) shows the deduced amino acid sequence of CKI1 aligned with the receiver domains. Asterisk at Asp^{1050} indicates a putative phosphoryl group acceptor. Boxes surround the highly conserved regions in the receiver domains (*16*). (**D**) Schematic representation of predicted structure of the CKI1 protein in comparison with the ETR1 protein. Open boxes represent histidine kinase domains, open ovals represent receiver domains, and filled boxes represent stretches of hydrophobic residues characteristic of membrane-spanning sequences. Putative histidine and aspartate phosphorylation sites are indicated.

similar in structure, the putative input domains of CKI1 and ETR1 differ, suggesting separate functions. The simplest explanation of the *cki1* phenotype is that CKI1, acting as a cytokinin receptor, when overexpressed confers the ability on the expressing cells to sense low concentrations of endogenous cytokinin that is normally unable to trigger growth and shoot formation. Obviously, further work is required to define the function of CKI1, but it and ETR1 could represent members of a gene family that diverged to respond to different phytohormones.

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6. The screenings for cytokinin-independent mutants were done as follows. Calli transformed with pPCVICEn4HPT were cultured on basal medium (*5*) supplemented with hygromycin (40 mg/liter), cefotaxime (150 mg/liter), and vancomycin (100 mg/liter), at 23°C under continuous light (5000 lux) for 3 weeks. Calli that proliferated rapidly, turned green, and produced shoots were selected and subcultured on the same medium. These lines were maintained by subculturing on basal medium (*5*).
7. T. Kakimoto, data not shown.
8. DNA isolated from the *cki1-1* line was digested with Spe I, self-ligated, and used to transform *Escherichia coli* (DH10B) by electroporation, and the plasmid (pC1S1) was purified from the ampicillin-resistant *E. coli* cells as described elsewhere [C. Koncz *et al.*, in *Plant Molecular Biology Manual*, S. B. Gelvin and R. A. Schilperoort, Eds. (Kluwer Academic, Dordrecht, Netherlands, 1994), section B2].
9. A Spe I digest of pC1S1 was ligated to the Xba I fragment of pPCVICEn4HPT (*4*), which carries the T-DNA right border sequence, the vegetative (oriV), the conjugational (oriT) DNA replication origins of plasmid RK2, and the T-DNA left border sequence, to yield Ti plasmid pC1S1Ti.
10. RNA from the *cki1-1* line was reverse-transcribed with the sequence-specific primer 5′-AGGCGTCCATTCCAGCTTGAATGG. The cDNA was amplified by polymerase chain reaction with a 5′ primer (5′-AGATCGCACCATTGTTGTTTGTAGC) and a 3′ primer (5′-CACACAAACCATACAGGCCAACCG), whose sequences were based on the genomic sequence. The amplified DNA was used to screen a cDNA library that had been constructed in Lambda ZAP II (Stratagene), in accordance with the manufacturer's instructions, with polyadenylated [poly(A)$^+$] RNA from shoot tissue of the *cki1-1* line. Eighteen positive plaques were identified among 2×10^5 plaques, and both ends of the five longest clones were sequenced. All of the sequences determined from the 5′ ends were found in pC1S1. Of the five clones, cCKI1-16 was used for subsequent experiments. Both strands of cCKI1-16 were sequenced, and the complete sequence of cCKI1-16 was present in pC1S1 with the exception of the 3′ noncoding region. cCKI1-16 contained 186 bp of 5′ noncoding sequence, 3366 bp of coding sequence, and 143 bp of 3′ noncoding sequence including poly(A). The DNA Data Bank of Japan accession number for the nucleotide sequence of the cDNA is D87545.
11. For construction of the Ti plasmid carrying *CKI1* cDNA linked to the CaMV 35*S* RNA promoter, 157 bp of the 5′ noncoding sequence of cCKI1-16 was removed by treatment with exonuclease III, and all of the 3′ noncoding sequence was removed by digestion with Spe I (the stop codon is at the Spe I site). The remaining sequence, together with the 34-bp vector sequences flanking the 5′ end of the cDNA, was blunt-end cloned into the Sma I site of pMON530 [S. G. Rogers, H. J. Klee, R. B. Fraley, *Methods Enzymol.* **153**, 253 (1987)] next to the CaMV 35*S* RNA promoter, and the plasmid that contained the insert in the sense orientation was selected and designated p35ScCKI1.
12. Five micrograms of poly(A)$^+$ RNA was used for each lane.
13. The DNA region from the 5′ end of the Pst I site at nucleotide 2652 was used as a probe. Hybridization was done in 5× SSPE (750 mM NaCl, 5 mM EDTA, 50 mM sodium phosphate, pH 7.4) that contained sheared salmon sperm DNA (200 μg/ml) and 0.2% SDS at 68°C; washing was done in 0.5× SSPE containing 0.2% SDS at 65°C.
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26. 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.

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Quantitative Image Analysis of HIV-1 Infection in Lymphoid Tissue

Ashley T. Haase,* Keith Henry, Mary Zupancic, Gerald Sedgewick, Russell A. Faust, Holly Melroe, Winston Cavert, Kristin Gebhard, Katherine Staskus, Zhi-Qiang Zhang, Peter J. Dailey, Henry H. Balfour Jr., Alejo Erice, Alan S. Perelson

Tracking human immunodeficiency virus–type 1 (HIV-1) infection at the cellular level in tissue reservoirs provides opportunities to better understand the pathogenesis of infection and to rationally design and monitor therapy. A quantitative technique was developed to determine viral burden in two important cellular compartments in lymphoid tissues. Image analysis and in situ hybridization were combined to show that in the presymptomatic stages of infection there is a large, relatively stable pool of virions on the surfaces of follicular dendritic cells and a smaller pool of productively infected cells. Despite evidence of constraints on HIV-1 replication in the infected cell population in lymphoid tissues, estimates of the numbers of these cells and the virus they could produce are consistent with the quantities of virus that have been detected in the bloodstream. The cellular sources of virus production and storage in lymphoid tissues can now be studied with this approach over the course of infection and treatment.

Viral burden is a critical measure of the progress of HIV-1 infection (*1*), but there is as yet little information about the magnitude of infection in cellular compartments in lymphoid tissue (LT) where virus is produced and persists (*2–4*). A portion of the viral load in LT is associated with mononuclear cells (MNCs) such as CD4$^+$ T lymphocytes, monocytes, and macrophages in which viral RNA is readily detected. Virus production, and loss of CD4$^+$ T cells from the cytopathic effects of viral replication or elimination by immune surveillance, can be directly attributed to this population, and it is this population that should diminish in response to current antiretroviral treatments that block