pared from young adult male Sprague-Dawley rats. Slices were submerged in a stream (flow rate, 210 ml/hour) of artificial cerebrospinal fluid (ACSF) (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM glucose) maintained at room temperature (22° to 25°C) and gassed with 95% O_2 and 5% CO_2. The initial slope (1 to 2 ms) of field excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the Schaffer collateral–commissural afferents (once every 15 s) was measured in the stratum radiatum at a depth of 100 to 150 μm below the slice surface. The application and storage of the neurotrophic factors were as previously described (*4*). Anisomycin and cycloheximide were maintained as 40 mM stock solutions in ethanol at 4°C. The final concentration (0.1%) of ethanol to which the slices were exposed had no detectable effect on synaptic transmission. All experiments with protein synthesis inhibitors were paired with a same-day control experiment in which BDNF or NT-3 potentiated synaptic transmission. Inhibitors were applied at least 30 min before the addition of neurotrophin. BDNF and NT-3 potentiated synaptic strength at least 30% in 84.4 and 83.7% of control experiments, respectively. The percent of baseline measurements indicated in the text were obtained 170 to 180 min after the application of neurotrophin, unless otherwise noted. Ensemble average plots represent group means of each EPSP slope, for all experiments, aligned with respect to the time of neurotrophin application. Statistical significance was assessed by paired *t* tests or one-way analysis of variance; a *P* value of < 0.05 was considered statistically significant.

6. In vitro assays of protein synthesis inhibition in hippocampal slices were performed basically as previously described (*2*). Slices were individually maintained in 200 μl of ACSF in a 24-well tissue culture dish for 1 hour at room temperature. Either anisomycin (40 μM) or cycloheximide (40 μM) was added to each well, and, after 30 min, [^{3}H]leucine (20 μCi/ml) was introduced and the slice was incubated for an additional hour. The inhibitor and [^{3}H]leucine were washed out with ice-cold ACSF and the slices were homogenized. Protein synthesis was measured by incorporation of [^{3}H]leucine into trichloroacetic acid–precipitable material. When compared to controls, anisomycin and cycloheximide inhibited protein synthesis by 71.9 ± 5.2 and 71.2 ± 6.0%, respectively ($n = 4$ for each inhibitor).
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CRINKLY4: A TNFR-Like Receptor Kinase Involved in Maize Epidermal Differentiation

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The maize *crinkly4* (*cr4*) mutation affects leaf epidermis differentiation such that cell size and morphology are altered, and surface functions are compromised, allowing graft-like fusions between organs. In the seed, loss of *cr4* inhibits aleurone formation in a pattern that reflects the normal progression of differentiation over the developing endosperm surface. The *cr4* gene was isolated by transposon tagging and found to encode a putative receptor kinase. The extracellular domain contains a cysteine-rich region similar to the ligand binding domain in mammalian tumor necrosis factor receptors (TNFRs) and seven copies of a previously unknown 39–amino acid repeat. The results suggest a role for *cr4* in a differentiation signal.

The surface of plant organs is defined by a specialized epidermal cell layer. The leaf epidermis has essential functions in development, gas exchange, water retention, and defense against pathogens. In grass seeds, the endosperm contains an epidermis-like layer called aleurone, which is an important source of hydrolytic enzymes required for remobilization of stored starch and protein during germination. The recessive *cr4* mutation of maize affects the differentiation of both epidermis and aleurone. It was identified in a line containing *Mutator* transposable elements and mapped to chromosome 10S (*1*).

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Mutant *cr4* plants are short in stature and have crinkly leaves with a rough texture and dull luster (Fig. 1A). The leaves adhere to one another, which inhibits unfurling as the leaves grow, causing contortion of the plant. Floral organs including glumes and anthers are also affected, but the roots appear normal. Histological analysis indicated that the defect was predominantly epidermal. Normal epidermis comprises a variety of cell types arranged in longitudinal files (2) (Fig. 1, B and D). Epidermal cells are rectangular with a smooth surface and interlocking crenulate sidewalls. Stomata occur in files, and prickle hairs and macrohairs are associated with rows of bulliform cells. The mutant contains a nearly normal array of epidermal cell types but displays irregular cellular morphology (Fig. 1C). The cells are abnormally large and have irregular surfaces covered with a granular material. Adjacent cells interlock less, and the crenulations sometimes occur on the surface wall. In some plants, areas of abnormal cells are interspersed with normal areas. Stomates are often in crevices formed by the overexpansion of neighboring cells.

The internal anatomy was relatively unaffected in mutant leaves (Fig. 1E) except when leaves were severely distorted. In some places the epidermis was more than one cell thick, and in regions of adherence, interconnecting bridges of tissue appeared to arise from the fusion and proliferation of epidermal cells (Fig. 1F). Two epidermal attributes that normally prevent organ fusions during development include restriction of cell division to the anticlinal plane such that daughter cells remain in the same cell layer (3), and terminal differentiation that prevents epidermal dedifferentiation or participation in graft unions (4). Both characteristics

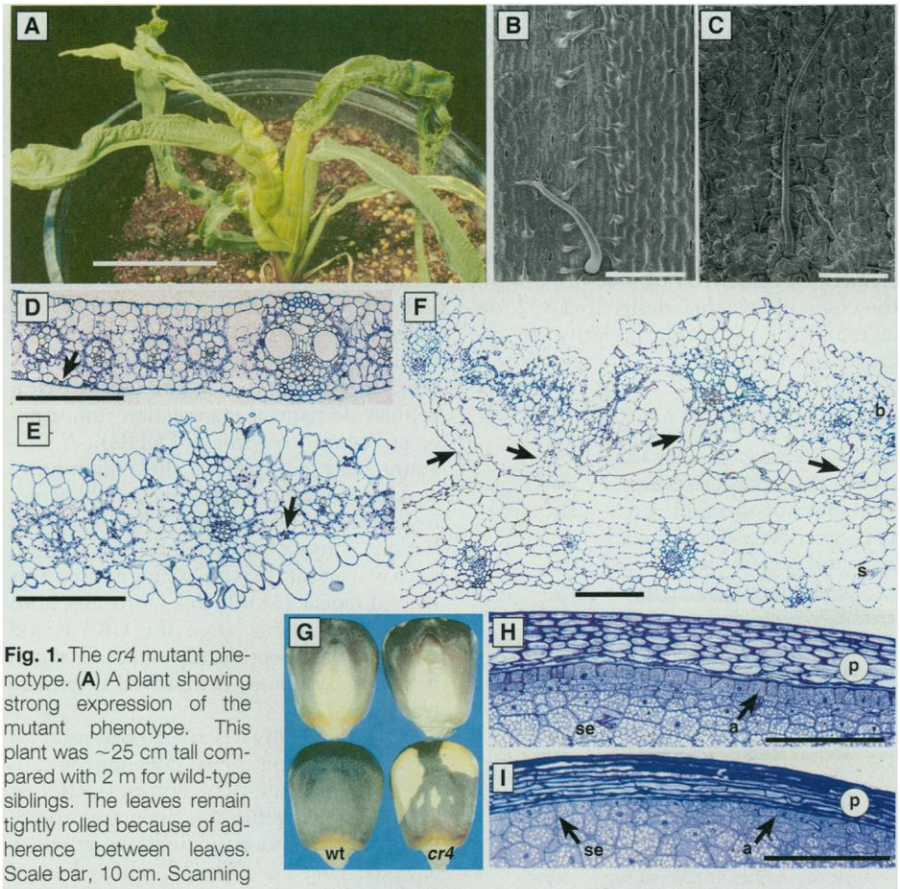

Fig. 1. The *cr4* mutant phenotype. (**A**) A plant showing strong expression of the mutant phenotype. This plant was ~25 cm tall compared with 2 m for wild-type siblings. The leaves remain tightly rolled because of adherence between leaves. Scale bar, 10 cm. Scanning electron micrographs of the adaxial epidermis of (**B**) wild-type and (**C**) *cr4* mutant leaves. The mutant contains a normal complement of cell types but displays irregular cellular morphology. (**D**) Transverse section of a wild-type leaf. The internal tissue displays typical kranz anatomy. The epidermis is an even layer of small, thick-walled, cuboidal cells, covered by a cuticle and interspersed with specialized cells such as stomates (arrow). (**E**) A section through a leaf showing a strong mutant phenotype. The internal tissue of the mutant is relatively normal, whereas the epidermal cells are abnormally large and irregularly shaped with thin cell walls and cuticle. In places the epidermis is more than one cell thick. The arrow denotes a stomate located internal to several epidermal cells. (**F**) Transverse section through a region of adherence between the sheath portion of one leaf (s) below and the blade portion of the subsequent leaf (b) on top. The epidermises of the two organs have fused and grown interconnecting bridges of tissue (arrows). Scale in (B) to (F), 225 μm. (**G**) Kernel phenotype. On the left are germinal (top kernel) and abgerminal (bottom kernel) views of wild-type kernels. The purple aleurone forms a continuous layer over the endosperm except at the embryo. On the right are two *cr4* mutant kernels displaying abgerminal mosaicism. (**H**) Section of wild-type endosperm. The kernel is covered by maternal pericarp tissue (p). Most of the endosperm is starchy endosperm (se) composed of large irregularly shaped cells with prominent starch grains (unstained bodies). The aleurone (a) is a single layer at the surface of the endosperm with densely staining, cuboidal cells that lack starch grains. (**I**) A section from a *cr4* mutant kernel at the border between pigmented and unpigmented regions. The pigmented area on the left contains cells characteristic of aleurone. Cells at the endosperm surface of the unpigmented region on the right are characteristic of starchy endosperm (se). Scale bar in (H) and (I), 225 μm.

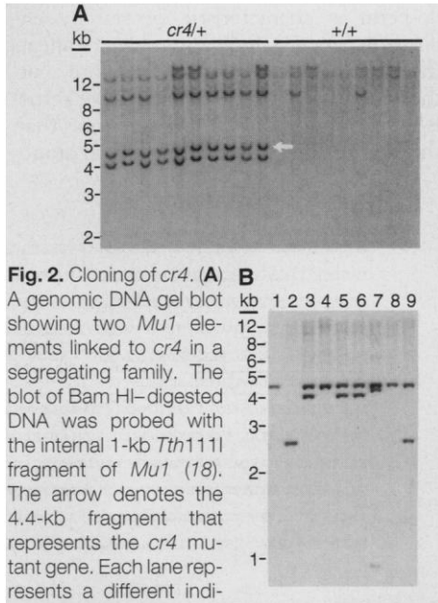

Fig. 2. Cloning of *cr4*. (**A**) A genomic DNA gel blot showing two *Mu1* elements linked to *cr4* in a segregating family. The blot of Bam HI–digested DNA was probed with the internal 1-kb *Tth*111I fragment of *Mu1* (18). The arrow denotes the 4.4-kb fragment that represents the *cr4* mutant gene. Each lane represents a different individual. (**B**) Genomic DNA gel blot of isolated *cr4* alleles. DNA was digested with Bam HI, and the blot was probed with a genomic fragment flanking the *Mu1* insert in λ37. Six of the seven mutants had detectable DNA alterations relative to the wild-type progenitor. The other linked clone detected no alterations (not shown), indicating that λ37 represents the *cr4* locus. Lane 1 contains DNA from the original *cr4-R* mutant male parent, lane 2 is the female *Mu* line containing a wild-type *cr4* allele, and lanes 3 through 9 represent the seven mutant F_1 plants. The male and all the F_1 plants contained a 4.4-kb fragment corresponding to the *cr4-R* allele. The *Mu* line contained a 2.5-kb fragment representing the wild-type progenitor. The *cr4-30* (lane 3), *cr4-58* (lane 5), and *cr4-60* (lane 6) alleles contained a ~1.4-kb insertion, and *cr4-98* (lane 7) has a 2.6-kb insertion with an internal Bam HI site. Alleles *cr4-41* and *cr4-121* (lanes 4 and 8) are deletions. No difference has yet been detected between the *cr4-127* (lane 9) allele and the progenitor, suggesting that it is a point mutation or small alteration.

appear compromised in the mutant, indicating that *cr4* is important for the acquisition of these traits in epidermal cells. Thus, *cr4* is required for proper specification of the surface of leaf-like organs. Epidermal fusions occur in several other mutants (*5*) and in specialized situations such as postgenital carpel fusion (*6*), and it will be interesting to test whether *cr4* is involved.

In kernels, aleurone differentiation is marked by a transition to exclusively anticlinal cell division in the outer cell layer. This transition initiates near the embryo and progresses over the endosperm surface for a period of several days (*7*). In appropriate genotypes, the aleurone accumulates anthocyanin pigments late in seed development (Fig. 1G). About 10% of the *cr4* mutant seed are mosaic for pigmented and unpigmented regions (Fig. 1G). The unpigmented regions do not contain aleurone cells, and the outer cell layer of the endosperm is characteristic of starchy endosperm (Fig. 1I), indicating that *cr4* affects the acquisition of aleurone cell fate. Furthermore, the abgerminal face of the kernel is much more likely to lack aleurone than the germinal face. Thus, the pattern of mosaicism reflects the temporal pattern of aleurone differentiation and is distinct from the cell lineage pattern commonly revealed by clonal sectors (*8*).

The *cr4* mutation arose in a line containing active *Mutator* (*Mu*) transposons. From genomic DNA blot analysis of segregating populations we identified two *Mu1* elements that cosegregated with the mutation (Fig. 2A). Attempts to isolate recombinants between either element and the *cr4* mutation were unsuccessful, so genomic fragments containing each element were cloned. A directed transposon mutagenesis was performed to identify the fragment representing the *cr4* gene. The original *cr4* mutant (*cr4-R*) was crossed as a male to wild-type *Mu* lines and the F_1 generation screened for mutant seedlings. Mutant F_1 plants indicated a new mutant *cr4* allele inherited from the *Mu* female. We isolated seven mutant plants and subjected them to genomic DNA blot analysis using fragments flanking the cloned *Mu1* elements (Fig. 2B). The probe from one of the cloned fragments, designated λ37, detected DNA alterations relative to the progenitor allele in six of the seven cases, indicating that this cloned fragment corresponds to the *cr4* locus. Furthermore, the new alleles display both aleurone and epidermal phenotypes, confirming that both phenotypes are conferred by a single gene and not closely linked mutations (*9*).

The same probe recognized a 3.4-kb transcript on RNA gel blots from shoot tissue (*9*) and was used to isolate cDNAs from a seedling shoot library. A 3.1-kb cDNA contained the entire coding sequence. A 2.2-kb clone was truncated at the 5′ end but was polyadenylated about 300 bases 3′ of the first. Together the two cDNAs represent 3.4 kb, a nearly complete transcript. Stop codons indicate that no coding sequence occurs 5′ to the designated start codon.

The cDNA sequence revealed a 901–amino acid open reading frame (Fig. 3). Homology searches showed that the CR4 protein has characteristics of a receptor kinase. The carboxy domain shows strong homology to the catalytic domain of serine-threonine protein kinases. The presumptive extracellular domain contains seven copies of a 39–amino acid repeat motif. Proximal to the repeats is a cysteine-rich region that resembles the extracellular domain of mammalian tumor necrosis factor receptors (TNFRs). A 26–amino acid motif within this region has a striking similarity to the second cysteine-rich repeat of mammalian TNFR (*10*). All but one contact between TNFR and tumor necrosis factor (TNF) occur within this second repeat (*11*). If this motif functions similarly in plants, then the CR4 ligand may also be a peptide. One peptide signal in plants, systemin, is involved in disease response (*12*).

Most previously described plant receptor kinases have extracellular domains containing either leucine-rich repeats (LRRs) or homology to the *Brassica* S-locus glycoprotein (*13*). Exceptions include the ethylene receptor (*14*) and PRO25, which has an epidermal growth factor repeat (*15*). With its novel extracellular domain, CR4 identifies another class of receptor kinases. Although receptor kinases are known to regulate development in animals (*16*), CR4 is one of few known to function in plant development (*17*).

There are two possible relations between *cr4* function in the leaf epidermis and the aleurone. Both tissues occur on the surface of their respective organs, grow by anticlinal cell division, and contain morphologically similar cells with cuboidal shape and thick walls. The mutant phenotype affects aspects of these characteristics in both tissues; thus, *cr4* might function in a pathway common to both tissues. Alternatively, CR4 could perform different regulatory

A

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MDHVPALVLAGCCFLALLPGWACGLGSMSSIAVSYGEDGPVFCGLNSDGSHLVACFGADASVLYGAPPNIPFLGL    75
TAGDGFVCGLLLDTRQPYCWGSNSYVKSGVPQPMVEGARYSELSAGDNHLCALRAAQDGGRGSSAATSLIDCWGY   150
NMTATHAVDEAVSTVSAGSVFNCGLFARNRTVFCWGDETVSGVVGLAPRDLHFQSIGAGGYHVCGVLENAQVFCW   225
GRSLEMQQVVPSSAIGDGDVNIVPMDAMSTVVGGRFHACGIRSLDHQVACWGFTLHNSTSPPKGLKMYALVAGDY   300
FTCGVPAETSLMPRCWGNSGPLALPMAVPPGICVPTACSHGYYEYVNHGEVGSIKVCKPANSRICLPCSTGCPEG   375
LYESSPCNATADRVCQFDCLKCVTDECLSFCLSQKRTKSRKLMAFQMRIFVAEIVFAVVLVLSVSVTTCLYVRHK   450
LRHCQCSNRELRLAKSTAYSFRKDNMKIQPDMEDLKIRRAQEFSYEELEQATGGFSEDSQVGKGSFSCVFKGILR   525
DGTVVAVKRAIKASDVKKSSKEFHNELDLLSRLNHAHLLNLLGYCEDGSERLLVYEFMAHGSLYQHLHGKDPNLK   600
KRLNWARRVTIAVQAARGIEYLHGYACPPVIHRDIKSSNILIDEDHNARVADFGLSILGPADSGTPLSELPAGTL   675
GYLDPEYYRLHYLTTKSDVYSFGVVLLEILSGRKAIDMQFEEGNIVEWAVPLIKAGDIFAILDPVLSPPSDLEAL   750
KKIASVACKCVRMRGKDRPSMDKVTTALEHALALLMGSPCIEQPILPTEVVLGSSRMHKVSQMSSNHSCSENELA   825
DGEDQGIGYRAPSWITFPSVTSSQRRKSSASEADIVGRRATDGRNVGSSIGDGLRSLEEEIAPASPQENLYLQHN   900
F                                                                               901
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B

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          VSYGEDGPVF-CGLNSDGSHLVACFGADASV--LYGAPP
          FLGLTAGDGFVCGLLLDT-RQPYCWGSNSYV--KSGVPQ
          LCALRAAQDGGRGSSAATS-LIDCWGYNMTA--THAVDE
          VSTVSAGSVFNCGLFA-RNRTVFCWG-DETV---SGVVG
          FQSIGAGGYHVCGVLE--NAQVFCWGRSLEM---QQVVP
          MSTVVGGRFHACGIRSLD-HQVACWGFTLHN---STSPP
          MYALVAGDYFTCGVPAETSLMPRCWGNSGPLALPMAVPP
Consensus: VS.L.AG.VF.CGL.A.TS..V.CWG....V---SGVPP
          F  V    Y    V                      V
          M
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C

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TNFR-#1   KCGGHDYEKDGLCCAS-CHPGFYASRLCG-PGSNTVCS
CR4       ----------GICVPTACSHGYYEYVNHGEVGSIKVC-

TNFR-#2   PCEDGTFTASTNHAPACVSCRGPCTGHLSESQPCDRTHDRVC
CR4       ---------KPANSRICLPCSTGCPEGLYESSPCNATADRVC

TNFR-#3   NCSTGNYCLLK-GQNGCR-ICAPQTKCPAGYGVSGHTRAGDTLCE
CR4       ----QFDCL-KCVTDECLSFCLSQKRTSRK--------------
```

Fig. 3. (**A**) Predicted amino acid sequence of the CR4 protein (GenBank accession number U67422). The NH_2-terminus contains a 21–amino acid predicted signal peptide (italics), and a 24–amino acid transmembrane domain occurs between residues 427 and 450 (double underlined). From residue 481 to 778 is a 298–amino acid region with homology to the catalytic domain of protein kinases (bold). The putative extracellular domain contains seven repeats of a previously unknown 39–amino acid motif [single underline, see (B)] and a region with similarity to the cysteine-rich repeats of TNFR [see (C)]. The solid boxed region is the 26–amino acid motif that was detected in BLAST searches as similar to TNFR, and the dashed boxes denote the extended region of TNFR similarity. (**B**) Alignments of the extracellular 39–amino acid repeats. Highly conserved amino acids are shown in bold, and consensus amino acids are below. (**C**) Homology between CR4 and the cysteine-rich repeats (TNFR-#1, #2, and #3) of Shope fibroma virus TNFR. Conserved cysteines are shaded. 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.

functions in the two tissues, as occurs with receptor kinases in other systems (*16*). The findings that *cr4* encodes a receptor kinase and that the mutant affects the nonclonal progression of aleurone differentiation imply that cell interactions are involved in the differentiation of aleurone and epidermis and suggest *cr4* may function in a differentiation signal. The mutant phenotype and the similarity to a known ligand binding domain will provide advantages for identifying other components of the *cr4* signal transduction chain.

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Similarity of a Chromatic Adaptation Sensor to Phytochrome and Ethylene Receptors

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Complementary chromatic adaptation in cyanobacteria acts through photoreceptors to control the biosynthesis of light-harvesting complexes. The mutant FdBk, which appears black, cannot chromatically adapt and may contain a lesion in the apparatus that senses light quality. The complementing gene identified here, *rcaE*, encodes a deduced protein in which the amino-terminal region resembles the chromophore attachment domain of phytochrome photoreceptors and regions of plant ethylene receptors; the carboxyl-terminal half is similar to the histidine kinase domain of two-component sensor kinases.

The phycobilisomes (PBS), macromolecular complexes in cyanobacteria that are peripherally associated with thylakoid membranes, harvest light energy in the visible region of the electromagnetic spectrum between 540 and 660 nm and efficiently transfer that energy to the photosynthetic reaction centers within the thylakoid membranes (*1*). The PBS are composed of two types of proteins, pigmented phycobiliproteins and nonpigmented linker polypeptides. In the filamentous cyanobacterium *Fremyella diplosiphon*, the major species of phycobiliproteins are allophycocyanin (AP), phycocyanin (PC), and phycoerythrin (PE). Linker polypeptides associate with phycobiliproteins and are important for assembly, stability, and efficient energy transfer within the PBS and to the photosynthetic reaction centers (*2*).

As noted almost a century ago (*3*), the pigmentation of cyanobacteria can change in response to light quality; these changes reflect an altered pigment-protein composition of the PBS. In *F. diplosiphon*, red light causes the synthesis of large amounts of the blue chromoprotein PC and small amounts of the red chromoprotein PE, making the color of the organism blue-green. Conversely, in green light the organism has small amounts of PC and large amounts of PE, making it red in color. This change in PBS pigment-protein composition in response to different wavelengths of light is called complementary chromatic adaptation (CCA); it affords the cyanobacterium an adaptive advantage as the light quality of the environment changes because PC effectively absorbs red light and PE effectively absorbs green light. The differences in composition of the PBS in cells grown in red compared with green light are mostly a reflection of differential transcriptional activities of a specific set of red light–inducible PC genes, designated *cpcB2A2* (the protein designation is PCi, for inducible PC) and green light–inducible PE genes, designated *cpeBA* (*4*). The genes encoding linker polypeptides associated with PCi and PE are also regulated by light quality (*5*).

Classes of CCA mutants (*6–10*) that appear red (FdR), blue (FdB), green (FdG), or black (FdBk) have been characterized. FdR strains always express PE and cannot express PCi; they are always red. FdB strains appear bluer than wild-type cells and are less sensitive to light quantity; it takes a greater fluence rate to suppress PCi synthesis in FdB strains than in wild-type cells (*11*). In FdG strains PCi expression is normal, but PE genes are never activated. Finally, FdBk mutants have moderate amounts of both PE and PCi; these amounts are constant in red and green light (*10*).

The gene *rcaC* (regulator of chromatic adaptation) (*9*), which complements some FdR mutants, encodes a polypeptide with similarity to a bacterial response regulator. Response regulators are elements of two-component regulatory systems, which control a wide range of responses to environmental cues in both prokaryotes and eukaryotes (*12, 13*). The RcaC polypeptide (73 kD) is larger than most response regulators and has two conserved receiver domains, one at the COOH-terminus and the other at the NH_2-terminus, each with a putative aspartate phosphorylation site.

Here we identify the DNA fragment (on plasmid pDK1) capable of complementation of one of the FdBk mutants (Fig. 1). It includes open reading frames (ORFs) I, II, and III. ORFIII is truncated in the region encoding the NH_2-terminus of the putative protein. Subclones were modified (Fig. 1) from pDK1 and used for further complementation tests.

Results of complementation studies with each of the modified clones are shown in Fig. 2. The FdBk strain has the same pigmentation in red and green light. When transformed with pDK2 or pDK4, normal CCA is restored. pDK3 cannot complement the mutant phenotype, although it rescues another class of CCA mutants and therefore must produce functional ORFII protein (*14*). Spectral measurements demonstrate

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