and variable abundances. The HREE patterns are relatively flat, and, although the overall abundance changes, the patterns remain similar within an individual mine. However, in South Crofty the HREE are apparently concentrated in the later fluids, whereas at Wheal Jane the converse is true, the explanation of which is currently unclear.

The variation in REE patterns cannot be attributable to a mixing of fluids from different sources in the vein systems because this would result in variable initial ϵ_{Nd} values. It is also difficult to envisage why the HREEs should maintain such consistent patterns if mixing is responsible for changes in the LREEs. Leaching of uraninite or other HREE-rich minerals would not only fractionate the REEs but would also increase the relative abundance of all HREEs in the mineralizing fluid. Similarly, leaching of LREE-enriched phases, such as monazite, could not produce the depletion in the LREEs observed in the paragenetically later fluorite.

These relations lead us to conclude that the most likely mechanism for producing the observed change in the Sm-Nd composition of the fluid is the coeval precipitation of an unidentified LREE-enriched phase, analogous to monazite, in the hydrothermal system. The dramatic crossover in the REE element patterns is good evidence for the importance of such a process at South Crofty. The extent to which such a model can be extended to account for the Sm-Nd fractionation observed in many other kinds of fluorite-bearing mineralization (*7, 9, 23, 24*), such as MVT deposits, is uncertain.

Regardless of the exact mechanism, REE fractionation in fluorites is commonly observed. The large range in Sm/Nd ratios, the low uncertainty in age, and resistance to late hydrothermal effects render the Sm-Nd dating technique a powerful geochronometer even for relatively young mineralization.

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25. Standard ion-exchange procedures were used to separate the REEs (*26*). All concentration measurements were performed by isotope dilution at the University of Michigan on a VG sector mass spectrometer (*26*). An average $^{143}Nd/^{144}Nd$ ratio of 0.511862 ± 10 was obtained for the La Jolla standard. Ages were calculated with model 1 regressions on Isoplot (*27*). The decay constant $\lambda\ ^{147}Sm = 6.54 \times 10^{-12}$ year^{-1} was used for age calculations. We calculated the ϵ_{Nd} values using present-day values for bulk earth of $^{143}Nd/^{144}Nd = 0.512636$ and $^{147}Sm/^{144}Nd = 0.1967$.
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29. We thank M. Owen, P. Gribble, and S. Speed of Carnon Consolidated Limited for access to the South Crofty and Wheal Jane mines and for geologic expertise during sample collection. S. Nakai provided expertise during REE analysis. We thank T. J. Shepherd, W. C. Kelly, S. F. Kesler, K. Mezger, J. R. O'Neil, and D. P. F. Darbyshire for discussion and critical review of the original manuscript. Research was supported by National Science Foundation grants EAR 8616061, 8804072, and 9004413 to A.N.H. This paper is published with the approval of the Director of the British Geological Survey (Natural Environment Research Council).

13 November 1990; accepted 28 February 1991

A Calcium-Dependent Protein Kinase with a Regulatory Domain Similar to Calmodulin

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Calcium can function as a second messenger through stimulation of calcium-dependent protein kinases. A protein kinase that requires calcium but not calmodulin or phospholipids for activity has been purified from soybean. The kinase itself binds calcium with high affinity. A complementary DNA clone for this kinase has been identified; it encodes a protein with a predicted molecular mass of 57,175 daltons. This protein contains a catalytic domain similar to that of calmodulin-dependent kinases and a calmodulin-like region with four calcium binding domains (EF hands). The predicted structure of this kinase explains its direct regulation via calcium binding and establishes it as a prototype for a new family of calcium-regulated protein kinases.

IN PLANTS, FREE CALCIUM (Ca^{2+}) HAS been implicated as a second messenger in diverse processes including cytoplasmic streaming and signal transduction (*1*). Increased concentrations of intracellular Ca^{2+} have been shown to regulate stomatal closure (*2*). In animals, Ca^{2+} can regulate signal transduction pathways by activating protein kinases dependent on Ca^{2+} and calmodulin (Ca^{2+}/calmodulin) (*3*). Calmodulin is present in plant cells (*4*) and some evidence that plant cells also contain Ca^{2+}/calmodulin-dependent kinase activity has been presented (*5*). However, a Ca^{2+}-dependent serine-threonine protein kinase has been purified from soybean (*Glycine max* L. cv Wayne) that does not require calmodulin. This Ca^{2+}-dependent protein kinase (CDPK) shows half-maximal stimulation at 2 μM Ca^{2+} and the enzyme itself contains a high affinity Ca^{2+} binding site or sites (*6, 7*).

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We have identified and sequenced cDNA clones encoding CDPK. Peptides from purified CDPK were sequenced and used to design degenerate DNA oligomers (Fig. 1). These oligomers were used as polymerase chain reaction (PCR) primers to amplify a 151-bp (base pair) DNA fragment from the soybean genome. This fragment was cloned and labeled for use as a hybridization probe to identify two partial cDNA clones, SK2 and SK5, from a soybean (*G. max* L. cv Williams) cDNA library (*8*). To obtain the full-length cDNA sequence for the SK5 clone (Fig. 1), the missing 3′ end was amplified by PCR and the sequence was verified from three independent clones.

The predicted SK5 protein sequence (Fig. 1) was identified as the longest open reading frame spanning the cDNA sequence. Termination codons are present upstream of the predicted initiation codon in all three reading frames. The chosen initiation codon is preceded by a short open reading frame encoding a predicted peptide of 12 residues. Short upstream reading frames have been observed in several plant genes (*9*) and may be important for translational regulation (*10*). The molecular mass of the SK5 encoded protein is predicted to be 57,175 daltons which is close to the molecular mass of 52,000 to 55,000 daltons determined by chromatographic and electrophoretic analysis of purified CDPK (*7*).

Correspondence of the full-length SK5 cDNA to soybean CDPK is based on similarity between the predicted amino acid sequence of the SK5 protein and the amino acid sequence of peptides obtained from purified CDPK. An alignment between SK5 and three proteolytic peptides from CDPK shows a correspondence of 7 of 14 identical amino acids for a peptide located outside the catalytic domain, and 15 of 20 and 23 of 33 identical amino acids, respectively, for two peptides located within the predicted kinase domain. The strong, but less than complete correspondence, suggests the presence of multiple CDPK isozymes. Isozymes of Ca^{2+}/calmodulin–dependent protein kinase II and protein kinase C are also encoded by distinct genes (*11*). Further evidence of multiple CDPK isoforms in soybean is provided by the isolation of another related cDNA clone, SK2. The predicted amino acid sequence of SK2 contains regions with similarity to the proteolytically derived peptide sequences, but the substitutions occur at different positions from those in the SK5 sequence (*12*). Southern blot analysis of soybean genomic DNA reveals multiple fragments hybridizing with a DNA probe derived from the SK5 cDNA (Fig. 2) and is consistent with the presence of multiple CDPK isoforms in soybean. A cDNA from *Arabidopsis* has also been characterized and shows strong similarity to the same CDPK peptide sequences and corroborates the same overall structure seen in SK5 and SK2 (*12*).

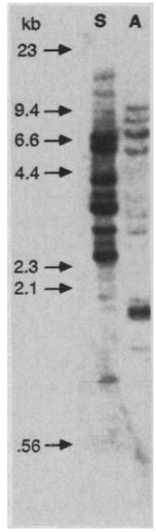

Fig. 2. Southern blot analysis of genomic DNA. Samples (5 μg) of genomic DNA from *G. max* L. cv Williams (lane S) and *Arabidopsis thaliana* cv Columbia (*9*) (lane A) were digested with Bam HI and Eco RI and subjected to electrophoresis through a 1% (w/v) agarose gel. DNA was transferred, and hybridizations were done with high stringency as described (*9*). The hybridization probe was a ^{32}P-labeled PCR fragment corresponding to nucleotides 230 to 1319.

```
                                                          CGGTTAATTTG   11
TTGAGAGAGAGCAATCAAAAACATTCATGGCGTCTTCCAAGGAGAGAGAAGAAAACACCA   71
                                M  A  S  S  K  E  R  E  E  N  T  N
ATTAACCACCACCTGATAGGCTGATACAATACAATCTCCTTTCCTTTTTCGACAACAAGA  131
ATGGCTGCGAAATCTAGTTCGAGTTCGACAACAACAAACGTGGTGACTCTGAAGGCGGCG  191
M  A  A  K  S  S  S  S  S  T  T  T  N  V  V  T  L  K  A  A
TGGGTTCTCCCGCAGCGCACGCAGAACATCCGTGAGGTGTACGAGGTTGGGCGGAAGCTC  251
W  V  L  P  Q  R  T  Q  N  I  R  E  V  Y  E  V  G  R  K  L
                  k  D  P  N  I  R  D  L  Y  T  L  G  R  K
GGGCAGGGCCAATTCGGGACCACCTTCGAGTGCACGCGCCGTGCGAGTGGTGGGAAGTTC  311
G  Q  G  Q  F  G  T  T  F  E  C  T  R  R  A  S  G  G  K  F
GCGTGCAAGTCGATTCCGAAGCGGAAGCTGCTGTGCAAGGAGGACTACGAGGACGTGTGG  371
A  C  K  S  I  P  K  R  K  L  L  C  K  E  D  Y  E  D  V  W
                                       k  -  d  V  E  D  V  R
CGGGAGATTCAGATAATGCACCACTTGTCGGAACACGCCAACGTTGTCCGCATCGAAGGG  431
R  E  I  Q  I  M  H  H  L  S  E  H  A  N  V  V  R  I  E  G
R  E  I  Q  I  M  H  H  L  A  G  H  K                  k  -
ACGTACGAGGATTCCACGGCCGTGCACCTGGTCATGGAGTTGTGCGAGGGTGGAGAGTTG  491
T  Y  E  D  S  T  A  V  H  L  V  M  E  L  C  E  G  G  E  L
a  Y  E  D  P  L  Y  V  H  I  V  M  E  L  C  F  G  R  E  L
TTTGACAGGATCGTGCAGAAGGGACACTACAGCGAGAGACAGGCGGCGAGGTTGATAAAG  551
F  D  R  I  V  Q  K  G  H  Y  S  E  R  Q  A  A  R  L  I  K
F  D  R  I  I  Q  R  G  H  Y  T  E
ACGATTGTTGAGGTTGTTGAGGCGTGTCACTCGCTAGGGGTCATGCATAGGGACCTTAAG  611
T  I  V  E  V  V  E  A  C  H  S  L  G  V  M  H  R  D  L  K
CCTGAGAATTTCTTGTTTGATACCATTGATGAGGATGCTAAGCTTAAGGCCACCGATTTC  671
P  E  N  F  L  F  D  T  I  D  E  D  A  K  L  K  A  T  D  F
GGCTTGTCTGTTTTTTACAAGCCTGGTGAATCCTTTTGTGATGTTGTCGGGAGCCCATAC  731
G  L  S  V  F  Y  K  P  G  E  S  F  C  D  V  V  G  S  P  Y
TATGTCGCACCAGAGGTCTTGCGCAAGCTCTATGGACCTGAATCAGATGTTTGGAGTGCA  791
Y  V  A  P  E  V  L  R  K  L  Y  G  P  E  S  D  V  W  S  A
GGGGTTATTCTGTACATCTTATTAAGTGGGGTGCCACCATTTTGGGCCGAATCTGAACCG  851
G  V  I  L  Y  I  L  L  S  G  V  P  P  F  W  A  E  S  E  P
GGGATCTTCCGACAGATTTTACTAGGAAAACTTGATTTTCATTCTGAGCCTTGGCCTAGC  911
G  I  F  R  Q  I  L  L  G  K  L  D  F  H  S  E  P  W  P  S
ATTTCAGACAGTGCCAAGGATCTAATTCGGAAAATGCTTGATCAAAATCCAAAAACAAGG  971
I  S  D  S  A  K  D  L  I  R  K  M  L  D  Q  N  P  K  T  R
CTTACAGCACATGAAGTACTCCGCCACCCATGGATTGTTGATGACAACATTGCACCTGAT 1031
L  T  A  H  E  V  L  R  H  P  W  I  V  D  D  N  I  A  P  D
AAACCTCTTGATTCTGCAGTTTTATCACGTCTGAAACAATTCTCTGCCATGAATAAATTG 1091
K  P  L  D  S  A  V  L  S  R  L  K  Q  F  S  A  M  N  K  L
AAAAAGATGGCATTGCGTGTTATTGCTGAGAGGCTATCTGAAGAAGAAATTGGTGGACTG 1151
K  K  M  A  L  R  V  I  A  E  R  L  S  E  E  E  I  G  G  L
AAAGAGTTATTCAAGATGATTGACACAGACAACAGTGGAACCATAACGTTTGATGAGTTA 1211
K  E  L  F  K  M  I  D  T  D  N  S  G  T  I  T  F  D  E  L
AAAGATGGTTTGAAGCGAGTAGGATCTGAACTTATGGAGTCTGAAATCAAGGATCTTATG 1271
K  D  G  L  K  R  V  G  S  E  L  M  E  S  E  I  K  D  L  M
GATGCTGCGGATATTGATAAAAGTGGGACAATTGATTATGGTGAATTCATTGCTGCCACT 1331
D  A  A  D  I  D  K  S  G  T  I  D  Y  G  E  F  I  A  A  T
GTTCATTTAAATAAGCTGGAGAGAGAGGAAAACCTAGTGTCGGCCTTCTCCTATTTTGAC 1391
V  H  L  N  K  L  E  R  E  E  N  L  V  S  A  F  S  Y  F  D
AAAGATGGCAGTGGTTACATAACCCTTGATGAGATACAACAAGCTTGTAAGGACTTTGGT 1451
K  D  G  S  G  Y  I  T  L  D  E  I  Q  Q  A  C  K  D  F  G
TTAGATGATATCCATATTGACGACATGATCAAGGAAATTGATCAAGATAACGATGGGCAA 1511
L  D  D  I  H  I  D  D  M  I  K  E  I  D  Q  D  N  D  G  Q
ATAGATTATGGGGAATTTGCTGCCATGATGAGAAAGGGCAATGGAGGAATTGGAAGGCGA 1571
I  D  Y  G  E  F  A  A  M  M  R  K  G  N  G  G  I  G  R  R
ACTATGAGAAAAACACTAAATTTAAGAGATGCTCTTGGATTAGTAGACAATGGGTCCAAT 1631
T  M  R  K  T  L  N  L  R  D  A  L  G  L  V  D  N  G  S  N
CAAGTTATTGAGGGCTACTTTAAGTAATAATTACTTGGAGACAAAATTGACCAAATATTA 1691
Q  V  I  E  G  Y  F  K  .  .
ATTTAGTAAATTTCTACGTTGTTTGTAGTTGCTTGACCAAATTTTGCGTATCTTTTAAAT 1751
TATAGACCCACTTCAGG  1768
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Fig. 1. Nucleotide sequence of cDNA and predicted protein sequence for soybean CDPK isoform SK5. Amino acid sequence of three proteolytic peptides derived from CDPK are shown in bold letters under the predicted SK5 protein sequence. Lower case letters are tentative assignments. CDPK was purified from *G. max* L. cv Wayne as described (*7*). Purified CDPK (800 pmol) was cleaved at lysine residues with *Achromobacter* protease I (*23*). The digest was *S*-pyridylethylated and fractionated by size exclusion high-performance liquid chromatography (HPLC) on a TSK G3000 PW column equilibrated in a solution of trifluoroacetic acid (0.1%) and acetonitrile (45%) in water. Peptides were resolved further by reversed-phase HPLC on a RP-300 column and sequenced by Edman degradation. A 1319-bp cDNA encoding the 5′ region of SK5 was identified from a library of *G. max* cv. Williams cDNA (*8*) by hybridization (*9*) with a 151-bp fragment of soybean CDPK that was amplified by PCR with two degenerate primers based on the underlined peptide sequence. The 1319-bp cDNA was subcloned and called pSK5. The pSK5 contains the T3 promoter followed by an Eco RI linker, the 5′ end and sequence encoding the first 399 amino acid residues, an Eco RI site truncating the 3′ end, and the T7 promoter. The missing 3′ end of the SK5 cDNA was isolated by PCR amplification (*24*) with cDNA template from *G. max* L. cv Mandarin RNA. The primers used were oligo(dT) with an Xho I linker (*24*) and a DNA sequence between nucleotides 1239 and 1263 with a Bam HI linker. The 3′ sequence was based on clone p899. Two additional clones showed polyadenylate termination sites at positions 1718 and 1730, respectively. All three clones showed 100% DNA sequence identity to pSK5 over a 56-bp region. Both strands were sequenced (Sequenase Kit, U.S. Biochemical). The translation start codon begins at nucleotide position 132; the 508 residue protein sequence is shown below. A short upstream reading frame from nucleotides 38 to 76 is underlined and the predicted 12-residue peptide indicated below.

The deduced SK5-encoded protein sequence contains conserved sequences diagnostic of protein kinase catalytic domains (*13*) between residues 41 and 328. This domain includes a nucleotide binding site and two regions characteristic of serine-threonine kinases (Fig. 3). A search of the GenBank database revealed that this kinase domain is most closely related (39% identity in a 296–amino acid overlap) to the catalytic domain of the β-subunit of the Ca^{2+}/calmodulin–dependent kinase II from rat brain (Fig. 3) (*14*).

Adjacent to the kinase domain is a calmodulin-like sequence with 39% amino acid identity to calmodulin from spinach (Fig. 3) (*16*). The calmodulin-like region contains four putative EF-hand Ca^{2+}-binding motifs (Fig. 3) (*15*), each of which contains all the requirements of such Ca^{2+}-binding sites: six oxygen-containing ligands at positions 1, 3, 5, 7, 9, and 12, an invariant glycine residue at position 6, and a conserved aliphatic residue at position 8. Each of the four Ca^{2+}-binding sites is flanked by residues predicted to form helices (*16*), as expected in a Ca^{2+}-binding EF hand. The presence of four such Ca^{2+}-binding sites explains the observation that Ca^{2+} directly binds to CDPK and regulates its activity.

There are several distinctions between the amino acid sequence of the CDPK calmodulin-like domain and plant calmodulin. CDPK has a tyrosine rather than a phenylalanine residue at position 428, and a threonine instead of a cystine residue at position 356. The CDPK substitutions are characteristic of animal calmodulins (*17*). Also distinct is the spacing between the last three Ca^{2+}-binding sites in CDPK which are shifted by an apparent deletion of residues corresponding to positions 75, 114, and 115 in the calmodulin sequence. The deletion corresponding to residues 114 and 115 removes a lysine residue that is normally trimethylated in plant and animal calmodulins (*17*). These differences between CDPK and calmodulin may affect Ca^{2+} binding and protein-protein interactions.

Fig. 4. Diagramatic representation of the soybean CDPK structure based on the full-length SK5 cDNA sequence.

The two central domains that presumably encode the catalytic and Ca^{2+}-binding functions are flanked by NH_2-terminal and COOH-terminal domains of 40 and 35 amino acid residues, respectively (Fig. 4). The flanking regions were examined for similar sequences in the GenBank database, but no significant matches were found and no functional assignments have been proposed.

The CDPK provides the second example of a calmodulin-like domain fused to a catalytic domain and establishes this arrangement as a structural theme. The analogous structure is found in the 80-kD subunit of calpain, a Ca^{2+}-dependent protease from animal cells (*18*). Both enzymes contain a protein-modifying catalytic domain with an adjoining calmodulin-like regulatory domain. The calmodulin-like domain of CDPK is more highly conserved with respect to plant or animal calmodulin than that of calpain in both sequence identity (39% for CDPK versus 12 to 15% for calpain) and the spacing between the four Ca^{2+}-binding sites.

It is possible that the CDPK gene evolved as a fusion of two preexisting genes. Sequence comparisons suggest that the CDPK kinase domain originated from the fusion of a gene encoding a Ca^{2+}/calmodulin–dependent kinase with a calmodulin-like gene. The proposed fusion junction interrupts a putative ancestral calmodulin-binding domain corresponding to position 295 to 315 in the Ca^{2+}/calmodulin–dependent kinase from rat brain (*14*). This junction region also contains a potential pseudosubstrate site (CDPK residues 319 to 328), a sequence rich in basic residues that is proposed to bind to the active site of protein kinases in the absence of activators (*19*). CDPK may be capable of a faster or more versatile response to Ca^{2+} because direct Ca^{2+} binding relieves limitations due to the relatively slow diffusion of a larger Ca^{2+}-calmodulin complex.

Although CDPK is one of the best characterized plant kinases, its function and in vivo substrates are still unknown. The CDPK enzyme is found throughout the plant, from leaves to roots (*20*). Within a cell, immunocytological localization with a monoclonal antibody to the soybean CDPK indicates an association with an actin microfilament system (*20*). Biochemical fractionation studies have also indicated an association of an oat CDPK with the plasma membrane (*21*). However, hydropathy analysis of the SK5 isoform did not reveal any clear membrane spanning domains. The role of CDPK isoforms in specific signal transduction pathways remains uncertain.

Our results suggest a reexamination of studies that implicate calmodulin in Ca^{2+}-dependent processes. Because of the similarity of the CDPK Ca^{2+}-binding domain to calmodulin, CDPK can be expected to share many of the characteristic properties of calmodulin. CDPK copurifies with calmodulin through DEAE and phenyl-Sepharose chromotography and is sensitive to the calmodulin inhibitor W-7 (*6, 7, 22*). The similarity between these proteins may undermine the interpretation of studies that use inhibitors and assays of crude extracts to evaluate the function of calmodulin.

```
       1
CDPK   MAAKSSSSSTTTNVVTLKAAWVLPQRTQNIREVYEVGRKLGQGQFGTTFE
CaMPK                      MATTVTCTRFTDE*QLYEDI*K*A*SVVRR
                           1                      a
       51
CDPK   CTRRASGGKFACKSIPKRKLLCKEDYEDVWREIQIMHHLSEHANVVRIEG
CaMPK  *VKLCT*HEY*A*I*NTK**SARDH-QKLE**AR*-CR*LK*S*I**LHD
       101
CDPK   TYEDSTAVHLVMELCEGGELFDRIVQKGHYSERQAARLIKTIVEVVEACH
CaMPK  SISEEGFHY**FD*VT*****ED**AREY***AD*SHC*QQ*L*A*LH**
       151
CDPK   SLGVMHRDLKPENFLFDTIDEDAKLKATDFGLSVFYKPG-ESFCDVVGSP
CaMPK  QM**V*********L*LASKCKG*AV*LA****AIEVQGDQQAWFGFA*T*
              b
       200
CDPK   YYVAPEVLRK-LYGPESDVWSAGVILYILLSGVPPFWAESEPGIFRQILL
CaMPK  G*LS******EA**KPV*I*AC********V*Y****D*DQHKLYQ**KA
          c
       249
CDPK   GKLDFHSEPWPSISDSAKDLIRKMLDQNPKTRLTAHEVLRHPWIVDDNIA
CaMPK  *AY**P*PE*DTVTPE**N**NQ**TI**AK*I****A*K***VCQRSTV
       299                            328
CDPK   PDKPLDSAVLSRLKQFSAMNKLKKMALRVI--------------------
CaMPK  ASMMHRQETVEC**K*N*RR***GAI*305 >> 542
                                       329
CDPK   ------------------------------AERLSEEEIGGLKELFKMI
CaM                                  **E*TD*Q*AEF**A*SLF
                                       1
       350   356
CDPK   DTDNSGTITFDELKDGLKRVGSELMESEIKDLMDAADIDKSGTIDYGEFI
CaM    *K*GD*C**TK**GTVMRSL*QNPT*A*LQ*MINEV*A*GN****FP**L
          I                                     II
       400                            428
CDPK   AATVH-LNKLEREENLVSAFSYFDKDGSGYITLDEIQQACKDFG--LDDI
CaM    NLMARKMKDTDS**E*KE**RV****QN*F*SAA*LRHVMTNL*EK*T*E
            75                         III             114
       447
CDPK   HIDDMIKEIDQDNDGQIDYGEFA-AMMRKGNGGIGRRTMRKTLNLRDALG
CaM    EV*E**R*A*V*G****N*E**VKV**A*148.
                   IV
       496
CDPK   LVDNGSNQVIEGYFK508..
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Fig. 3. Alignment of amino acid sequences from the predicted SK5 protein (CDPK) with Ca^{2+}/calmodulin–dependent kinase II (CaMPK) and calmodulin (CaM). The 508–amino acid sequence for the SK5 encoded CDPK isoform is aligned with the first 305 residues of Ca^{2+}/calmodulin–dependent protein kinase II from rat brain (GenBank accession number A26464) and the entire 148 residues of calmodulin from spinach (GenBank accession number A03024). Identical residues are indicated by a (*). The CDPK sequence is divided at the boundary between the kinase and calmodulin-like domains. Boxes a, b, and c are diagnostic kinase sequences and boxes I, II, III, and IV are calcium-binding sites. DNA and amino acid sequence comparisons were performed with DNASTAR (Madison, Wisconsin) software.

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25. We thank S. Kumar and L. Manney for technical assistance with purification of peptides and cDNA sequencing, respectively, M. J. Cormier for fostering the early work on CDPK, R. Amasino for soybean RNA, and K. Walsh for advice and helpful discussions. Supported by grants from the USDA (M.R.S. and A.C.H.), DOE (M.R.S.), and NSF (A.C.H.) and a grant to K. Walsh from NIH (GM15731).

13 November 1990; accepted 7 March 1991

Cloning of a Factor Required for Activity of the Ah (Dioxin) Receptor

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The aryl hydrocarbon (Ah) receptor binds various environmental pollutants, such as polycyclic aromatic hydrocarbons, heterocyclic amines, and polychlorinated aromatic compounds (dioxins, dibenzofurans, and biphenyls), and mediates the carcinogenic effects of these agents. The complementary DNA and part of the gene for an 87-kilodalton human protein that is necessary for Ah receptor function have been cloned. The protein is not the ligand-binding subunit of the receptor but is a factor that is required for the ligand-binding subunit to translocate from the cytosol to the nucleus after binding ligand. The requirement for this factor distinguishes the Ah receptor from the glucocorticoid receptor, to which the Ah receptor has been presumed to be similar. Two portions of the 87-kilodalton protein share sequence similarities with two *Drosophila* proteins, Per and Sim. Another segment of the protein shows conformity to the consensus sequence for the basic helix-loop-helix motif found in proteins that bind DNA as homodimers or heterodimers.

THE AH RECEPTOR IS DETECTABLE IN many tissues and organs. The best understood activity of the receptor concerns its role in the induction of cytochrome P450IA1. Complexes between the Ah receptor and ligand bind to specific DNA sequences upstream of the P450IA1 gene, termed xenobiotic responsive elements (XREs), and stimulate transcription of the gene (*1, 2*). The receptor also mediates induction of cytochrome P450IA2 and several other enzymes of xenobiotic metabolism. The enzymatic activities of P450IA1 (aryl hydrocarbon hydroxylase, or AHH) and P450IA2 are important in the metabolism of polycyclic aromatic hydrocarbons (found in cigarette smoke and smog) and certain heterocyclic amines (found in cooked meat) to carcinogenic intermediates (*3*). The pathological effects of the polychlorinated aromatic compounds also depend on the action of the Ah receptor, but the mechanism of pathogenesis is unknown (*4*).

The Ah receptor is a soluble protein complex of ~280 kD. The ~95-kD ligand-binding subunit, which has not been cloned, and the 90-kD heat shock protein (Hsp90) are both components of this complex (*5*). After cells are treated with ligand, receptor molecules become tightly bound in the nucleus. However, the location of the receptor before ligand binding has not been fully resolved (*6*). After conventional subcellular fractionation, the unoccupied receptor is found in the cytosol. Operationally, therefore, ligand treatment leads to "nuclear translocation" of the receptor. The Ah receptor resembles the steroid hormone receptors (*7*), and this resemblance has led to the suggestion that the ligand-binding subunit of the Ah receptor may be a member of the steroid receptor superfamily.

The mouse hepatoma cell line Hepa-1 shows P450IA1 inducibility. Mutants of Hepa-1 cells defective in induction have been isolated, and those mutants that are recessive have been assigned to four complementation groups (*8, 9*). Mutations in groups B, C, and D affect functioning of the Ah receptor (*9, 10*). In the group C mutants, the receptor is present in normal amounts but does not translocate to the nucleus after binding ligand. We now describe the isolation of part of the human C gene [termed the Ah receptor nuclear translocator gene (*arnt*)] and the isolation and

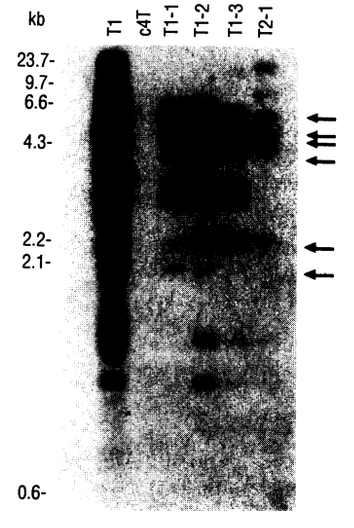

Fig. 1. DNA blot analysis of transfectants. Genomic DNA (10 μg) was digested with Pst I, subjected to agarose gel electrophoresis, and transferred to nitrocellulose. Hybridization was performed in 50% formamide, 5× SSC (standard saline citrate), 20 mM sodium phosphate (pH 6.5), 10% dextran sulfate, 1× Denhardt's solution, and denatured salmon sperm DNA (250 μg/ml) at 42°C. After hybridization, the filter was washed in 0.1× SSC (standard saline citrate) plus 0.1% SDS at 65°C. The probe was the Bam HI, Alu-containing fragment recloned from pBLUR8 into M13mp8 and isolated from the latter. Arrows indicate bands common to all secondary transfectants. Numbers at the left margin indicate sizes in kilobases.

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