Project #: G-32-638  
Center #: 10/24-6-R7846-0A0  
Contract#: MCB-9305321  
Prime #:  
Subprojects ?: Y  
Main project #:  
Cost share #:  
Center shr #:  
Rev #: 7  
OCA file #:  
Work type : RES  
Document : GRANT  
Contract entity: GTRC  
CFDA: 47.074  
PE #: N/A  
Mod #: NO COST EXT  
Active

Project unit: BIOLOGY  
Project director(s): CHOI J H  
Unit code: 02.010.134  
(404)894-3701

Sponsor/division names: NATL SCIENCE FOUNDATION  
Sponsor/division codes: 107  
Awards to 970630 (performance) 970928 (reports)

Sponsor amount  
Contract value 0.00  
Funded 0.00  
Total to date 238,005.00  
Cost sharing amount 0.00  
New this change 0.00  
Does subcontracting plan apply ?: N

Title: AN ATYPICAL CALCIUM-DEPENDENT PROTEIN KINASE HOMOLOGUE IN CARROT & ...

PROJECT ADMINISTRATION DATA

OCA contact: Michelle A. Starmack 894-4820  
Sponsor technical contact  
BARBARA K. ZAIN (703)306-1442  
Sponsor issuing office  
GRACIELA NARCHO (703)306-1218  
NATIONAL SCIENCE FOUNDATION  
4201 WILSON BLVD.  
ARLINGTON, VA 22230

Security class (U,C,S,TS) : U  
Defense priority rating : N/A  
Equipment title vests with: Sponsor  
ONR resident rep. is ACO (Y/N): N  
NSF supplemental sheet  
GIT X

Administrative comments -  
ISSUED TO GRANT NO COST EXTENSION THOROUGH 6/30/97 IAW OPAS FORM VIA FASTLANE.  
FINAL REPORT NOW DUE 9/28/97.
PROJECT CLOSEOUT - NOTICE

Closeout Notice Date 23-SEP-1997

Project Number G-32-638

Center Number 10/24-6-R7846-0A0

Project Director CHOI, JUNG

Project Unit BIOLOGY

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Division Id 3393

Contract Number MCB-9305321

Prime Contract Number

Contract Entity GTRC

Title AN ATYPICAL CALCIUM-DEPENDENT PROTEIN KINESE HONOLOGUE IN CARROT & ...

Effective Completion Date 30-JUN-1997 (Performance) 28-SEP-1997 (Reports)

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Comments

LETTER OF CREDIT APPLIES. 98A SATISFIES PATENT REPORT.

Distribution Required:

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The grant was initiated September 1, 1993. The experimental goals for the first year of the project were:

1) preparation of DNA and antibody probes for carrot CDPK and CRK;
2) sequencing of Arabidopsis genomic clones; and
3) expression and purification of CDPK and CRK in bacteria or in insect cells.

To date (March 17, 1994) we have made progress on all three objectives.

1) DNA probes comprising the 3' ends of the carrot CDPK and CRK genes have been tested and found to hybridize to unique bands on Southern blots of carrot genomic DNA. Preliminary Northern blots indicate that CRK (PK421) transcripts accumulate maximally in somatic embryos, whereas CDPK (PK431) transcripts appear at equal levels in somatic embryos and undifferentiated suspension cells.

Polyclonal antisera have also been generated to a synthetic peptide corresponding to the junction domain of CRK. These antisera will be affinity purified and tested on Western blots of carrot cells and somatic embryos, carrot plants and Arabidopsis plants.

2) The Arabidopsis genomic clones have been fully sorted. Subcloning efforts are now underway prior to sequencing.

3) The missing 5' end of carrot CDPK (PK431) has been amplified by PCR amplification of cDNA, following 5'-RACE protocols. The sequencing is nearly complete, and it should be possible to piece together or reamplify the full coding sequence in the next few months.

The C-terminal domains of CRK and CDPK have been expressed in E. coli and tested for calcium binding by gel mobility shift assays. The CDPK C-terminal domain displays a calcium-induced mobility shift comparable to calmodulin upon SDS-PAGE. However, the CRK C-terminal domain shows no change in mobility with 5 mM calcium. More sensitive tests are planned using native gels and blots with 45Ca.
Plans for Next 12 Months:

1) Following the timetable in the original proposal, antibodies will be generated to the C-terminal tail of CDPK, again from a synthetic peptide, and tested for isozyme specificity. The antibodies and gene-specific DNA probes will be used to investigate the expression of CDPK and CRK isoforms in carrot cells and somatic embryos, carrot plant tissues, and Arabidopsis plant tissues.

2) Sequencing of Arabidopsis genomic clones will proceed. In addition, we plan to screen an Arabidopsis cDNA library with a carrot CRK probe. An ROA supplement request has been submitted regarding this work. An Arabidopsis cDNA homologous to CRK can be sequenced more quickly than the genomic clones.

3) The initial effort to express full-length CRK and CDPK in bacteria should be complete by this summer. If the bacterially-expressed enzymes have catalytic activity, then biochemical characterization can proceed.

4) The advent of the 2-hybrid genetic screen for protein interactions in yeast (Fields and Song, 1991) provides an opportunity to quickly identify and isolate genes for potential CDPK and CRK substrates or regulators. A kit containing all the yeast strains and vectors is available from a commercial vendor, Clontech. The great outstanding question concerning CDPKs in plants is their function; the in-vivo substrates are unknown. Given the potential for rapid progress and the relatively low risk in terms of cost and personnel time, we believe the yeast 2-hybrid system is worth trying. Therefore, we plan to devote part of this spring and summer to construct a carrot cDNA library in the yeast GAL4 activation-domain fusion vector and screen for clones that interact with CDPK and CRK fused to the GAL4 DNA-binding domain.

Educational Impact:

Two Ph.D. students working on this project are supported with NSF funds as research assistants. Eric Lindzen is in his 4th year of graduate study and should finish his degree in June of 1995. Paul Farmer is in his 2nd year and joined my lab last fall (fall 1993).

In addition, two undergraduate students, both juniors, have worked on this project: David Simon, who wants to enroll in an MD-PhD program, and Joshua Hayes, who intends to apply to graduate school for a Ph.D. Both students have been in my lab for a year. I have recruited two other students, both currently sophomores, for similar long-term research commitments: Courtney Artman and Todd.

An ROA supplemental request has been submitted for Dr. Cynthia Galloway to work on isolating a CRK homologous cDNA from Arabidopsis this summer. Dr. Galloway is an Assistant Professor at Texas A&M, Kingsville. Also, a supplement request has been submitted to the NSF Directorate for Education and Human Resources for a local high school biology teacher to work on this project this summer, under the aegis of the GIFT (Georgia Industrial Fellowships for Teachers) program run by Georgia Tech.
ANNUAL NSF GRANT PROGRESS REPORT

NSF Program: Cell Biology
NSF Award Number: MCB 9305321

Pl Name: Jung H. Choi
Period Covered By This Report: Sep 1, 1993 – Mar 17, 1994

Pl Institution: Georgia Tech
Date: March 18, 1994

Pl Address: School of Biology, Georgia Tech, Atlanta, GA 30332-0230

☐ Check if Continued Funding is Requested

Please include the following information:

1. Brief summary of progress to date and work to be performed during the succeeding period;
2. Statement of funds estimated to remain unobligated —if more than 20%— at the end of the period for which NSF currently is providing support (not required for participants in the Federal Demonstration Project);
3. Proposed budget for the ensuing year in the NSF format, only if the original award letter did not indicate specific incremental amounts or if adjustments to a planned increment exceeding the greater of 10% or $10,000 are being requested;
4. Current information about other research support of senior personnel, if changed from the previous submission;
5. Any other significant information pertinent to the type of project supported by NSF or as specified by the terms and conditions of the grant;
6. A statement describing any contribution of the project to the area of education and human-resource development, if changed from any previous submission; and
7. Updated information on animal care and use, Institutional Biohazard Committee and Human Subject Certification, if changed substantially from those originally proposed and approved.

Progress report attached.

I certify that to the best of my knowledge (1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and (2) the text and graphics in this report as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I understand that the willful provision of false information or concealing a material fact in this report or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001.)

P.I. Signature: [Signature]

NSF Form 1328 (1/94)
### ANNUAL NSF GRANT PROGRESS REPORT

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<th>Cell Biology</th>
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<td>MCB 9305321</td>
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<tr>
<td>PI Name:</td>
<td>Jung H. Choi</td>
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<tr>
<td>PI Institution:</td>
<td>Georgia Tech</td>
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<td>PI Address:</td>
<td>School of Biology, Georgia Tech, Atlanta, GA 30332-0230</td>
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Check if Continued Funding is Requested

Please include the following information:

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2. Statement of funds estimated to remain unobligated— if more than 20%— at the end of the period for which NSF currently is providing support (not required for participants in the Federal Demonstration Project);
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7. Updated information on animal care and use, Institutional Biohazard Committee and Human Subject Certification, if changed substantially from those originally proposed and approved.

I certify that to the best of my knowledge (1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and (2) the text and graphics in this report as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I understand that the willful provision of false information or concealing a material fact in this report or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001.)

P.I. Signature: [Signature]

NSF Form 1328 (1/94)
Progress Report for NSF Project No. MCB-9305321

Period Covered: March 1994-March 1995

The objectives for the second year of the project were:

1) Developmental Expression of CRK and CDPK
2) Expression of CRK and CDPK in E. coli & Biochemical Characterization
3) Sequencing of Arabidopsis genomic clones.

1) This objective is progressing well. A cDNA fragment encoding the N-terminal domain of CDPK431 has been subcloned into an expression vector (pRSET) and expressed in E. coli with a polyhistidine tag. After affinity purification on nickel resin, this N-terminal peptide was used to raise a polyclonal antiserum in rabbits. This antiserum recognizes a protein of approximately 60 kDa on immunoblots of crude homogenates from carrot somatic embryos and suspension cells. We are currently attempting to affinity purify this crude antiserum.

Quantitative Northern analyses are underway, using the N-terminal domain fragment of CDPK431 and the C-terminal domain fragment of CRK421 as isoform-specific probes. A newly acquired departmental Ambis 4000 direct radioisotopic imager enables precise quantitation and comparison of relative probe hybridization levels. Initial experiments have shown that CDPK431 levels remain constant in carrot suspension cells maintained in 2,4-D. When embryogenesis is initiated by removing 2,4-D, the levels drop to 20% within 5 days and then increase as somatic embryos mature. A more detailed time course of somatic embryo development is underway to verify this observation. Protein and total RNA will be extracted from the same samples for dual comparisons of CDPK431 and CRK421 expression.

2) This objective is progressing well for CDPK431, but expression of CRK421 has been delayed. A full-length CDPK431 cDNA has been cloned into pRSET so that the native protein will be expressed in E. coli without foreign sequences attached to either the N-terminal or C-terminal ends. Although we have successfully obtained high level expression in small volume (50 ml) cultures, attempts to scale up to large cultures (1-2 liters) for large scale purification have resulted in very low levels of expression. We are now transferring the clone into a different expression vector, pLEX, that has tighter repression of uninduced expression.
To study N-terminal myristoylation, the full-length CDPK431 has been coexpressed in E. coli cells along with yeast N-myristoyl transferase (nmt). Labeling these E. coli cells with $^3$H-myristate resulted in labeling of a prominent band of circa 65 kDa that is absent in control cells expressing yeast nmt alone. These results indicate that the putative N-myristoylation signal of carrot CDPK431 can be recognized and myristoylated by yeast nmt. We plan to label carrot somatic embryos with $^3$H myristate and immunoprecipitate with antibodies to CDPK431 and CRK421 to try to determine whether they are myristoylated in carrot cells.

Studies on calcium-binding with the C-terminal domain of CRK421 expressed in E. coli has revealed that this domain shows neither a gel-mobility shift in the presence of calcium or EGTA, nor detectable binding of $^{45}$Ca on blots. In contrast, the C-terminal domain of CDPK431 shows both gel mobility shift and binding of $^{45}$Ca. Thus CRK may not be activated by calcium, although we cannot eliminate the possibility that it may yet respond to calcium in vivo in association with other proteins.

The expression of a full-length CRK421 in E. coli has not yet been accomplished. Because of a lack of suitable restriction sites, the assembly of this clone from smaller fragments has been difficult. However, we expect that the full-length clone will be constructed and expressed in E. coli by June, 1995.

3) This objective is on track. One of the Arabidopsis genomic clones has been subcloned and partially sequenced. However, the sequence data obtained thus far has been uninformative. More sequence is needed to evaluate whether this clone encodes a CRK homologue.

**Plans for Next 12 Months:**

All three objectives will be pursued.

1) Now that the isoform specific probes and antisera have been generated, we will examine expression of CRK421 and CDPK431 during embryogenesis, and examine subcellular and tissue-specific localization using RNA and protein blots. The new departmental Ambis 4000 direct radioisotopic imager will be used to quantitate relative levels of mRNA accumulation in various tissues and at various stages of development. Changes in expression in response to phytohormones, calcium ionophores and calcium channel blockers will be examined.

2) The most urgent priority here is the expression of full-length CRK421 in E. coli. In the meantime, however, we can and will investigate the effects of N-myristoylation on the biochemical activity and/or the membrane association of CDPK431. Similar studies will be performed with CRK421 once the full-length construct is available. Moreover, the junction domain peptide for CRK421 has been synthesized and will be used to test for phosphorylation by CDPK431, cyclin-dependent kinase, and eventually, CRK421.

3) Sequencing of Arabidopsis genomic clones will continue.

In addition to the above three objectives, we will construct a yeast GAL4 activation domain fusion library of cDNA from carrot somatic embryos for use in a 2-hybrid screen, using
CDPK431 and CRK421 as bait. The two-hybrid system has the potential of not only identifying substrates for the kinases, but other proteins that may interact with them. We have obtained the vectors and strains for the 2-hybrid system.

**Educational Impact**

Two Ph.D. students have been supported with NSF funds. Eric Lindzen is in his final year of graduate study. He presented an oral paper at the Southern Section ASPP meeting in Knoxville, March 18-20, 1995, and received an Outstanding Student Paper award. Paul Farmer is in his 3rd year, and will present a poster at the Plant Signal Transduction meeting in Hilton Head, S.C., March 29-April 4, 1995.

Four undergraduate students have worked on this project and been paid as part-time student assistants. Josh Hayes graduated in March, 1995 and has applied to Ph.D. programs in genetics. Josh was responsible for sorting the Arabidopsis genomic clones and subcloning one of them. David Simon is a senior and applying to both graduate and medical schools. He expressed the C-terminal domain of CRK421 and tested it for calcium binding. Courtney Artman, a rising senior, has been named a Howard Hughes undergraduate research intern for Winter and Spring quarters of 1995 and is performing the bulk of the expression studies. She presented a poster at the Southern Section ASPP meeting in Knoxville, Tennessee, March 18-20, 1995. Tobi Todd, also a rising senior, is learning DNA sequencing. He is 50% African-American, 50% Asian-American.

Last summer, Ms. Barbara Williams, a high school teacher, was recruited through the GIFT program and supported by a supplement from the NSF Directorate for Education and Human Resources. She performed some antibody characterization and immunoblot analyses with antisera to the junction domain of CRK421. She has since used the gel electrophoresis techniques in the Advanced Placement Biology classes. Tobi Todd and I have also visited her class to talk about our research.

Dr. Cynthia Galloway, an Associate Professor at Texas A&M University in Kingsville, Texas, visited my lab last summer, sponsored by an ROA supplement. She screened a cDNA library, came up with some putative positive clones, and will return this summer to further characterize these clones.

**Publications**

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
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<th>Investigator:</th>
<th>Jung H. Choi</th>
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**Current and Pending Support**

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*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

NSF Form 1239 (1/94) USE ADDITIONAL SHEETS AS NECESSARY
September 7, 1997

Sherrie B. Green
Program Manager, ARI
Science and Technology Infrastructure
National Science Foundation
4201 Wilson Boulevard
Arlington, VA 22230

Dear Dr. Green:

Described below is information requested in your July 18, 1997 letter with regard to the National Science Foundation grant STI-9313425 to Georgia Institute of Technology. The grant was for the renovation of space to create research laboratories in the Cherry Emerson Biology Building. Three pictures of the renovated laboratories are also enclosed, and the drawings of the floor plan. If further information is needed please let me know.

Sincerely yours,

Roger M. Wartell
Chair and Professor
INFORMATION REQUESTED FOR FINAL REPORT ON
NATIONAL SCIENCE FOUNDATION FACILITIES GRANT
STI-9313425

"RENOVATION OF HIGH BAY AREA OF CHERRY EMERSON BUILDING
TO RESEARCH LABORATORIES

Principle Investigator: Roger M. Wartell
Institution: Georgia Institute of Technology

1. Summary of Project Activities

A high bay area, approximately 50 x 23 sqf in floor space and two stories high- at the west end of the rectangular Cherry Emerson Building- was converted to research laboratories. This space, and a 770 sqf mezzanine area on the second floor level were previously used for equipment storage. Design and engineering plans of the project were developed by the Institute's Facilities Office. Bids were solicited and the company Interstate School Construction Co. was awarded the bid. The first step of the construction was to install a floor at the second floor level of the high bay. This was connected to the mezzanine area and created a complete second floor in the west end of building. Three laboratory rooms, approx, 550 sqf each were created on the newly constructed second floor area. A large single laboratory 1058 sqf was created on the ground floor. To enhance the ground floor research space, an unnecessary fume hood was removed in a nearby lab and a new lab bench was added. Enclosure of an open but roofed area near the building’s loading dock created an equipment storage room (12 x 10 sqf). An area under a stairwell was also converted into another equipment storage space to replace storage space lost in the renovation. Offices were also created adjacent to the laboratories to replace offices that were demolished in the construction. Construction of the latter used only Georgia Tech funds.
2. Final Actual Cost of Renovation $343,173.

Charges Assigned to Matching Funds

Interstate School Construction Co. (Lab Construction) $165,000
Interstate School Construction Co. (Construction-offices) $35,000
Interstate School Construction Co. (Construction-modification) $5,000

Total $205,000

Charges Assigned to NSF Grant

Georgia Tech Plant Operations (Design and Engineering) $19,703
Interstate School Construction Co. (Lab Construction) $91,463
VWR (Lab benches and cabinets) $5,972
Williams Russell & Jones (Construction-Storage areas) $7,815
Lab. Operations Clearinghouse (lab hood removal, new bench) $5,220
Georgia Tech Plant Operations- HVAC adjustments, Sec. Floor) $1,000

Total $138,173.

3. Project Completion/Occupancy Date

Construction of the research laboratories was completed by Spring, 1996. Completion of storage areas construction, HVAC adjustments for research laboratories, and upgrade of first floor research space adjacent to new laboratory was completed by January 10, 1997. Two of the second floor laboratories were fully occupied by Professor Thomas DiChristina's group in Spring 1996 for microbiology research. The ground floor laboratory was also occupied in late Spring 1996 by Professor Choi's and Professor Chernoff's groups for molecular biology research. The remaining second floor laboratory was occupied by Professor Patricia Sobecky in Fall 1997.

4. Expected Impact of Project

The renovation funded by NSF has provided a highly cost effective way to create new laboratory space for the expansion of research in the School of Biology. It has already had a positive impact on the ability of the School's faculty and students to carry out research, and to recruit new faculty.

Since the proposal was submitted, the biological sciences at Georgia Tech has experienced increased research and educational activity. The number of biology majors in the undergraduate program has increased from 274 to 360 during the past three years. The faculty
increased from 14 to 17, sponsored research has increased by about 30%, and the School received a GAANN graduate fellowship grant to support five additional graduate students. Next year construction will begin on a new building adjacent to the Cherry Emerson Building that will house faculty from Biology, Chemistry and Biochemistry, and Bioengineering. The Biology faculty is anticipated to grow to a size of 25 to 30. The new research space that was created in the Cherry Emerson Building has been crucial for enabling the School to recruit new faculty now, prior to the completion of the new facility. It will continue to be essential later to accommodate the growth of activity within the School of Biology.

The second floor area is providing space for research by two faculty in environmental microbiology. Graduate and undergraduate students carrying out research with Drs DiChristina and Sobecky are utilizing the new facilities. The ground floor laboratory is currently being shared by Professors Choi and Chernoff and their laboratory groups. It is anticipated that it will become the main research laboratory of a new faculty member in molecular biology who we will be recruiting this year. The additional space has enabled the School to accommodate an increased number of undergraduates as well as graduate students in laboratory research.

The new laboratories are being utilized by four faculty members, fifteen graduate students and two postdoctoral associates, as well as twelve undergraduates. Three to five additional student and postdoctoral research associates are expected to utilize the new labs within the next year.