Annotated NIAID SBIR Phase I Grant Application

Last updated on August 21, 2003.

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Introduction

We are indebted to Dr. John Trawick and Elitra Pharmaceuticals for permitting us to show his outstanding Phase I and Phase II SBIR applications. Dr. Trawick submitted his Phase I application as a new investigator in December 1999, and his Phase II application in April 2002.

To avoid confusing current applicants, we have updated his original SBIR Phase I form pages onto those of the PHS 398 which are required today. We have also made some minor changes in personnel names and deleted confidential salary information. Other than these minor changes, the applications are exactly as submitted.

We have also included the Summary Statement and Notice of Grant Award for each application. Further, we've added annotations to explain how these application reflect much of the advice we give in our "Advice on SBIR and STTR Grant Applications."

NIAID annotations are in yellow boxes, like this one.

If you have questions or comments, please contact:

Gregory Milman, Ph.D.
Director, Office for Innovation and Special Programs
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases, NIH, DHHS
6700-B Rockledge Drive; Room 2140
Bethesda, MD 20892-7610 (US Mail)
Rockville, MD 20817-7610 (Delivery Services)
Tel (301) 496-8666 Fax (301) 402-0369
Email gmilman@niaid.nih.gov

SBIR Phase I and II applications must use PHS398 form pages

Form Approved Through 05/2004 OMB No. - 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed 56-character length restrictions, including spaces.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT
Dominant expression for new targets in Candida albicans

Title describes project.

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PHS 2003-2 Title: Phase I SBIR

Indicates response to SBIR solicitation.

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

New Investigator ☐ **No** ☒ **Yes**

3a. NAME *(Last, first, middle)*
Trawick, John D.

3b. DEGREE(S)
Ph.D.

3c. POSITION TITLE
Senior Research Scientist

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Elitra Pharmaceuticals, Inc.
3510 Dunhill Street
San Diego, CA 92121

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 858-410-3019 FAX: 858-410-3090

E-MAIL ADDRESS:
jtrawick@elitra.com

4. HUMAN SUBJECTS RESEARCH
☒ No
☐ Yes

4a. Research Exempt ☐ No ☐ Yes
If "Yes," Exemption No.

4b. Human Subjects Assurance No.

4c. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☐ Yes

5a. If "Yes," IACUC approval Date

5b. Animal welfare assurance no

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
07/01/00	01/01/02	$94,466	$100,000		

9. APPLICANT ORGANIZATION
Name Elitra Pharmaceuticals, Inc.
Address 3510 Dunhill Street
San Diego, CA 92121

Institutional Profile File Number (if known)

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☒ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER

DUNS NO. *(if available)* 33-0779254
Congressional District 46

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name
Title
Address

Tel FAX
E-Mail

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Harry F. Hixson
Title President and CEO
Address Elitra Pharmaceuticals, Inc.
3510 Dunhill Street
San Diego, CA 92121

Tel 858-410-3030 FAX 858-410-3090
E-Mail hhixson@elitra.com

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.	SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*	DATE
15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)*	DATE

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

A screen for dominant negative genes will be used to identify targets and pathways in Candida albicans. The genes and pathways identified will be developed as new antifungal targets. An expression vector system suitable for screening libraries in C. albicans has been devised. Phase I of this project includes final construction and optimization of the expression vector and construction of cDNA libraries capable of identifying dominant negative mutants. Preliminary screening will begin in Phase I. Phase II of the project will entail identifying essential genes and processes by dominant negative mutagenesis and to develop screens for new antifungals based on these essential genes. The method proposed for identifying essential genes is ideal for C. albicans, a diploid human pathogen not normally amenable to genetic analysis, and can be automated.

Kept very simple. Avoids description of confidential information. Shows linkage with Phase II. Would be better with a list of specific aims.

PERFORMANCE SITE(S) *(organization, city, state)*

Elitra Pharmaceuticals, Inc.
3510 Dunhill Street
San Diego, CA 92121

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below.
Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
John D. Trawick, Ph.D.	Elitra Pharmaceuticals, Inc.	Principal Investigator
J. Gordon Foulkes, Ph.D.	Elitra Pharmaceuticals, Inc.	Co-Investigator
Sherry Nouraini	Elitra Pharmaceuticals, Inc.	Postdoctoral Fellow
Carlos Zamudio	Elitra Pharmaceuticals, Inc.	Collaborator
William A. Fonzi, Ph.D.	Georgetown University	Consultant

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☒ **Yes** ☐ **No**

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

BUDGET JUSTIFICATION PAGE MODULAR RESEARCH GRANT APPLICATION				
Initial Budget Period	**Second Year of Support**	**Third Year of Support**	**Fourth Year of Support**	**Fifth Year of Support**
$94,446				
Total Direct Costs Requested for Entire Project Period				$94,446

Personnel

John D. Trawick, Ph.D., P.I. (50% effort) - will supervise and coordinate the experimental studies, including vector optimization and validation and library construction and essential gene identification.
J. Gordon Foulkes, Ph.D., Co-P.I. (5% effort) - will assist Dr. Trawick with these responsibilities. In addition, he will provide considerable expertise required for the development of drug screens for new antimicrobials.
Shahrzad Nouraini, Research Associate (70% effort) - will assist in the vector and library construction projects and essential gene identification.
Carlos Zamudio, - Director of Bioinformatics at Elitra Pharmaceuticals (5% effort) - will assist in the bioinformatic identification and prioritization of targets.

Consortium

William A. Fonzi, Ph.D., general consultant for Elitra - will provide advice and materials for design of the vector to be used and provide advice and expertise Candida albicans molecular biology and pathogenesis.

Fee (SBIR/STTR Only)

A fee of 6% of direct costs is requested. This contributes to the capitalization of Elitra Pharmaceuticals, Inc., and provides

A Biographical Sketch PDF form was used in this application but applicant could have included same information in word processed document using similar format.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
John Douglas Trawick	**Senior Research Scientist, Elitra Pharmaceuticals**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Gustavus Adolphus Coll., St.Peter, MN	B.A	1976	Biology
Northern Illinois University, Dekalb IL	M.S.	1979	Biological Sciences
University of Minnesota, Minneapolis, MN	Ph.D.	1984	Microbiology

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

1984-1985 Postdoctoral Fellow, Mayo Foundation, Rochester, MN. Research in actin gene expression in mammalian cells.

1985-1990 Postdoctoral Research Associate, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO. Expression and regulation of cytochrome oxidase genes in the yeast, *Saccharomyces cerevisiae*, nuclear-mitochondrial interactions in *S. cerevisiae*.

1990-1992 Assistant Professor, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-1996 Adjunct Assistant Professor, Department of Biology, San Diego State University. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-present Adjunct Assistant Professor, Department of Biology, San Diego State University.

1996-1997 Course instructor, Biology Departments San Diego State University and University of San Diego.

1997-present Senior Research Scientist, Drug Development Dept., Elitra Pharmaceuticals. Target evaluation and validation *in E. coli* and *Staphylococcus aureus*. Target discovery in *Salmonella typhimurium*, target discovery in *Candida albicans*, vector development and improvement, and new organism evaluation.

Patent applications

Genes identified as required for proliferation in Escherichia coli. 2000 Inventors: Zyskind, J. W., Ohlsen, K.L., **Trawick, J.D.,** Forsyth, R. A., Froelich, J. M., Carr, G. J., Yamamoto, R. T., Xu, H. **WO 00/44906**

Identification of essential genes in prokaryotes. 2001. Haselbeck, R., Ohlsen, K. L., Zyskind, J. W., Wall, D., **Trawick, J. D**., Carr, G. J., Yamamoto, R.T., Xu, H. H. **WO 01/70955**

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

Kline, B., Seelke, R. and **Trawick, J.** Replication and incompatibility functions in mini-F plasmids. *In* Levy, S.B., Clowes, R.L., and Koenig, E.L., eds., Molecular biology, pathogenicity, and ecology of bacterial plasmids, pp. 317-326. Proceedings of the International Plasmid Conference on Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, January 5-9, 1981, Santo Domingo, Dominican Republic, Plenum Press, NY.

Kline, B.C., Seelke, R.W., **Trawick, J.D.,** Levy, S.B. and Hogan, J. Genetic studies on the maintenance of mini-F plasmids. *In* Proceedings of the Third Tokyo Symposium on Mechanisms of Antibiotic Resistance, October, 1981, Tokyo, Japan 1984.

Seelke, R.W., Kline, B.C., **Trawick, J.D.,** and Ritts, G.D. 1982. Genetic studies of F plasmid maintenance genes involved in copy number control, incompatibility and partitioning. *Plasmid* **7:** 163-179.

Kline, B.C. and **Trawick, J.** 1983. Identification and characterization of a second copy number control gene in mini-F plasmids. *Molec. Gen. Genet.* **192:** 408-415.
Trawick, J.D. and Kline, B.C. 1985. A two-stage molecular model for control of mini-F replication. *Plasmid* **13:** 59-69.
Wright, R.M., **Trawick, J.D.,** Trueblood, C.E., Patterson, T.E., and Poyton, R.O. Organization and expression of nuclear genes for yeast cytochrome c oxidase. *In*: Cytochrome systems: Molecular biology and bioenergetics, pp. 49-56. ed. S. Papa. 1987. Plenum Press, NY.
Trawick, J.D., Wright, R.M., and Poyton, R.O. 1989. Transcription of yeast COX6, the gene for subunit VI of the cytochrome c oxidase of S. cerevisiae, is dependent on heme and on the HAP2 gene. *J. Biol. Chem.* **264:** 7005-7008.
Trawick, J.D., Rogness, C.R., and Poyton, R.O. 1989. Identification of an upstream activation site and other cis-acting elements required for transcription of COX6 from Saccharomyces cerevisiae. *Mol. Cell. Biol.* **9:** 5350-5358.
Farrell, L.E., **Trawick, J.D.,** and Poyton, R.O. Mitochondrial-nuclear interactions: transcription of nuclear COX genes in yeast is reduced in cells that lack a mitochondrial genome. *In*: Structure, function, and biogenesis of energy transfer systems, pp.131-134, ed. E. Quaglieriello, S. Papa, F. Palmieri, and C. Saccone. 1990. Elsevier Press.
Trawick, J.D., Simon, F.R., Kraut, N.,and Poyton, R.O. 1992. Regulation of Yeast COX6 by the General Transcription Factor ABF1 and Separate HAP2 and Heme Responsive Elements. *Mol. Cell. Biol.* **12:** 2301-2314.
Leighton, J.K., Dueland, S., Straka, M.S., **Trawick, J.,** and Davis, R.A. 1991. Activation of the silent endogenous cholesterol-7-alpha-hydroxylase gene in rat hepatoma cells: A new complementation group having resistance to 25-hydroxycholesterol. *Mol. Cell. Biol.* **11:** 2049-2056.
Davis, R.A., Dueland, S. and **Trawick, J.** 1992. Bile Acid Synthesis and the Enterohepatic Circulation: Processes Regulating Total Body Cholesterol Homeostasis. *In* Molecular Genetics of Coronary Heart Disease and Stroke. Lusis, A., Rotter, J. and Sparkes, R.S., eds. Karger Press.
Thrift, R., Drisko, J. Dueland, S., **Trawick, J.D.**, and Davis, R.A.,. 1992. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line.*Proc. Natl. Acad. Sci. USA*. **89:**9161-9165.
Dueland, S., **Trawick, J.D.,** Nenseter, M.S., MacPhee, A.A., and Davis, R.A. 1992. Expression of 7alpha-hydroxylase in non-hepatic cells results in liver phenotypic resistance of the low density lipoprotein receptor to cholesterol repression. *J. Biol. Chem.* **267:** 22695-22698.
Trawick, J. D.,Lewis, K.D., Moore, G.L., Simon, F.R., and Davis, R.A. 1996. Rat hepatoma L35 cells, a liver-differentiated cell line, display resistance to bile acid repression of cholesterol 7 alpha-hydroxylase. *J. Lipid Res.* **37:** 588-599.
Moore, G. L., Drevon, C. A., Machleder, D., Lusis, A. J., **Trawick, J. D.**, Unson, M. A., McClelland, A., Roy, S., Lyons, R., Jambou, R., and Davis, R.A. 1997. Expression of human cholesterol 7 alpha-hydroxylase in atherosclerosis-susceptible mice via adenovirus infection. *Biochem. J.* **324:** 863-867.
Dueland, S., France, D., Wang, S.-L., **Trawick, J. D.,** and R. A. Davis. 1997. Cholesterol-7alpha-hydroxylase influences the expression of hepatic Apo AI in two inbred mouse strains displaying different susceptibilities to atherosclerosis and in hepatoma cells. *J. Lipid Res*. **38:** 1445-1453.
Trawick, J. D., Shui-Long Wang, David Bell, and R.A. Davis.1997. Transcriptional induction of 7 alpha-hydroxylase by dexamethasone in L35 hepatoma cells requires sulfhydryl reducing agents. *J. Biol. Chem*. **272:** 3099-3102.
R. A. Forsyth, R. J. Haselbeck, K. L. Ohlsen, R. T. Yamamoto, H. Xu, **J. D. Trawick**, D. Wall,L. Wang, V. Brown-Driver, J. M. Froelich, Kedar G. C., P. King, M. McCarthy, C. Malone, B. Misiner, D. Robbins, Z Tan, Z.-y. Zhu, G. Carr, D. A. Mosca, C. Zamudio, J. G. Foulkes & J. W. Zyskind. 2002. A genome-wide strategy for the identification of essential genes in *Staphylococcus aureus Molec. Microbiol*. **43 (6):** 1387-1400.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

Task Force Leader of a task force in Elitra collaboration with Merck. 2001-present. Organize and carry out efforts to build proprietary Elitra genetic system for identification of cellular targets "hit" by active compounds in the human pathogen, *S. aureus*. Co-leader of task force, designed principle genetic tools in effort and have overseen efforts of several person team in implementing these genetic tools.

Task Force leader of target validation for *E.coli* and *S. aureus* genes. 2000-present. Organized and executed efforts to validate essential gene targets recognized in Elitra genetic screening. Responsible for Elitra validation of essential gene targets in both of these organisms.

Member of team, Collaboration between Elitra and LG Chem. 2000-present. Responsible for functional evaluation of targets presented to LG Chem (Republic of Korea) as part of Elitra collaboration. Has evolved into responsibility for functional (i.e., biological role) of potential antibacterial targets in Elitra collaborations with other pharmaceutical firms.

Leader of team for essential gene identification in *Salmonella enterica* Typhimurium. 1999. Led effort to apply Elitra genetic technology to *Salmonella enterica*. Led effort to improve genomic library construction and genetic screening.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Shahrzad (Sherry) Nouraini	Post Doctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto, Toronto, Canada	Ph. D.	1997	Yeast Genetics
University of Toronto, Toronto, Canada	M. Sc.	1992	Yeast Genetics
York University, Toronto, Canada	B. Sc.	1989	Biology/Chemistry

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

J. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

1997-2001 Post doctoral fellow, Burnham Institute, San Diego, CA
2001-present Post doctoral fellow, Elitra Pharmaceuticals, San Diego, CA

Honors

1998-2000 University of California Tobacco Related Disease Research Program Fellowship
1992-95 National Cancer Institute of Canada Steve Fonyo Studentship
1990-92 Medical Research Council of Canada Studentship
1990 Ontario Graduate Scholarship
1989 University of Toronto Open Master's Fellowship
1989 Dean's Honor list
1988 Book prize award of Chemical Institute of Canada
1988 NSERC summer studentship
1986 NSERC summer studentship

K. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

1-The putative pore-forming domain of Bax regulates mitochondrial localization and interaction with Bcl-X_L. **Shahrzad Nouraini**, Emmanuelle Six, Shigemi Matsuyama, Stainslaw Krajewski, and John C. Reed (2000), Mol. Cell. Biol. 20: 1604-1615.

2-Yeast as a tool for apoptosis research. Shigemi Matsuyama, Shahrzad Nouraini, and John C. Reed (1999), Current Opinion in Microbiology 2: 618-623.

3-Genetic evidence for selective degradation of RNA polymerase subunits by the 20S proteasome in *Saccharomyces cerevisiae*. **Shahrzad Nouraini**, Deming Xu, Sue Nelson, Marcus Lee, and James D. Friesen (1997) Nucl. Acids. Res. 12: 3570-3579.

4-An RNA-dependent ATPase associated with U2/U6 snRNA in pre-mRNA splicing. Deming Xu, Shahrzad Nouraini, Deborah Field, Shou-Jiang Tang, and James D. Friesen (1996) Nature 381: 709-713.

5-Rpo26p, a subunit common to yeast RNA polymerases, is essential for the assembly of RNA polymerases I and II and for the stability of the largest subunits of these enzymes. **Shahrzad Nouraini**, Jacques Archambault, and James D. Friesen (1996), Mol. Cell. Biol. 16: 5985-5996.

6-Mutations in an Abf1p binding site in the promoter of yeast RPO26 shift the transcription start sites and reduce the level of RPO26 mRNA. **Shahrzad Nouraini**, Jim Hu, Linda D. B. McBroom, and James D. Friesen (1996) Yeast 12: 1339-1350.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
J. Gordon Foulkes	Executive Vice President, Research and Development

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Dundee, Scotland	Ph. D.	1979	Biochemistry
University College Cardiff, Wales	B. Sc.	1976	Biochemistry

A. Positions and Honors

Positions and employment

1980 – 1981 Postdoctoral fellow, University of Colorado, Denver, Colorado
1982 – 1984 Senior fellow, Massachusetts Institute of Technology, Massachusetts
1984 – 1987 Tenured Member of the Scientific Staff, The Medical Research Council (MRC), National Institute for Medical Research, London, U.K
1987 - 1990 Director of Therapeutics, Oncogene Science, Inc., New York, NY
1990 – 1992 Vice President and Director of Therapeutics, Oncogene Science, Inc., New York, NY
1992 – 1995 Vice President and Chief Scientific Officer, Oncogene Science, Inc., New York, NY
1994 – 1996 Appointed to the Office of the Chief Executive and the Board of Directors, Oncogene Science, Inc., New York, NY
1996 – 1998 Chief Technical Officer and Member of the Board of Directors, Aurora Biosciences Corporation, San Diego, CA
1999 – Present Executive Vice President, Research and Development, Elitra Pharmaceuticals, San Diego, CA

Publications:

Over 50 major publications and reviews prior to joining Oncogene Science in 1987. Examples:
Discovery and characterization of mammalian protein-tyrosine phosphatases: J. Biol. Chem. 258, 431-438; FEBS Lett. 130, 197-200.
Discovery in transformed cells of tyrosine phosphorylated nuclear proteins: Nature 325, 552-554.
Development of the first bacterial expression system for purification of a tyrosine kinase: J. Biol. Chem. 260, 8070-8077.
Identification of serine/tyrosine protein kinase cascade systems. Proc. Natl. Acad. Sci. U.S.A. 82, 272-276; EMBO J. 4, 3173-3178; Proc. Natl. Acad. Sci. U.S.A. 84, 4408-4412.
Identification of protein phosphatases in translational control. Proc. Natl. Acad. Sci. U.S.A. 82, 272-276; Proc. Natl. Acad. Sci. U.S.A. 79, 7091-7096; J. Biol. Chem. 258, 1439-1443.
Discovery of a new human oncogene. Nature 325, 635-637.
Cloning of TGF-β3. Proc. Natl. Acad. Sci. USA 85, 4715-4719.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
William A. Fonzi	Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Texas A&M University, College Station, TX	B.S.	1975	Zoology
Texas A&M University, College Station, TX	Ph.D.	1981	Microbiology
University of California, Irvine, CA	Post-Doc	1981-1985	Molecular Genetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Positions:
1998 - present Associate Professor Georgetown University
10/94-1998 Assistant Professor Georgetown University
7/94- 10/94 Associate Adjunct Prof. UC Irvine
9/89-6/94 Asst. Adjunct Prof. UC Irvine
9/85-9/89 Res. Associate UC Irvine

Awards and Other Professional Activities:
1997 Burroughs Wellcome Scholar of Molecular Pathogenic Mycology
1998 to present Editorial Board, Revista IberoAmericana de Micologia
1999 to present Editorial Board, Infection and Immunity
2000-2002 Editorial Board, Journal of Bacteriology
1999 external reviewer, International Institute of Genetics and Biophysics, Naples, Italy
2000 ad hoc reviewer, NIH Bacteriology Mycology II
2000 ad hoc reviewer, Research Grants Council of Hong Kong
2000 ad hoc reviewer, North Carolina Biotechnology Center

Research Projects Ongoing or Completed During the Last 3 Years:
"Regulation of Dimorphism in Candida albicans"
Principal Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: R01 (GM47727) Period: April 1, 1997 to March 31,2001
The aims of this project were to define the functions of the pH-regulated genes PHR1 and PHR2 and the mechanism of their pH-dependent regulation. The long term objective is to understand how dimorphism, a potential virulence attribute, is controlled and how this developmental process contributes to virulence.

"Environmental signals and virulence of Candida albicans"
Principal Investigator: William A. Fonzi
Agency: Burroughs Welcome Fund
Type: Scholar Award Period: July 1, 1997 to June 30, 2003
The aims of this project are to develope a method of isolating regulatory mutants using URA3 as a selectable reporter gene. The long term objective is to define the way in which environmental signals are integrated to control dimorphism.

"Niche-specific pathobiology of Candida albicans"
Principal Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: RO1 (AI46249) Period: August 1, 1999 to July 31,2003
The specific aims of this project are to define the function of the cell surface protein encoded by HWP1, to delineate the promoter elements controlling its developmental expression and to examine the relevance of these elements to expression during infection. The long-term objective is to understand the control of gene expression during infection.

"New approaches to target-specific antifungal agents"
Principal Investigator: Ronald L. Cihlar
Co-Investigator: William **A.** Fonzi
Agency: National Institutes of Health
Type: RO1 (CA88456-01) Period: March 24,2000 to June 30,20004
The specific aims of this project are to examine the potential of various proteins of Candida albicans as drug targets.

"Candida Albicans Microarrays"
Principal Investigator: Greenspan, John BDS
Co-Investigator: William **A.** Fonzi
Agency: National Institutes of Health
Type: PO1 (DE07946-14S1) Period: 05/01/00-04/30/02
The aim of this project is to develop a microarray containing all open readingframes of the Candida albicans genome.

Relevant Publications (Partial listing):

1. Donovan, M., J. J. Schmuke, W. A. Fonzi, **S.** L. Bonar, K. Gheesling-Mullis, *G*. S. Jacob, V. J. Davisson, and S. B. Dotson. 2000. Virulence of an ADE2 deficient Candida albicans strain in an immune-suppressed murine model of systemic candidiasis. Infect. Immun. In press.
2. Mouyna, I., T. Fontaine, M. Vai, M. Monod, W. A. Fonzi, M. Diaquin, L. Popolo, R. P. Hartland, and J. P. Latge. 2000. Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. J. Biol. Chem. 275(20): 14882-14889.
3. Yesland, K., and W. A. Fonzi. 2000. Allele-specific gene targeting in Candida albicans results from heterology between alleles. Microbiology. 146(9):2097-2104.
4. Barkani, A. E., **O**. Kurzail, W. A. Fonzi, A. M. Ramon, A. Porta, M. Frosch, and F. **A.** Mühlschlegel. 2000. Dominant active alleles of RIM101/PRR2 bypass the pH restriction on filamentation of Candida albicans. Mol. Cell. Biol. 20(13):4635-4647.
5. Heinz, W. J., **O**. Kurzai, **A.** A. Brakhage, W. A. Fonzi, H. C. Korting, M. Frosch, and F. A. Muhlschlegel. 2000. Molecular responses to changes in the environmental pH are conserved between the fungal pathogens Candida dubliniensis and Candida albicans. Int. J. Med. Microbiol. 290(3):231-238.
6. Tsuchimori, N., L. L. Sharkey, W. **A.** Fonzi, S. W. French, J. E. Edwards, Jr., and S. G. Filler. 2000. Reduced virulence of HWP 1-deficient mutants of Candida albicans and their interactions with host cells. Infect. Immun. 68(4): 1997-2002.
7. Fonzi, W. A. 1999. PHR1 and PHR2 of Candida albicans encode putative glysocidases required for proper cross-linking of b-1,3- and b-1,6-glucans. J. Bacteriol. 181(22):7070-7079.
8. Ramon, A. M., A. Porta, and W. A. Fonzi. 1999. Effect of environmental pH on morphological development of Candida albicans is mediated via the PacC-related transcription factor encoded by PRR2. J. Bacteriol. 181(24):7524-7530.
9. Sharkey, L. L., M. D. McNemar, **S.** M. Saporito-Irwin, P. **S.** Sypherd, and W. A. Fonzi. 1999. HWP1 functions in the morphological development of Candida albicans downstream of EFG1, TUP1 and RBF1. J. Bacteriol. 181(17):5273-5279.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Carlos Zamudio	Vice president, Drug Discovery Informatics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Los Angeles	B. Sc.	1979	Computer Science and Mathematics

A. Positions and Honors

Positions and Employment

1979-1984 Technical Staff, Defense Systems Group, TRW, Los Angeles, CA
1984-1986 Technical Staff, Jet Propulsion Laboratories, Pasadena, CA.
1986-1988 Product Development Manager, Technology Systems Organization, TRW, Los Angeles, CA
1988-1993 Group Leader, DNA Technology Cosnortium, Applied Biosystems Inc./Perkin Elmer, Foster City, CA
1993-1997 Director, Bioinformatics and Engineering, Sequana Therapeutics, La Jolla, CA.
1997-1999 Vice President, Bioinformatics, Axiom Biotechnologies Inc., San Diego, CA
1/99-10/99 President, Drug Discovery Informatics Consulting, San Diego, CA
1999-2002 Director of Drug Discovery Informatics, Elitra Pharmaceuticals, San Diego, CA
2002-Present Vice President, Drug Discovery Informatics, Elitra Pharmaceuticals, San Diego, CA

B. Selected peer-reviewed publications

N/A

C. Research Support

Appointments

. Member of the National Center for Biotechnology Information (NCBI) Board of Scientific Counselors, 1994 - 1997
. Ad-hoc member of the industry advisory committee to NCBI
. Ad-hoc member of industry advisory committee to the National Center for Genome Resources (NCGR)

Invited Talks

· Genome Sequencing Conference, Hilton Head, 1990, "Applications of the Fast Data Finder Systolic Array in Sequence Analysis"
. Japanese Genome Informatics, Tokyo, 1991, "Fast Data Finder and Sequence Analysis"
. Genome Sequencing Conference, Hilton Head, 1992, "Reconstruction of Very Large Sequencing Projects"
. CHI Conference on Bioinformatics, San Francisco, CA., 1994, "Automated Genome Analysis in Gene Hunting"
. CHI Conference on Bioinformatics, Baltimore, MD., 1995, "Automation for Genetic and Genome Analysis for Drug Discovery"
. American Chemical Society, Annual Meeting, Las Vegas, 1997, "Drug Discovery Informatics"
. Third Annual Meeting, The Society for Bioinolecular Screening, 1997, "Integrated Drug Discovery Informatics – Bioinformatics, Cheminformatics and Pharmacoinformatics"
. 1997 Annual Fall Meeting of the Biomedical Engineering Society, 1997, "Integrated Drug Discovery Informatics – Bioinformatics, Cheminformatics and Pharmacoinformatics"
. Association for Laboratory Automation – Lab Automation ' 98, 1998, "Integrated Drug Discovery Informatics – Bioinformatics, Cheminformatics and Pharmacoinformatics"
. Maximizing Discovery-Stage Lead Selection and Functional Validation, Oct 15-16, 1998, Philadelphia, Global Business Research LTD, "Pharmacoinformatics: Informatics for High Throughput Hit-to-Lead Analysis "
. Screen ' 99: Advances in High Throughput Screening Technologies, August 9-13, 1999, San Diego, Institute for International Research, "Knowledge management and decision support for HTS"

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Elitra Pharmaceuticals' laboratories are located in La Jolla, CA, where many biotech companies, UCSD, the Salk Institute, and Scripps Research Institute are also located. The Elitra facilities occupy 11,900 square feet. The laboratories contain all necessary standard equipment standard for molecular biology including incubators, cold room, warm room, centrifuges, freezers, chemical hood, a biosafety containment level 2 facility, electroporators, phase contrast microscope, spectrophotometer, autoclaves, 5 PCR machines, ice machine, ABI 377 and 3700 DNA sequencers, chemical balances, etc. Also included is equipment for automated picking and replica gridding of bacterial and fungal colonies. We have added an automated chemical screening system that was in place in October of 1999. Informatics support includes an internal TCP/IP network connected to the Internet through a high-bandwidth connection, and both multi-processor Windows NT and Sun Microsystems' Solaris computers serving as computer and file servers.

Computer:

Office:

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

ABI 377 and 3700 DNA Sequencers
2 Colony pickers that each pick bacterial colonies at a rate of 1800/hour
2 Gridders that can move bacterial cells from microtiter plates containing liquid or solid media into liquid or solid media
Biomek robots that carry out a variety of tasks including serial dilutions, plasmid minipreps, setting up of PCR reactions for sequencing, and PCR product cleanup

RESEARCH PLAN

A. Specific Aims

Identifies market.

The yearly world market for antimicrobial drugs is over $22 billion, making this the third largest pharmaceutical market. An important and growing component of this market is in the antifungal area. Fungal pathogens such as *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* cause increasingly problematic diseases in healthy and immunocompromised hosts.

A critical problem in combating fungal infections is that many of the existing antifungal drugs target eukaryotic processes common to both the fungi and mammals. For example, of the several different antifungal drug classes, three target sterol synthesis or directly target plasma membrane sterols (32). The potential and reality of drug toxicity are readily apparent because these drug targets are homologous between fungi and mammals. Picking new targets can be complicated by the many other similarities among all eukaryotes at the levels of cell biology and biochemistry. These facts point to a need to identify and utilize new fungal targets that are absent in humans for the development of more effective and safer antifungal agents.

The most important human fungal pathogen is *Candida albicans*. Capable of switching between yeast and mycelial forms, *C. albicans* causes both topical and systemic infections in humans. Though *C. albicans* is closely related to the well understood model organism, *Saccharomyces cerevisiae*, there are many differences in biology between the two, including the pathogenicity of *C. albicans,* that point to a need for identifying targets and processes within *C. albicans* (26, 31, 32, 40).

One tool that has proven powerful in identifying critical targets is screening for dominant negative phenotypes. Dominant negative screening for genes and phenotypes associated with essentiality will be used to obtain new antifungal targets in *C. albicans*. This fungus has been difficult to study experimentally because of its asexual diploid nature and variant genetic code. The phase I goals are:

Clearly lists two measurable Phase I goals.

1. Construction and optimization of a *C. albicans* site-specific integrating expression vector. A suitable vector has already been constructed and subjected to preliminary testing. Phase I work will be initiated by cloning the *C. albicans* MET3 promoter into this vector and comparing the induction and repression in *C. albicans* of MET3 promoter or MAL2 (already constructed) promoter driven variations of this vector using a reporter gene. Further optimization and testing of alternative promoters will extend and complete this aim. The vector is a site-specific integrating *C. albicans* expression vector allowing exchange of alternative promoter cassettes. This is to construct the best vector possible for expression library screening and to test this with known dominant lethal genes.

2. This vector will be utilized for production and identification of dominant negative mutants with cDNA libraries; the phenotype to be tested is growth/viability. Libraries will be constructed, mutated, and used in large high-throughput screens to identify *C. albicans* genes that are potential antifungal targets because of their dominant negative phenotype. During this phase, all of the cDNA libraries will be screened to saturation and also subjected to chemical, PCR-based, and deletion mutagenesis and screened to saturation using the high-throughput capabilities at Elitra. As targets are identified, they will be evaluated in terms of their presence in other fungi and in mammals.

Item #3 is not a Phase I goal and probably should have been stated in a separate paragraph.

3. The ultimate goal in phase II will be to take the new *C. albicans* targets that have been found during phase I, prioritize these based on essentiality in this pathogenic fungus and not in mammals and employ the targets in the cell based assays developed at Elitra. Hits will then be validated and developed into leads for new antifungal drugs.

B. Significance

Describes project's importance.

Candida albicans is the single most important fungal pathogen in humans (31). In particular, *C. albicans* causes oral and systemic candidiasis in immunocompromised patients and vulvovaginal candiadiasis (VVC) in women. Candidiasis is an extremely important problem in HIV-infected patients, 84 % of who exhibited oropharyngeal colonization by *Candida* spp. in a 1994 study (45). VVC is extremely widespread and a significant medical problem. According to the CDC, some 75 % of women in the USA will have at least one episode of VVC in their lives, 40 % will have two, and a smaller number (~5 %) will have the recurrent form (45). Taken together, this information demonstrates the significant medical and economic importance of *C. albicans* pathogenesis.

There is an increasing need for safer and more effective antifungal agents. Some of the more effective antifungal agents, amphotericin B and the azoles (e.g. fluconazole, itraconazole) have toxicity problems because their cellular targets have homologues in mammalian cells. The azoles inhibit lanosterol 14 α–demethylase, a cytochrome P450 enzyme critical for sterol synthesis in fungi and mammals; the azoles are also effective inhibitors of many cytochrome P450 reactions and

because of this are useful tools in mammalian cell biology (27). Amphotericin B targets plasma membrane sterols and is nephrotoxic (32). Additionally, *C. albicans* strains resistant to the azoles have been on the increase in recent years (32).

C. albicans exhibits a complex life cycle dependent upon in vitro and in vivo growth conditions. Normally, *C. albicans* grows as a yeast cell or blastospore at 30^{o} C and with glucose as a carbon source. However, when cultured in the presence of serum, with carbon sources such as N acetyl-glucosamine, at elevated (37^{o} C) temperatures, or at altered (higher) pH, *C. albicans* switches to a predominantly hyphal form (40). The transitions between the yeast and hyphal forms appear to be essential for virulence. Non-hyphal *C. albicans* strains are avirulent (15) as are obligately hyphal *C. albicans.*

Despite the importance of *C. albicans* to human disease, work on this organism has often been hindered due to its asexual diploid nature and variant CTG codon (in *C. albicans* CTG encodes serine instead of leucine; 28,46). The more facile organism for molecular biology, *S. cerevisiae,* is able to transition between haploid and diploid forms, and is suited to mutagenic analysis of gene function using knock-outs. Though fast and reliable gene disruption methods have been described for *C. albicans* (17,36,60) the lack of sexual cycle along with some manner of inducing meiosis and sporulation means that essentiality of a gene must usually be inferred from negative results. Much work has focused on using *Saccharomyces cerevisiae* as a surrogate model for *C. albicans* genetics and biology, however the many significant genetic, developmental, and pathogenic differences between the two organisms show that much more can be learned by developing methods to study *C. albicans* directly.

Describes innovative approach.

There is a critical need to identify new and better targets in *C. albicans* and other pathogenic fungi that can be exploited for antifungal drug development. It is important to perform research directly in *C. albicans* to understand and exploit the unique characteristics of this organism. *C. albicans* is ideally suited to the dominant negative approach because of its diploid nature, complex development, and variant genetic code (20, 33). Dominant genetic methods work because some gene products, often those involved in important regulatory processes, will become trans-dominant inhibitors when the gene is mutated or overexpressed. This methodology is attractive for *C. albicans* because it can be applied to diploid organisms or organisms that lack means for conventional approaches such as targeted gene disruption. In theory, gene products with multiple sites, such as catalytic and regulatory domains that interact with other polypeptide or nucleic acids are potentially susceptible to trans-dominant analysis (20, 49,51). This approach has proven very valuable in a number of systems without conventional genetics (33,38,51), such as mammalian somatic cell culture, and has even proven useful in the classical genetic model, *S. cerevisiae* (42).

In bakers' yeast, *S. cerevisiae*, results of several large-scale screens for dominant negative mutants have been published (30, 43), and have identified genes involved in growth (1, 42), mating type regulation (58), and other processes. Moreover, *S. cerevisiae* has been successfully used as a surrogate background for analysis of *C. albicans* libraries by a dominant negative approach (58), one indication of the similarity of biological functions between the two organisms. Surprisingly, this screen identified a number of dominant genes that interfere with *S. cerevisiae* mating type control of the cell cycle (58). Though lacking a sexual cycle, *C. albicans* does have homologues of the *S. cerevisiae* mating-type genes (21) as well as homologues for the regulators of these genes (13) though the *C. albicans* functions encoded by these genes may vary considerably from their *S. cerevisiae* equivalents.

Refers to published studies that suggest likelihood of success.

Dominant negative gene analysis works in *C. albicans*. Both a directed dominant negative (7) and identification of a filamentation-causing dominant gene from a library screen (6) have been reported. Recently, a mutant allele of the Ca-SEC4 gene was overexpressed in *C. albicans* (33). The targeted gene, SEC4, is a Ras-like GTPase that appears to be essential in *C. albicans* and was mutated to mimic a well-characterized trans-dominant mutation in mammalian Ras. The dominant negative allele of SEC4 was successfully used to demonstrate the functional role of SEC4 in cell growth and protein secretion (33).

In *C. albicans*, it is probable that overexpression of some genes can lead to a dominant phenotype (24,33). Some genes in *C. albicans* appear to be uniquely sensitive to minor copy number alterations (22, 39). Regulation of the sorbose utilization gene, SOU1, appears to be through a regulated shift in copy number of *C. albicans* chromosome 5, since monosomic strains assimilated sorbose while non-assimilating disomic strains did not (22). Selection for fluconazole resistant strains of *C. albicans* also resulted in chromosomal copy number shifts (39). Results such as these suggest that there is a distinct possibility that many *C. albicans* genes are regulated through dosage effects and would likely be susceptible to dominant screens involving overexpression.

Describes preliminary studies.

To identify genes regulating filamentous growth in *C. albicans*, a library was introduced using the REMI method (6). A putative transcription factor with a zinc-finger domain was isolated. Taken together, the two very recent reports (6) of dominant gene isolation in *C. albicans* demonstrate very dramatically the possibilities inherent in working directly *in C. albicans*. Therefore, it would seem likely that larger-scale analysis are possible with suitable vectors and promoters. The goals of this project are to optimize a *C. albicans* expression vector and to exploit the unique characteristics of this vector in dominant negative phenotype screening of *C. albicans* genes involved in growth control and viability of the organism.

Details company capabilities.

The new genes identified in this research will be included in Elitra's unique relational database of both targets and drug screens for all the major gene/protein targets across multiple pathogens. This database will markedly enhance the ability of Elitra and its corporate partners to make informed decisions on which novel targets to pursue. Drug screens will be developed for these novel targets and used to identify new antifungals with high-throughput screening of chemical libraries. Elitra has assembled a team of scientists with extensive experience in developing drug screens for a wide range of targets. Elitra's current library is in excess of 130,000 compounds and can screen at rates in excess of 20,000 compounds per day.

C. Relevant Experience

The principle investigator for the proposed research project will be Dr. John D. Trawick. Dr. J. Gordon Foulkes will assist the project as co-investigator. Dr. William Fonzi will participate as a consultant. Experimental design and execution will be assisted by Mr. Trung Phuong. The qualifications of the investigators are listed below.

Principal Investigator

Shows experience to accomplish proposed study.

Dr. Trawick has over 20 years of experience in molecular biology, genetics, and microbiology in yeasts, bacterial, and mammalian systems. This background has given him extensive experience in the control of gene expression and in the construction and use of plasmid vectors. He graduated cum laude from Gustavus Adolphus College, St. Peter, Minnesota with a B. A. in Biological Sciences. After obtaining a M. S. degree in Biological Sciences from Northern Illinois University, Dekalb, Illinois, he enrolled in the Ph.D. program at the University of Minnesota, Mayo Graduate School of Medicine. Research for his doctoral dissertation, "Control of mini-F plasmid DNA replication" was carried out in the laboratory of Dr. Bruce C. Kline at the Mayo Clinic in Rochester, Minnesota. His research focussed on elucidating the transcriptional interactions involved in plasmid copy number control and DNA replication. He received his Ph.D. in Microbiology from the University of Minnesota in 1984. After obtaining his Ph.D. degree, Dr. Trawick worked in the laboratory of Dr. Michael Getz at the Mayo Clinic under a cancer training grant.

In 1985 Dr. Trawick moved to the laboratory of Dr. Robert O. Poyton in the Department of Molecular, Cellular, and Developmental Biology at the University of Colorado, Boulder, Colorado. In Dr. Poyton's laboratory, he studied the control of cytochrome oxidase gene regulation and nuclear-mitochondrial interactions in the yeast, *Saccharomyces cerevisiae*. As part of this effort he conducted an extensive study of COX6 gene promoter structure and of the transcription factors acting on this promoter.

In 1990, Dr. Trawick became an Assistant Professor (Adjunct) in the Department of Medicine, University of Colorado Health Sciences Center, Denver. At the Health Sciences Center Dr. Trawick studied the regulation of cholesterol metabolism and bile acid synthesis in mammalian cells within the Hepatobiliary Research Center under Dr. F. R. Simon and Dr. R. A. Davis. From 1992 to 1996, he was an Adjunct Assistant Professor in the Biology Department at San Diego State University Foundation also working on cholesterol metabolism in mammals and collaborating with Dr. R. A. Davis. Dr. Trawick is currently still an Adjunct Research Professor in the Department of Biology at San Diego State University, San Diego, California. While working at the Health Sciences Center and at San Diego State University, Dr. Trawick received grants from the University of Colorado, the American Heart Association of Colorado, and the American Heart Association of California.

In 1997, Dr. Trawick was hired by Dr. Judith Zyskind, founder of Elitra Pharmaceuticals, as the first employee of Elitra Pharmaceuticals; Dr. Trawick is currently head of the yeast (*Candida albicans*) gene identification program.

Co-Principal Investigator

Dr. Foulkes has over 20 years of research experience in signal transduction, molecular biology, and drug discovery. He has managed research teams of over 120 scientists and research budgets of $38MM annually. He trained in several of the world's top research laboratories including Professor Sir Philip Cohen (Univ. of Dundee), Professor Raymond Erikson (now at Harvard) and Professor David Baltimore (currently President of California Institute of Technology), before running his own laboratory as a tenured member of the Medical Research Council, U.K. For the last 12 years, he has headed research efforts in 3 biotechnology companies, Oncogene Science, Aurora Biosciences, and now at Elitra Pharmaceuticals. Previous industrial experience with the SBIR program led to multiple successes, including major collaborations in the anti-infective area with both Biochem Pharma and Sankyo, in addition to internal successes in moving programs forward. His experience also includes advanced engineering automation and drug discovery, through to moving drug candidates into Phase 2 clinical trials.

Research Associate

Trung Phuong has extensive practical experience in a wide range of molecular biology techniques and has expertise that is particularly applicable to this research proposal. He gained experience in expression vector development and optimization while working at Chiron, Inc. Since moving to Elitra Pharmaceuticals in 1998, Mr. Phuong has had extensive experience in vector development and expression library construction and high throughput screening in several different systems. Mr. Phuong has worked with yeast, bacterial systems, and mammalian systems.

D. Experimental Designs and Methods

***Development and optimization of a *C. albicans* expression vector**

This proposal requires a vector system for introduction of inducible promoters controlling the expression of *C. albicans* DNA fragments that allows efficient and stable genetic transformation of *C. albicans*. For library-scale transformations and screening, standard plasmid vectors have proven unreliable in *C. albicans* due to the lack of stable replicons. Vectors from other yeast species such as *S. cerevisiae* will not work in this organism. Therefore, a site-specific integrating *C. albicans* expression vector was chosen to facilitate the dominant negative analysis to be proposed in this application.

Shows proposal builds on previous work.

Previous work in this laboratory has focused on the development of just such an integrating vector system. The site-specific integrating vector pEF/SceTARGET constructed by W. Fonzi (personal communication) was chosen as a backbone for this vector. It contains an I-Sce I meganuclease site that also has been placed within the genome of *C. albicans* strain CYZ-1 at a disrupted chromosomal copy of the Ca-URA3 gene. When this vector is linearized at the I-Sce I site and transformed, insertion at the described chromosomal locus within the Ca-URA3 gene is favored. (Fig 1). This vector and all of its derivatives are *C. albicans-E. coli* shuttle vectors with pUC19 replication origins and ampicillin resistance markers to facilitate plasmid DNA production, library or clone construction, and analysis of clones.

Several episomal plasmids for use in *C. albicans* have been constructed and can be useful when a single gene is to be expressed in *C. albicans* (8, 25, 41) but use of these in expression library-based methods is difficult because of general plasmid instability. Therefore, a site-specific integrating *C. albicans* expression vector is required for ectopic expression of dominant-negative clones in this organism. To construct the needed vector, several components have been assembled and tested (Figures 1and 2 and Tables 1 and 2, below). This *C. albicans* expression vector can efficiently and reliably integrate into a neutral site (Table 1), show highly regulated expression controlled by a *C. albicans* promoter, and will facilitate retrieval of sequences or of the whole vector for characterization or subsequent experiments. When 0.5 μg of pEF/SceTARGET DNA was transformed into the correct host, CYZ1, around 10^3 transformants per μg resulted, ten-fold better than the number of transformants into the non-specific integrating host, CAI4 (16)(Table 1). This result shows that a site-specific integrating vector can work much more efficiently in *C. albicans* than a randomly integrating vector. This improvement in overall transformation efficiency is necessary for library-scale expression projects in this organism. Though transformation efficiencies for *C. albicans* are still well below levels achievable with *S. cerevisiae*, 10^3 transformants per μg is sufficient for library transformation and even more efficient methods are becoming available (14). Other methods to increase site-specificity of integration, such as restriction enzyme mediated integration (REMI), have also been employed in *C. albicans* for the same purpose (7).

Optimization of the components to the overall technology will likely be required to insure efficient transformation and characterization of *C. albicans* recombinant clones. Preliminary tests and screens using the proposed technology indicate that we will be able to obtain large numbers of *C. albicans* transformants and to screen these for a set of desired phenotypes.

Clear figures, tables and legends help reviewers understand the project.

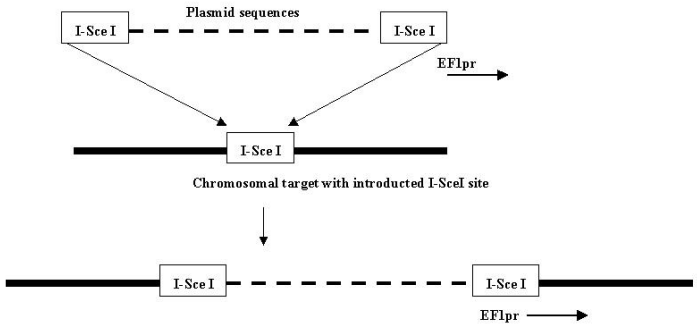

***Figure 1. Site-specific integration using I-Sce I.** The plasmid vector of choice has a single I-Sce I meganuclease site within a region of several hundred base pairs of homology to the chromosomal target (open boxes). To enhance the recombination frequency, a strong, constitutive promoter, Ca-EF1pr (translation elongation factor 1 gene) is oriented towards the I-Sce I site. The plasmid is linearized with I-Sce I and transformed into a host with an identical target region, i.e. the several hundred base pairs of sequence identity, the I-Sce I site, and the promoter. The final integrant should be in a single copy and the Ca-EF1 promoter oriented outwards from the integrated plasmid sequences. The *C. albicans* vector, pEF/SceTARGET has this configuration with the complete Ca-URA3 gene for selection of uridine prototrophy in *C. albicans* and an *E. coli* shuttle vector for propagation in bacteria.

***TABLE 1, Transformation of *C. albicans* with pEF/SceTARGET**

***C. albicans* strain**	**Colonies on SD –ura plates**
CYZ-1	**402, 408**
CAI4	**29, 33**

C. albicans strains CYZ-1 and CAI4 are both uridine auxotrophs because both copies of the native URA3 gene have been disrupted (17); in CYZ-1 one of the deleted alleles of the URA3 gene has been replaced with phage λ DNA into which a I-Sce I meganuclease (5'-TAGGGATAA/CAGGGTAAT-3') site has been inserted. No I-Sce I sites are present in the deleted URA3 alleles of CAI4 (17). Into cells of each, 0.5 μg of pEF/SceTARGET were transformed by the Candida-lithium acetate/PEG one-step transformation protocol (10). Transformant colonies were selected for growth on synthetic dextrose drop-out medium lacking uridine (52). Results are from duplicate transformations.

Figures and tables located in the body of the text rather than at the section's end makes reading easier for reviewers.

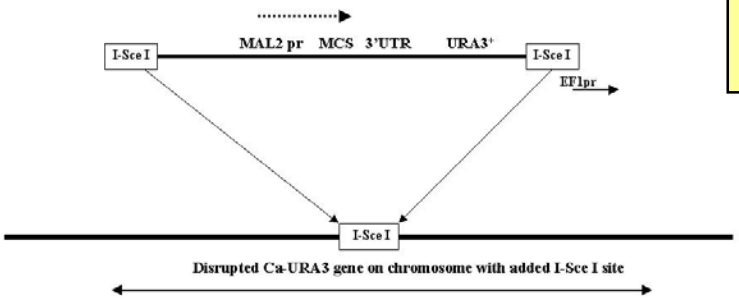

Figure 2. Schematic diagram of pEF/SMAL2. This plasmid has the basic structure and is used as shown in Figure 1. The plasmid features the Ca-MAL2 promoter region (500 bp) or the MET3 promoter region (9). A multiple cloning site, and a yeast 3'-UTR region in addition to the URA3 selectable marker for *C. albicans* and the *E. coli* shuttle sequences for bacterial propagation are also present(but not depicted).

Promoter choice and requirements.

The best possible properties of a promoter for an expression vector are repressibility to zero background and strong inducibility, with induction and repression mediated by factors which do not otherwise affect growth or differentiation. Due to the variant genetic code, it hasn't been possible to adapt promoters requiring transcription factors derived from other species, so *C. albicans* molecular genetics has lagged. Very recently, the MET3 promoter from *C. albicans* has been cloned and demonstrated to by nearly completely repressed when cells are grown in the presence of methionine or cysteine (9). When both of these amino acids were present at 0.5 mM the MET3 promoter was fully repressed (9). Furthermore, when these amino acids were removed from the culture medium, an induction of 85 fold occurred (9). Switching the MET3 promoter on or off by removal or addition of methionine or cysteine does not affect *C. albicans* growth or morphology and control can be accomplished in host strains prototrophic for these amino acids. Therefore, the MET3 promoter sequences will be isolated and cloned as described (9) into the pEF/SceTARGET vector. The minimal length of the MET3 promoter appears to be 1362 bp (9). The MET3 promoter fragment will replace a cassette that contains the 500 bp Ca-MAL2 promoter to create pEF/SMET3, in addition to pEF/SMAL2. This will result in two different expression vectors, one with the MET3 promoter and the other with the maltase promoter (7, 12, 18). The MAL2 promoter controls the maltase gene and is repressed when glucose is added to the growth medium, and induced when maltose is added. The resulting plasmid also contains a multiple cloning site, and the 3' UTR and transcriptional termination region of the Sc-CYC1 gene. The latter will insure proper termination of transcription, cleavage and polyadenylation. Transcriptional terminators and cleavage and polyadenylation regions are generally interchangeable among the yeasts and fungi (19, 23), therefore it is likely that these *S. cerevisiae*-derived transcription termination elements will work in *C. albicans*. In the unlikely event that proper termination

and mRNA processing does not occur with this region, a substitute from an endogenous *Candida albicans* gene such as the maltase gene will be cloned into the vector. (Fig 2). While the MET3 promoter driven expression vector will be the first choice and the MAL2 promoter vector, the second, a few alternative inducible promoters are available for *C. albicans*. These include HWP1, repressed by glucose and induced by serum and 37 C incubation (50, 56), PHR1, which is subject to pH-titratable control (37, 47), and the GAL1 promoter, though apparently not regulated as tightly as in *S. cerevisiae,* it is induced strongly by galactose (32,33).

The repressed state of a promoter on an expression vector should be complete to insure that leakage from the promoter does not cause loss of some clones from the library. However, most eukaryotic promoters do retain some small basal expression (55). This is why the MET3 promoter looks so attractive; no measurable growth occurred in *C. albicans* cells deleted for the native alleles of URA3 when a copy of URA3 controlled by the MET3 promoter was fully repressed (9). This repression was stronger than that seen for other regulated *C. albicans* promoters (9, 12). Prior to the characterization of the MET3 promoter, three *C. albicans* promoters were tested and compared (Table 2). Two of these (MAL2 and HWP1) were strongly repressed by a LacZ reporter assay and were strongly induced.

***TABLE 2, LacZ Assays in *C. albicans* CYZ-1**

Promoter	Glucose (repressing)	Maltose/Sucrose	Serum
MAL	**<0.3**	**1.73—4.2**	**n.d.**
ACT	**14.6**	**27**	**23**
HWP	**<0.1**	**n.d.**	**6-17**

LacZ fusions to maltase (Mal), actin (ACT), and HWP1 promoters were transformed into *C. albicans* CYZ-1 and URA+ transformants selected. Cultures were grown and assayed as suggested in Uhl and Johnson, 1999 and by Uhl (personal communication). The units are in β-galactosidase units from the ONPG assay (34). The variation is due to multiple experiments with variables in growth time and state.

Expression must be sufficient to produce enough of the gene product to interfere with normal activity of that enzyme. This need not be equivalent to the expression level of the native gene if the effect is dominant. Hypothetically, a single dominant negative polypeptide normally part of a homotetramer could, in principle, poison the active tetrameric complex. However, for overexpression-based gene identification, overall expression should be higher than total endogenous expression. The strongest described *C. albicans* promoters are probably those for genes such as actin (35), which is largely constitutive. The recently described MET3 promoter also appears to be very strongly induced (9), the 85 fold induction observed approaches the apparent maximum seen in microarray assays of the related organism, *S. cerevisiae* (48, 16).

To test whether or not our promoter/vector system is sufficient for this kind of gene identification, the CA-actin and/or tubulin genes will be cloned into the vector/promoter as outlined for libraries above and transformed into *C. albicans* CYZ-1. Overexpression of either actin or tubulin is sufficient to inhibit growth in *S. cerevisiae* (30). If overexpression of the *C. albicans* actin gene is sufficient to cause a growth defect, this will be observed under the inducing but not repressing conditions. Failure to overexpress actin or tubulin sufficiently to block growth or failure to overexpress TUP1 sufficiently to block filamentation will require use of an alternative promoter or mutagenesis of the existing promoter to increase expression.

Reporter gene for *C. albicans

The *Streptococcus thermophilus* lacZ gene (Material Transfer Agreement in place) functions as a reporter of gene expression in *C. albicans*. We have used this reporter to test the Ca-MAL2, Ca-HWP1 promoters, as well as alternative promoters such as the Ca-ACT1 promoter. To initiate the phase I study, the MET3 promoter will be cloned into pEF/SceTARGET as described (9). Then, the *S. thermophilus* lacZ gene will be used as a reporter to compare expression to other available *C. albicans* promoters. Preliminary results indicate that the MAL2 and HWP1 promoters are highly controllable, with strong repression and induction (up to several β-galactosidase units for single copy integrants) (Table 2). Judging from the published report (9), the MET3 promoter is expected to be completely repressed and more strongly induced. The site-specific integrating vector, pEF/SMAL2 has been constructed but a variant with the MET3 promoter needs to be constructed.

Cloning of lacZ reporter into the site-specific integrating vector. The appropriate restriction fragment of the *Streptococcus thermophilus* lacZ gene will be obtained by restriction digestion or PCR amplification and cloned into the multiple cloning region of pEF/SMET3 or pEF/SMAL2. After the structure and sequence of the recombinant plasmid has been confirmed, it will then be transformed into *C. albicans* strain CYZ-1 and transformants selected on minimal medium minus uridine (17, 52, a.k.a. SD –ura). The location and copy number of a small random collection of transformants will be examined by genomic DNA purification followed by PCR amplification. These recombinant *C. albicans* strains will be assayed for β-galactosidase activity when grown in +/- methionine-cysteine synthetic dextrose medium for repression or induction of MET3. The MAL2 promoter will be assayed in repressing (glucose) and inducing (maltose) medium over a

suitable time course. Alternatives to this strategy are: use of random site integrating vectors that allow efficient regulated expression but do carry the risk of causing mutations upon integration; alternative promoters: PHR1(37), induced by pH, and ACT1 (35) which is largely constitutive; construction of different site-specific integrating vectors that target another non-essential *C. albicans* allele.

Gene retrieval methodologies. Colony PCR from yeast colonies will be used to obtain the sequences of the cDNA giving the desired phenotype(s). Several efficient colony PCR methods from yeast, as well as bacterial colonies, are available (29, 53) and are standard methods in use at Elitra. Rescue of integrated plasmids by restriction digestion and ligation (44) will be employed to obtain the whole recombinant plasmid. The I-Sce I site within the vector and targeting site in the *C. albicans* genome will be exploited in this protocol. Genomic DNA from *C. albicans* that contains the integrated plasmid will be purified by standard means and completely digested with meganuclease I-Sce I. Then, the digested DNA will be ligated with T4 DNA ligase and electroporated into electrocompetent *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA) with an efficiency of 10^9 transformants per microgram. Rescued plasmids will be selected for ampicillin resistance and confirmed by restriction endonuclease digestion and DNA sequence analysis.

Identification of dominant negative genes in *C. albicans*. The vector/promoter system described and tested above, will be used to identify genes important to *C. albicans* growth and viability using a dominant negative approach. To identify dominant negative genes in *C. albicans*, full-length cDNA libraries will be constructed. These will be both unmutagenized and mutagenized (see below). A directed orientation cDNA library will be made by enriching poly A-containing RNA from total RNA using oligo dT cellulose according to standard methods (3). The first strand of cDNA will be made from purified poly A RNA by reverse transcriptase; second strand synthesis will be via DNA polymerase I. Commercial kits from supplies such as Life Technologies (Gaithersberg, MD) will be used to synthesize cDNA. Size selection of double-stranded cDNA will be performed to insure that predominantly full-length cDNA clones are synthesized. The cDNA library will be directionally cloned into pEF/SMET3 or pEF/SMAL2 by using primer/adapters for cDNA synthesis with specific restriction endonuclease sites to confer directionality (e.g. the 3', oligo-dT primer/adapter may have a NotI site and the 5' primer adapter a SalI site). To ascertain the quality of the cDNA library, random clones will be isolated and the DNA sequence of the inserts determined. A high quality library will be judged to contain fewer than 5 % vector lacking inserted clones, and the cDNA clones will be largely or entirely full-length and containing coding regions with total numbers of unique recombinant plasmids sufficient to saturate the genome. Identification of genes will be through BLAST searches of the Candida (http://sequence-www.stanford.edu/group/candida/ and http://alces.med.umn.edu/Candida.html), Saccharomyces (http://genome-www.stanford.edu/Saccharomyces/), and NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) public databases.

An advantage of cDNA libraries is that only coding regions, and, if size-selected, predominantly intact genes are cloned. This will force expression of clones containing genes expressed from their native start codons.

The cloned library DNA will be linearized within the vector using I-Sce I and then transformed into *C. albicans* strain CYZ-1 and integrants selected on minus-uridine medium. The extreme rarity of I-Sce I recognition sites means that it is unlikely that any cDNA inserts will contain a recognition sequence for the meganuclease. Transformant clones will be plated onto repressing medium (e.g. containing methionine and cysteine if the MET3 promoter is used). A high-throughput replica plating method will be used to identify dominant negative clones; i.e. clones that do not grow when induced. Colonies from a plated library will be picked and transferred to liquid repressing medium in 384 well plates with a robotic colony picker (GeneMachines, Inc, San Carlos, CA). This colony picking and inoculating device can aseptically identify and pick colonies from transformant plates at the rate of 2,000 colonies per hour; colonies are transferred to recipient plates containing liquid growth medium. After a growth period, these clones will then be robotically replica plated (BioGrid replica gridding device from BioRobotics, Ltd., Cambridge, U.K.) onto both repressing (+0.5 mM methionine/cysteine) and inducing (0 mM methionine/cysteine) plates. Robotic colony picking and replica plating enable the screening of 10,000 to 50,000 colonies per day at Elitra.

In screens for growth inhibitory genes, dominant negative clones will be those that grow on repressing medium and that fail to grow on inducing medium. Growth inhibition such as this may be very strong with a complete or nearly complete inhibition of growth. Alternatively, weaker growth inhibition might also be observed. This type of phenotype will be apparent on the robotically gridded plates but is subject to gridding artifacts. Plating serial dilutions of all putative growth inhibitors from the initial scoring of sensitives will be done to confirm the phenotype and score the relative strength of the phenotype.

As with all dominant gene technologies, the principle is to identify an essential process or interaction. Overexpressing a component of an essential multicomponent pathway may disrupt the whole pathway (20, 59). Several assayable phenotypes including growth (viability) and filamentation are suitable for screening by overexpression. For instance, the CA-TUP1 gene is a repressor of filamentation in *C. albicans* and is a factor in the control of this critical morphogenic pathway (5). One would predict that overexpression of TUP1 might block filamentation even under conditions known to induce TUP1-dependent filamentation such as serum and incubation at 37 C (5). In *S. cerevisiae*, genes that block growth (1, 30) or cause various phenotypic changes (43, 58) have been discovered by this method. Lethal overexpression

could also include events that interfere with viability by unbalancing concentration sensitive pathways such as actin and tubulin assembly

Shows investigators are prepared to test for artifacts.

The phenotype of clones sensitive to induction will be retested by regridding to confirm the identification of potential positive clones. Certain classes of false positive clones can be expected. *S. cerevisiae* transformations sometimes yield high rates of petite mutations. Such artifacts can be checked by comparing specific phenotypes, monitoring induction of the phenotype or by selecting for plasmid loss on counter-selective medium (5-fluourorotic acid containing plates will counter select URA3 genes in *C. albicans* as well as *S. cerevisiae*). Another type of artifact could be the illegitimate insertion of a plasmid into a gene, causing a mutation in cis. True sensitives will then be identified by PCR, inverse PCR, or plasmid rescue cloning, all followed by DNA sequencing. Artifactual production of mitochondrial petites may be much less likely with *C. albicans* than with *S. cerevisiae* because of the paucity of reports of petite and, therefore, respiration-deficient *C. albicans* strains compared to *S. cerevisiae* (2). All types of artifacts can be screened for by retransforming the identified clones into the parental *C. albicans* strain and confirming that the phenotype is due to the recombinant plasmid and not to some other factor.

Creation of mutagenized cDNA libraries. The approach outlined above depends on ectopic expression or overexpression of genes resulting in a dominant negative phenotype. There is considerable precedent for this phenomenon (30, 43). However, some dominant phenotypes require point mutations altering an enzymatic activity (33) while deletion mutations may favor other dominant phenotypes (20, 49, 51). To this end, cDNA will be made from *C. albicans* mRNA as described above. Then the DNA will be subjected to an in vitro mutagen. One such mutagen is hydroxylamine that hydroxylates cytosine residues and leads to a transition mutation after replication (54). The mutated DNA will be purified from the mutagen and then cloned into the vector, making a mutant genomic DNA library. Alternatively, mutagenic PCR amplification of the library may also provide a means of obtaining the desired mutants. This methodology greatly decreases the likelihood of multiple factors (e.g. promoter mutation and gene mutation) confounding the analysis. Transformation, selection, screening and gene identification will proceed as described above.

A cDNA library containing deletions will also be constructed. As discussed further above, this approach is more likely to identify membrane or secreted proteins that are toxic when overexpressed. These artifacts will be eliminated post-screening by using bioinformatic methods (GCG Wisconsin package programs) to identify domains of dominant negative clones. Deletion mutations will be constructed by synthesizing cDNA as described above and subjecting the cDNA to restriction enzyme digestion, DNAseI treatment, or mechanical shearing. In all cases the 5' (and therefore amino terminal) end will contain the SalI cloning site attached during second strand cDNA synthesis. Therefore, the library will mainly contain clones that will still initiate translation from their endogenous start codons. To insure proper termination of translation, stop codons in all three reading frames will be introduced 3' of the downstream cloning site and upstream of the transcription termination region.

As with any technology, there are limitations to the kinds and numbers of genes that can be identified. Essentiality or cell viability changes due to dominant negative expression may differ from effects seen in knockouts of the same genes. However, dominant negative screens, by their very nature (20, 38, 42, 49, 59), target essential reactions, complexes, or interactions. To determine if a particular gene in *C. albicans* is essential, null alleles of these genes will be created by the *C. albicans* URA-blaster technique (17). The limitation, due to the lack of a *C. albicans* sexual cycle, is that essentiality of a gene is seen as a negative result. Placing the gene under an inducible promoter and disrupting the normal alleles of the gene is the singular way to definitively demonstrate essentiality in *C. albicans* (4, 9). However, dominant negative mutants from mutagenized clones (deletions or point mutations) are more likely to produce a higher fraction of true essential genes. Another qualification to this type of technology is that it will not identify all possible essential genes nor will it identify all steps in a pathway. There is a theoretical requirement that a dominant mutant protein must have at least two domains—one of which will promote protein-protein or protein-nucleic acid interactions (49). However, the *C. albicans* genome (11, 26, 57) contains between 6,000 and 8,000 genes with ~15-17 % likely to be essential; a subset of these essential genes will be identified with the dominant negative approach.

Bioinformatics and target prioritization.

Targets in C. albicans that have been identified by this process will be included in the proprietary database of essential genes that Elitra Pharmaceuticals has been building. Each target will be evaluated against a set of criteria including presence or absence in other eukaryotic pathogens and presence or absence in the human or other mammalian genomes. The most desirable targets for antifungals might, in principle, be those that are found only in pathogens and do not have any human counterparts.

Summary

To identify both essential genes and new virulence targets in the human fungal pathogen, *C. albicans*, a dominant negative gene identification approach will be tested. Some preliminary work on transformation, testing of promoters and

reporters, and the construction of a site-specific integrating expression vector has already been accomplished. The focus of this phase I application will be to complete optimization of the vector, demonstrate the feasibility of dominant gene technology in *C. albicans*, construct the first libraries to be screened, and to identify as many targets as the technology will permit. Therefore, cDNA libraries will be screened to saturation, mutated, and those libraries screened to saturation. Multiple libraries will be generated to produce (*i*) intact, full-length cDNA clones for overexpression studies, (*ii*) deletion or partial length cDNA clones for dominant negative screens, and (*iii*) mutated libraries for dominant negative screens. Additionally, cDNA will be produced from mRNA isolated from *C. albicans* cells grown in a variety of growth condition and developmental phases.

Phase II

Tells how Phase I results will be expanded in Phase II.

Phase I of this SBIR proposal will accomplish a number of goals. These include validation of our *C. albicans* expression vector, development of the capability of large scale and high throughput handling of *C. albicans* molecular biology, and identifying dominant negative targets in *C. albicans*. The experimental approaches that I have described will clearly allow us to prove the efficacy of this methodology for *C. albicans* using cDNA libraries. An added benefit is that we will likely identify some potentially useful *C. albicans* targets and be ready for a much broader application of this methodology during Phase II of the SBIR. Envisioned are the identification of approximately 100 *C. albicans* essential targets during Phase I and Phase II. During Phase II, 3 to 5 of these targets will be validated and screened against ~150,000 compounds.

The new genes identified in this research will be included in Elitra's unique relational database of both targets and drug screens for all the major gene/protein targets across multiple pathogens. Using information gained from both the screens and bioinformatics, targets will be prioritized, drug screens will be developed using either a cell-based assay or a purified target assay to facilitate the screening of chemical libraries to obtain candidates for new antifungal drugs.

E. Human Subjects

No human subjects are involved in this project.

F. Vertebrate Animals: Not Applicable

No vertebrate animals are involved in this project.

G. Consultants

Well-known, knowledgeable academic investigator will act as consultant.

Professor William Fonzi will work as a consultant. His assistance will be important for the successful completion of the proposed project. A brief vita and a letter of consent are provided elsewhere in the proposal. William A. Fonzi, Ph.D., Associate Professor of Microbiology and Immunology, Georgetown University School of Medicine. Dr. Fonzi is one of the leading researchers in the molecular biology of *C. albicans*. He has developed important tools for the study of *C. albicans* genetics and aided in elucidating some of the principle pathways controlling *C. albicans* pathogenesis. His assistance in construction of expression vectors and screening in *C. albicans* will be invaluable.

H. Contractual Arrangements

None.

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December 12, 1999

Letter is included to show collaborator's agreement to participate in research.

John D. Trawick, Ph.D.
Senior Research Scientist
Elitra Pharmaceuticals, Inc.
3510 Dunhill Street
San Diego, CA 29121

Dear John,

This letter is to express my willingness to participate in the research project described in your NIH Phase I SBIR proposal, "Dominant Expression Libraries to Obtain New Targets in *Candida albicans*." My contribution will be as a paid consultant to Elitra Pharmaceuticals.

Sincerely,

William A. Fonzi

William A. Fonzi
Associate Professor

3900 Reservoir Road NW Washington, DC 20007-2197

Checklist

TYPE OF APPLICATION *(Check appropriate box{es}.)*

__X__ NEW application. *(This application is being submitted to the Public Health Service for the first time.)*

____ REVISION of previously-submitted application number _____

____ CHANGE of Principal Investigator *(if applicable)*
Name of former Principal Investigator _____

1. ASSURANCES/CERTIFICATIONS

The assurances/certifications set forth below are made and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (small business concern on the FACE PAGE of the application. Descriptions of individual assurances/certifications are found in application instructions under "**Checklist**." If unable to certify compliance with any item, provide an explanation and place it after this page.

· Human Subjects; · Vertebrate Animals; · Department and Suspension; · Drug-Free Workplace; · Delinquent Federal Debt; · Research Misconduct; · Civil Rights (Form HHS 690); · Handicapped Individuals (Form HHS 690); · Age Discrimination (Form HHS 690).

2. PROGRAM INCOME *(See discussion in application instructions under "**Checklist**.")*

All applications must indicate *(Yes or No)* whether program income is anticipated during the period for which grant support is requested.

X No Yes *(If "Yes," use the format below to reflect the amount and source(s) of anticipated program income.)*

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COSTS *(See discussion in application instructions under "**Checklist**."*

Insert the rate, if known. If the applicant organization does not have a currently negotiated rate with the Department of Health and Human Services (DHHS) or another Federal agency, it must estimate the amount of indirect costs allocable (applicable) to the proposed Phase I project. That amount should be inserted in the space provided below. The applicant organization should also be prepared to furnish financial documentation to support the estimated amount, if requested by the Public Health Service. An applicant organization may elect to waive indirect costs if it so desires.

____ DHHS agreement, dated: _____ . _____ % salary and wages or _____ % Total Direct Costs.

____ No DHHS agreement, but rate established with _____, dated: _____

____ Rate negotiation pending with the National Institutes of Health.

____ Indirect costs allocable (applicable) to this Phase I project are estimated to be $ _____

__X__ No indirect costs requested.

4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workplace and/or promote the non-use of tobacco products or have plans to do so?
__X__ Yes No *(The response to this question has no impact on the review or funding of this application.)*

Dennis M. Dixon, Ph.D.
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SUMMARY STATEMENT
(Privileged Communication)

Application Number: 1 R43 AI48345-01

ZRG1 SSS-K (1)
Review Group: CENTER FOR SCIENTIFIC REVIEW SEP

Meeting Dates: IRG: FEB/MARCH 2000 COUNCIL: MAY 2000 M31
Requested Start Date: 07/01/2000

TRAWICK, JOHN D, PHD
ELITRA PHARMACEUTICALS, INC. 3510 DUNHILL ST
SAN DIEGO, CA 92121

Project Title: DOMINANT EXPRESSION FOR NEW TARGETS IN CANDIDA ALBICANS
IRG Action: Priority Score: 118
Human Subjects: 10-NO HUMAN SUBJECTS INVOLVED
Animal Subjects: 10NO LIVE VERTEBRATE ANIMALS INVOLVED

PROJECT YEAR	DIRECT COSTS REQUESTED	DIRECT COSTS RECOMMENDED	ESTIMATED TOTAL COST
01	94,466	94,466	100,134
TOTAL	94,466	94,466	100,134

NEW INVESTIGATOR

Sometimes during discussion, the enthusiasm of the study section increases over that of the primary reviewer.

RESUME AND SUMMARY OF DISCUSSION: The objectives of this Phase I SBIR proposal from Dr. John D. Trawick are first, to construct and optimize a Candida albicans site-specific integrating expression vector, and second, to use this vector to isolate dominant negative mutants. The ultimate goal of the proposed study is to identify essential genes that might serve as new antifungal targets. This is an outstanding proposal from a new investigator who has assembled an impressive investigative team. The work is highly innovative and although the commercial potential may not be immediate, the utility of this work for the eventual identification of potential drug targets is highly significant. Despite some minor comments from one reviewer who expressed a concern that the experimental plan was overly ambitious and risky, enthusiasm increased during the discussion as the Study Section agreed that the overall quality of this proposal is excellent and important results will be obtained.

DESCRIPTION (Adapted from Applicant's Abstract): A screen for dominant negative genes will be used to identify targets and pathways in Candida albicans. The genes and pathways identified will be developed as new antifungal targets. An expression vector system suitable for screening libraries in C. albicans has been devised. Phase I of this project includes final construction and optimization of the expression vector and construction of cDNA libraries capable of identifying dominant negative mutants. Preliminary screening will begin in Phase I. Phase II of the project will entail identifying essential genes and processes by dominant negative mutagenesis and to develop screens for new antifungals based on these essential genes. The method proposed for identifying essential genes is ideal for C. albicans, a diploid human pathogen not normally amenable to genetic analysis, and can be automated.

CRITIQUE 1: Fungal infections are a serious and growing medical problem, and the number of effective antifungal treatments limited. Although there are many good research tools available for work in Saccharomyces cerevisiae, many do not work efficiently or at all within the pathogenic fungi, as pointed out in this proposal. Therefore there is a need to develop good molecular tools for working directly with pathogenic fungi.

Scientific Merit: The research plan as described in the proposal is thoughtfully presented and logical. Looking for dominant negative mutants of Candida albicans as method for the identification of potential new therapeutic targets is a reasonable approach. However, the plan is extremely ambitious, and it is unlikely that the researchers will be able to achieve the all aims enumerated in the proposal. It would have been more reassuring if some of the preliminary background experimentation had been done. Having said that, it will be very worthwhile to achieve even the first goal of developing a good, stable, integrative expression vector for C. albicans. The development of such a vector (or series of vectors) would allow many new types of experiments to be conducted in C. albicans that are currently not possible.

Reviewer thinks this is high-risk, high-return project. Phase II funding is likely if applicant is successful in achieving Phase I goals.

Another major challenge will be to construct a representative cDNA library in C. albicans, especially with the low transformation efficiency reported in the proposal. The researchers may want to spend some time and effort working on a more efficient transformation methodology. However, the identification and isolation of dominant negative C. albicans mutants does not necessarily require having a very complete cDNA library.

The experimental approach being designed in this proposal is very specific for C. albicans. Unfortunately the expression vector transformation and library generation methods will not be transferable to other major pathogenic fungi, like Aspergillus fumigatus or Cryptococcus neoformans. However, on the positive side, information about novel proteins that are potential targets for study, can be transferred across species lines within the database that the researchers are generating.

Finally, the experiments are being designed in a way that will be readily automated for high throughput, which is essential for the success of the screening effort, especially progressing into Phase II.

Innovation: The proposal has a very nice combination of novel applications of standard molecular techniques to be implemented in a new and complex system. The experiments have a high risk of failure, but are well constructed and controlled so the researchers should be able to evaluate their success or failure relatively quickly.

Application is seen as innovative but possible.

Qualifications of investigator, staff and consultants: The personnel associated with the application are most impressive. The Principal Investigator and Co-PI are well-qualified to conduct and direct the experimentation described. It is also a great benefit to have a Bioinformaticist associated with the project from its inception, which will facilitate the implementation of the high throughput screening efforts and the development of their database. The business experience of the principals at the company greatly enhances their chances of success, not only for the project described but for the company as commercial entity.

Reviewer considers researchers very capable of conducting project.

Facilities and Equipment: More than adequate for the project.

Commercial Potential: It is a long road from identifying potential antifungal drug targets to having marketable products. However, even if Elitra is successful only in the first step of developing a good integrative expression vector for C. albicans, the vector could have commercial value. Also, the clones generated and information gained will be helpful in developing diagnostic tools.

Reviewer recognizes significance of project.

Summary and Recommendations: There is a great need for new antifungal products, as well as novel approaches to studying pathogenic fungi. The project outlined is ambitious and risky, but can produce some interesting findings and useful molecular tools. It may not succeed but it seems ideal for a Phase I SBIR.

CRITIQUE 2: In this SBIR application the PI proposes to employ dominant negative screening for genes and phenotypes that are vital for survival or C. albicans in order to uncover new antifungal targets. There are two aims in this Phase I proposal. They are: (1) construct and optimize a C. albicans site-specific integrating

expression vector and (2) the vector will be used for production and identification of dominant negative mutants with cDNA libraries, the phenotype to be tested is growth/viability. The ultimate goal of this Phase I proposal will be to optimize a C. albicans expression vector and to exploit its unique characteristics in dominant negative phenotype screening to identify cellular targets involved in growth and survival of C. albicans. These genes will be included in Elitra's unique database of both targets and drug screens for all the major gene/protein targets across multiple fungal pathogens, which includes 130,000 compounds.

The PI emphasizes the fact that a critical factor in treating patients with the available antifungals is that many of these drugs target eukaryotic processes that are common to both the host and the parasite. Along with toxicity, many of the agents are fungistatic and require therapy for the life of the patient. Complicating the use of many of these agents is the growing list of fungi that have innate resistance or have developed tolerance to the available antifungals.

Of the pathogenic fungi that have been implicated in disease, Candida albicans is the most important causing a wide spectrum of clinical presentations. The biology and genetics of C. albicans pose a challenge in that it is a diploid organism with no known haploid state in addition to a variant chromosomal composition. For this and other reasons, several investigators have used Saccharomyces cerevisiae as a laboratory model to identify various cellular targets. However, the PI points out that, although C. albicans is closely related to S. cerevisiae, there are many differences in biology between the two, including the pathogenic potential of C. albicans. A powerful tool for identifying critical targets is such organisms is screening for dominant negative phenotypes.

There is precedence to show that dominant negative gene analysis works in C. albicans. Both a directed dominant negative and identification of a filamentous causing dominant gene from a library screen have been demonstrated. Furthermore, a mutant allele of the Ca-SEC4 gene, a Ras-like GTPase that appears to be essential in C. albicans was overexposed and was mutated to mimic a well characterized trans-dominant mutation in mammalian Ras. The dominant negative allele of SEC4 was successfully used to demonstrate the functional role of SEC4 in cell growth and protein secretion. Additional published data are described to support the notion that there is a distinct possibility that larger scale analyses are possible with suitable C. albicans vectors and promoters.

This is an innovative proposal that has scientific merit. The PI describes an experimental approach that appears feasible and has taken into consideration alternate procedures should he encounter pitfalls. Furthermore, the PI, a well-trained molecular geneticist, has brought together and excellent team of investigators to work with him, most notably is William A Fonzi of Georgetown University School of Medicine, who is one of the leading molecular biologists and authority on C. albicans and who has developed many of the techniques and probes relevant to this SBIR proposal.

BUDGET: The Study Section considers the requested budget to be reasonable and appropriate.

MEETING ROSTER

CENTER FOR SCIENTIFIC REVIEW SPECIAL EMPHASIS PANEL
CENTER FOR SCIENTIFIC REVIEW
ZRG1 SSS-K (01)
March 16, 2000 - March 17, 2000

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March 16, 2000 - March 17, 2000

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MEETING ROSTER

CENTER FOR SCIENTIFIC REVIEW SPECIAL EMPHASIS PANEL
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ZRG1 SSS-K (01)
March 16, 2000 - March 17, 2000

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GRANTS TECHNICAL ASSISTANT
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NATIONAL INSTITUTES OF HEALTH
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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

********** NOTICE OF GRANT AWARD **********
SMALL BUSINESS INNOVATION RESEARCH PROG Issue Date:02/19/2001
Department of Health and Human Services
National Institutes Of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
****

Grant Number: 1 R43 AI48345-01 (Revised)
Principal Investigator: TRAWICK, JOHN D PHD
Project Title: DOMINANT EXPRESSION FOR NEW TARGETS IN CANDIDA ALBICANS

MS. SHAWNA K. BOLES/EXECUTIVE AS
ELITRA PHARMACEUTICALS, INC.
3510 DUNHILL ST
SAN DIEGO, CA 92121

Budget Period: 08/15/2000 - 02/14/2002
Project Period: 08/15/2000 - 02/14/2002

This is a revised Notice of Award because this Phase I recipient needed to request a "no-cost" extension to complete the Phase I project. Because Phase I recipients often need this extra time, we recommend applicants request funding for one year instead of the "normal" six months.

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ELITRA PHARMACEUTICALS, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR PART 52 15 USC 638 and is subject to terms and conditions referenced below.

Acceptance of this award including the Terms and Conditions is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Award recipients are responsible for reporting inventions derived or reduced to practice in the performance of work under this grant. Rights to inventions vest with the grantee organization provided certain requirements are met and there is acknowledgement of NIH support. In addition, recipients must ensure that patent and license activities are consistent with their responsibility to make unique research resources developed under this award available to the scientific community, in accordance with NIH policy. For additional information, please visit http://www.iedison.gov.
.
If you have any questions about this award, please contact the individual(s) referenced in the information below.

Sincerely yours,

Laura Eisenman
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

See additional information below

SECTION I - AWARD DATA - 1 R43 AI48345-01 (Revised)

AWARD CALCULATION (U.S. Dollars):

Salaries and Wages	$44,425
Fringe Benefits	$8,885
Personnel Costs	$53,310
Consultant Services	$5,000
Supplies	$18,568
Travel Costs	$1,000
Other Costs	$16,568
Direct Costs	$94,446
APPROVED BUDGET	$94,446
Fee	$5,668
TOTAL	$100,114
AMOUNT OF THIS ACTION (FEDERAL SHARE)	+$0

FISCAL INFORMATION:
CFDA Number 93.856

EIN: 1330779254A1
Document Number: R3AI48345A
IC/ CAN / FY2000

AI/8425743/ 100,114

NIH ADMINISTRATIVE DATA:
PCC: M31 / OC: 41.4A /Processed: LEISENMAN 010216 0729

SECTION II - PAYMENT/HOTLINE INFORMATION - 1 R43 AI48345-01 (Revised)

For Payment and HHS Office of Inspector General Hotline Information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III - TERMS AND CONDITIONS - 1 R43 AI48345-01 (Revised)

This award is based on the application submitted to, and as approved by, the NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- The grant program legislation and program regulation cited in this Notice of Grant Award.
- The restrictions on the expenditure of federal funds in appropriations acts, to the extent those restrictions are pertinent to the award.
- 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(see NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

By accepting an award, recipients agree to these conditions. Review this information carefully.

This grant is excluded from Expanded Authorities.

"Excluded from Expanded Authorities" means that NIH permission is required to extend the grant period.

Treatment of Program Income:
Additional Costs

Revised award issued to extend 01 period in accordance with letter of 2/13/01 from G. Tibbitts/Elitra Pharmaceuticals. Supersedes Notice of Grant Award (NGA) issued 8/11/00. All terms and conditions cited on previous NGA still apply.

This award represents the final year of the competitive segment for this grant. Therefore, as stated in the NIH Grants Policy Statement, October 1998, part II, pages 83-84, a Financial Status Report (OMB 269) must be submitted within 90 days of the expiration date. In addition, unless an application for competitive renewal is funded, grant closeout documents consisting of a Final Invention Statement (HHS 568), (not applicable to training, construction, conference or cancer education grants) and a final progress report must also be submitted within 90 days of the expiration date.

The grantee is required to report inventions.

The Final Progress Report may be typed on plain white paper and should include, at a minimum, a summary statement of progress toward the achievement of the originally stated aims, a list of results (positive or negative) considered significant, and a list of publications resulting from the project as well as plans for further publications. An original and one copy are required.

The Final Progress Report and Final Invention Statement should be sent to the awarding component at the following address:

ATTENTION: CLOSEOUT
NIH, NIAID, Division of Extramural Activities
Grants Management Branch
Room 2200, 6700-B Rockledge Drive, MSC-7614
Bethesda, Maryland 20892-7614

The Financial Status Report should be submitted electronically to the Office of Financial Management, NIH. Other Financial Status Reports may be mailed to:

Government Accounting Branch
Office of Financial Management
National Institutes of Health
31 Center Drive, Room B1B05A
MSC 2050
Bethesda, MD 20892-2050

****

PAYMENT INFORMATION: The awardee organization will receive information and forms from the Payment Management System of the Department of Health and Human Services regarding requests for cash, manners of payment, and associated reporting requirements. Payment may be made on a cost- reimbursement or advance basis. Cost reimbursements may be requested monthly, quarterly, or at other periodic intervals. Advance payments may be requested on a monthly basis only. The telephone number for the Payment Management System Office is (301)

443-1660.

The fixed fee provided as part of this grant award is included in the maximum allowable total costs. An adjustment of the fee will be made in the event the grant is terminated. The fee is to be drawn down from the HHS Payment Management System in increments proportionate to the drawdown of funds for costs.

Any product resulting from this award should be manufactured in the United States.

Normally, the awardee organization retains the principal worldwide patent rights to any invention developed with United States government support. Under Title 37 Code of Federal Regulations Part 401, the Government receives a royalty-free license for its use, reserves the right to require the patent holder to license others in certain circumstances, and requires that anyone exclusively licensed to sell the invention in the United States must normally manufacture it substantially in the United States. To the extent authorized by Title 35 United States Code Section 205, the Government will not make public any information disclosing a Government-supported invention for a 4-year period to allow the awardee organization a reasonable time to file a patent application, nor will the Government release any information that is part of that application.

If equipment is purchased, it should be American made.

When purchasing equipment or products under this SBIR award, the grantee shall use only American-made items whenever possible.

Dennis M Dixon (DMID), Program Official 301-496-7728 dd24a@nih.gov
Patricia M Felner, Grants Specialist 301-402-6450 pf14h@nih.gov

SPREADSHEET
GRANT NUMBER: 1 R43 AI48345-01 (Revised)
P.I.: TRAWICK, JOHN D

INSTITUTION: ELITRA PHARMACEUTICALS, INC.

	YEAR 01
Salaries and Wages	44,425
Fringe Benefits	8,885
Personnel Costs	53,310
Consultant Services	5,000
Supplies	18,568
Travel Costs	1,000
Other Costs	16,568
TOTAL DC	94,446
TOTAL F&A	
TOTAL COST	94,446
FEE	5,668