

UNCLASSIFIED

AD NUMBER
ADB261128
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Sep 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.
AUTHORITY
USAMRMC ltr, 8 Jan 2003

THIS PAGE IS UNCLASSIFIED

AD _____

Award Number: DAMD17-99-1-9412

TITLE: Identification of Checkpoint Genes Involved in the
kar3 Cell Cycle Arrest

PRINCIPAL INVESTIGATOR: Robert M. Q. Shanks
Dr. Dean Dawson

CONTRACTING ORGANIZATION: Tufts University
Boston, Massachusetts 02111

REPORT DATE: September 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel
Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Sep 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20010102 148

DTIC QUALITY INSPECTED 4

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-99-1-9412
Organization: Tufts University
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Rath Mc 12/1/00

REPORT DOCUMENTATION PAGE			<i>Form Approved</i> OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggesting reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE September 2000	3. REPORT TYPE AND DATES COVERED Annual Summary (15 Aug 99 - 14 Aug 00)		
4. TITLE AND SUBTITLE Identification of Checkpoint Genes Involved in the <i>kar3</i> Cell Cycle Arrest			5. FUNDING NUMBERS DAMD17-99-1-9412	
6. AUTHOR(S) Robert M.Q. Shanks Dr. Dean Dawson				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tufts University Boston, Massachusetts 02111 E-MAIL: rshanks@opal.tufts.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Sep 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.			12b. DISTRIBUTION STATEMENT	
13. ABSTRACT (Maximum 200 Words) KAR3 encodes a kinesin-like motor protein that has a wide variety of roles in the budding yeast <i>Saccharomyces cerevisiae</i> . Genetic bypass studies and a mutant hunt are being done to discern the nature of the <i>kar3Δ</i> meiotic arrest and to find genes involved in the checkpoint function that mediates the arrest. Checkpoints are important regulatory mechanisms used by cells to prevent aberrant cell cycle divisions such as those observed in cancerous cells. In addition Kar3p functions are thought to be determined by interaction with a number of kinesin associated proteins (KAPs) including Cik1p and Vik1p. We are exploring the roles of these KAPs in meiosis.				
14. SUBJECT TERMS Cell cycle, checkpoints, Kar3, Cik1, Vik1, motor proteins, KAP			15. NUMBER OF PAGES 13	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....3

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....5-9

Reportable Outcomes.....10

Conclusions.....11

Appendices.....12

INTRODUCTION:

We use the budding yeast *Saccharomyces cerevisiae* as a model organism to study the meiotic cell cycle. Yeast cells deleted for a gene encoding a motor protein called Kar3 arrest during early meiosis in what we believe to be a checkpoint mediated fashion. The purpose of this research is to identify checkpoint genes involved in the initiation and maintenance of the *kar3* meiotic arrest. We are searching for mutations in genes that would allow *kar3* mutants to proceed through meiosis. In addition we are exploring the meiotic roles of genes known to mediate the function of Kar3p during other cellular processes.

BODY:

Training: Since the inception of the grant I have completed all of my required classes and have passed my qualifying exam. I have taught Molecular Biology Classes for first year medical students (as 'lead T.A.' 1998, 1999) and I have been a teaching assistant at the Cold Spring Harbor Yeast Genetics Course (1999). I have presented this research at yearly departmental research reports, and weekly lab meetings. In addition I regularly attend Boston Area Yeast Meetings and Departmental Seminars (thrice weekly). I attended the Gordon Research Conference on Meiosis this year at which I presented a poster.

Research: 1a. Identification of genes involved in the meiotic arrest of *kar3* mutants: Mutant Hunt. A mutant hunt described in the proposal was carried out. The Snyder Library (see Burns, et al. Genes Dev 1994 May 1;8(9):1087-105 Large-scale analysis of gene expression, protein localization, and gene in *Saccharomyces cerevisiae*) was used to generate 35,130 mutants (over five times the number of genes in the yeast genome) in a *kar3Δ* mutant strain. The library construct allows expression of the bacterial gene *lacZ* if the insertion is in frame with an upstream promoter. This allowed us to screen for colonies containing mutations in genes that are expressed on sporulation plates (containing X-gal). Since only 1/6 of insertions will be in the correct orientation and frame, the number of genomes screened is reduced to 0.9. These mutants were screened with fluorescent microscopy to identify mutations that allow nuclear division of *kar3Δ* cells during meiosis. Ten candidates were reproducibly allowed meiotic progression beyond the point of the *kar3* meiotic arrest (see Table 1). The location of the mutation in these candidates is currently being identified as specified by the Yale Genome Analysis Center (instructions are available on line at <http://ygac.med.yale.edu/>). Currently only candidates 27-16 and 27-80 have been identified. These insertions map to the uncharacterized open reading frame YJL017W and the nuclear import factor *KAP95* respectively. Both genes are expressed during meiosis according to the transcriptional program of sporulation web site (<http://cmgm.stanford.edu/pbrown/sporulation/>) see Chu S, et al. Science. 1998;282(5389):699-705. *KAP95* is an essential gene, the

insertion at codon 390 must allow for the formation of a functional truncated version of Kap95.

We plan to identify the location of the rest of the insertions and to reintroduce them in a fresh background to ensure there are no secondary mutations that account for the suppression phenotype.

Table 1. *kar3* meiotic suppressors

Candidate ^a	N ^b	% of non-mitotic Cells with 2 or more nuclear masses at 48 hours post meiotic induction ^c	% of cells forming asci ^d
No plasmid	79	2.5	0
pRS3 ^e	73	31.5	27.9
27-9	104	33.7	0
27-16	116	34.5	0.4
27-22	131	9.9	0.4
27-75	92	47.8	0.3
27-80	126	38.1	0
27-90	121	34.7	0.3
27-96	104	12.5	0
27-104	113	29.2	0.5
27-105	103	54.4	0
27-109	132	16.7	0

kar3 suppressor candidates were purified as diploids and then induced to undergo meiosis, at 48 hours post meiotic induction aliquots of cells were taken and microscopically assayed for meiotic progression. ^a Control strains are DJ1, while candidates are derivatives of DJ1 (see Bascom-Slack and Dawson, 1997). ^b N reflects the number of cells staining with DAPI (% of cells staining ranges from 49.2- 95.3 of total cells). ^c These cells were assayed microscopically for meiotic progression using the DNA dye DAPI. ^d Asci formation was assayed using aliquots of the same cells harvested at 80 hours post meiotic induction, and were viewed using standard light microscopy at 400X. ^e pRS3 is a CEN plasmid containing a wild type copy of *KAR3* made for this study.

1b. Mix and Match. We introduced mutations into genes in a *kar3* mutant strain to determine if these genes are important for the meiotic arrest. Genes chosen were known checkpoint genes or other genes involved in microtubule or meiotic regulation. We have tested *spo11* (a gene that encodes for the exonuclease that makes double stranded breads and effects meiotic timing and checkpoint activation), *spo13* (a meiotic regulatory gene), *rad17* (a recombination checkpoint), *mek1* (a synaptonemal complex component that is involved in meiotic regulation), *red1* (a synaptonemal complex component that is involved in meiotic regulation), and are currently in the process of *pch2* and *bim1* (a gene involved in microtubule dynamics and mitotic checkpoint control). Of these, only *spo11* allows a bypass of the *kar3* meiotic arrest. We are quite excited about *pch2* however, because it was isolated as a suppressor of a *zip1* meiotic arrest, and the *zip1* meiotic arrest is similar in many ways to the *kar3* meiotic arrest. *BIM1* is also interesting because it encodes for a microtubule binding protein that is homologous to EB1, a human protein that binds the adenomatous polyposis coli (APC) protein, a tumor suppressor.

From these data we conclude that *kar3* mutants are capable of meiotic division (*kar3 spo11* mutants form binucleate cells with normal looking spindles), and are likely to arrest because of a checkpoint rather than a physical inability to make a meiotic spindle. We do not know why *spo11* allows suppression of *kar3* mutants because mutations in *spo11* are known to have pleiotropic effects on meiosis. Also, since the *rad17 kar3* cells still arrest we are conclude that the lowered amounts of mature recombination products are not solely responsible for the *kar3* meiotic arrest.

2. Characterization of meiotic phenotypes of *cik1* and *vik1* mutants. *CIK1* and *VIK1* encode for similar proteins that mediate the functions of Kar3p during various cellular processes (Manning, et al., J. Cell Biol., V144, 1219-1233, 1999). It has been shown that Cik1p has a direct interaction with Kar3p that is essential for the activity of Kar3p during karyogamy (nuclear fusion in mating cells), and that *cik1* mutants share many phenotypes with *kar3* mutants in mitotic cells (Page BD, et al., J Cell Biol. 1994, 507-19) (Page BD and Snyder M., Genes Dev. 1992,

1414-29). Vik1p has been shown to interact with Kar3p during mitosis, but not during karyogamy. The roles of Cik1p and Vik1p during meiosis have not been thoroughly characterized, although both of these genes are expressed in meiosis (the Transcriptional Program of Sporulation, Chu, et al. 1998). We are in the process of exploring the phenotypes of *cik1* and *vik1* mutants in meiosis. We are assaying meiotic phenotypes that are commonly used to characterize meiotic defects.

-We have characterized meiotic phenotypes *cik1* mutants, and in most cases *cik1* mutants behave like *kar3* mutants in meiosis. I have briefly listed some of our findings below, all are similar to published data for *kar3* mutants (Bascom-Slack and Dawson, 1997), except where noted. *vik1* mutant phenotypes have also been noted.

-*cik1* mutants do not form spores.

-*cik1* mutants arrest in meiosis I with monopolar tubulin arrays.

-*cik1* mutants are severely deficient in meiotic recombination.

-*cik1* mutants show incomplete and abnormal synaptonemal complex formation.

-*cik1 rad17* mutants remain arrested in meiosis I.

-*cik1 spo13* mutants form binucleates at levels equal to isogenic *spo13* mutants. This is distinctly different than the *kar3 spo13* double mutants that remain arrested in meiosis I. This suggests Kar3p has Cik1p-independent functions during meiosis.

-Cik1-13xMyc and Kar3-13xMyc constructs both have similar localization patterns in meiosis I- colocalizing with the spindle pole body. Experiments to determining dependence of localization are underway.

-*vik1* mutants sporulate at wild type levels.

-*vik1* mutants have spore viability that is lower than isogenic wild type strains.

-*vik1* mutants do not seem to have elevated levels of aneuploidy compared with isogenic wild type strains. However, we have shown that they do have increased levels of mitotic chromosome loss that can lead to elevated levels of dead spores (A cell entering meiosis with only one copy of a chromosome will

yield four spores two of which will not inherit a copy of the lost chromosome, and will thus be inviable).

Reportable Outcomes:

As of this date no articles have been published from this study. However, one is in preparation and its working title is *The Kar3-interacting protein, Cik1p Plays a Critical Role in Passage through Meiosis I*. I am formatting it to be sent to the journal *Molecular Biology of the Cell*. I plan to submit this manuscript before January 1, 2001.

Conclusions:

Isolation of checkpoint proteins: We have isolated suppressors of *kar3* that are potential checkpoint genes, and are in the process of characterizing them. Known checkpoint genes are being tested to determine the nature of the *kar3* meiotic arrest.

Characterization of Kar3- interacting proteins: We have thoroughly characterized *cik1* mutants in meiosis, and they behave similarly to *kar3* mutants in meiosis, implying that Cik1p and Kar3p are involved in at least some of the same meiotic functions. We have used yeast genetics and cell biology to differentiate the *cik1* arrest from the *kar3* meiotic arrest and have genetic data consistent with the hypothesis that Kar3p has meiotic functions that are Cik1p-independent. Thus, Cik1p mediates a subset of meiotic Kar3p functions, but not all of them. We plan to investigate whether Vik1p mediates the Cik1p-independent Kar3p functions. We are beginning to investigate the phenotypes of *vik1* mutants. *vik1* mutants do not arrest during meiosis, although their viability is reduced. Vik1p is unlikely to be involved in the essential meiotic function of Kar3p.

Appendix 1

Abstract for the 1999 Gordon Research Conference on Meiosis

The Kar3-interacting protein, Cik1p Plays a Critical Role in Passage through Meiosis I

Robert Shanks and Dean Dawson Tufts University Department of Molecular Microbiology, Boston MA.

KAR3 encodes a kinesin-like motor protein that has a wide variety of roles in the budding yeast *Saccharomyces cerevisiae*. Kar3p functions are thought to be determined by interaction with a number of kinesin associated proteins (KAPs) including Cik1p and Vik1p. We are exploring the roles of these KAPs in meiosis. *cik1Δ* mutants display a meiosis I arrest with characteristics similar to those exhibited by *kar3Δ* mutants, including greatly reduced levels of heteroallelic recombination at the *ARG4* locus, and defects in homolog synapsis. In contrast *vik1Δ* mutants form spores at almost wild-type levels. Bypass studies have are being done to discern the nature of the *kar3Δ* and *cik1Δ* meiotic arrests.

Mutations in the recombination checkpoint gene *RAD17* have no effect upon the meiotic arrest conferred by *cik1Δ* or *kar3Δ* mutations, suggesting the arrest is not solely mediated by this checkpoint. However, we have found that *cik1Δ pch2Δ* double mutants are able to proceed through the first meiotic division at elevated levels compared to *cik1Δ* mutants, suggesting the arrest is checkpoint mediated. Finally have found that Kar3p may have meiotic roles that do not require Cik1p as *kar3Δ spo13Δ* double mutants fail to progress through meiosis, while *cik1Δ spo13Δ* mutants form spores at levels equivalent to *spo13Δ* mutants alone.

Further studies are being done to explore the roles of Kar3p, Cik1p and Vik1p in meiosis.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

8 Jan 2003

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed. Request the limited distribution statement for the enclosed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

A handwritten signature in black ink, appearing to read "Phyllis M. Rinehart".

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB265840
ADB279138
ADB264578
ADB281679
ADB281645
ADB261128
ADB261339
ADB273096
ADB281681
ADB259637
ADB256645
ADB262441
ADB281674
ADB281771
ADB281612

ADB266633
ADB251763
ADB281601
ADB258874
ADB281773
ADB281660
ADB259064
ADB266141
ADB281664
ADB258830
ADB266029
ADB281668
ADB259834
ADB266075
ADB281661

ADB282069
ADB265386
ADB282057
ADB258251
ADB264541
ADB241630
ADB281924
ADB281663
ADB281659