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14. ABSTRACT We proposed to study a potentially new function of the yeast homolog of neurofibromin, Ira1. It is suggested from the literature that a complete deletion of Ira1 is lethal to yeast while previous partial deletions of the same gene is viable. These seemingly conflicting results point to a possible essential function of the N-terminus of Ira1. However, we found that in our hands, a complete deletion of Ira1 is viable and tetrad analysis was also carried out in the strains provided by the Saccharomyces genome deletion consortium and we got the same result. Therefore, the N-terminus of Ira1 does not possess any essential function in the yeast strains we have tested.					
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INTRODUCTION:

Neurofibromatosis type I is defined by mutations in the gene encoding neurofibromin protein, which is conserved throughout evolution [1, 2]. The yeast *S. Cerevisiae* has 2 homologs, Ira1 and Ira2 [3]. These yeast Ira's not only have extensive sequence similarity with neurofibromin, but also can be functionally complemented by their mammalian counterpart [4]. Like mammalian neurofibromins, Ira's are GAPs (GTPase activating protein) for the Ras proteins. Loss of either or both Ira in yeast results in phenotypes such as heat shock sensitivity and inability to store glycogen, similar to those from activated Ras by point mutation. Recently, the Saccharomyces genome deletion consortium reported that deletion of *IRA1* is lethal to yeast [5]. This observation is seemingly contradictory to previous reports on the roles of *IRA* genes. However, there are two major differences between the two types of *ira1* deletion (*ira1D*) strains in the literature. First, the strain used by the consortium is deleted for the complete open reading frame of *IRA1*, from start to stop codon, whereas earlier deletions are all partial deletions roughly around the GAP domain, yet leaving a significant portion of the N-terminal sequence of *IRA1* still intact. Second, the strain background is different for each study.

The observation that deleting *IRA1* is lethal, but deleting its GAP domain is not, suggests that the N-terminal region of Ira1 may have an independent function that is essential to yeast. Since none of the deletion strains contains the GAP domain, this new function is not necessarily directly associated with Ras proteins.

We will identify the essential function of the N-terminus of Ira1 through the following 2 aims: 1. Determine the regions in Ira1, Ira2 and neurofibromin that are able to complement the *ira1D* lethal phenotype. 2. Isolate novel genes that can complement the *ira1D* phenotype through library screening.

BODY:

Task1. Determine the regions in Ira1, Ira2 and neurofibromin that are able to complement the lethal phenotype.

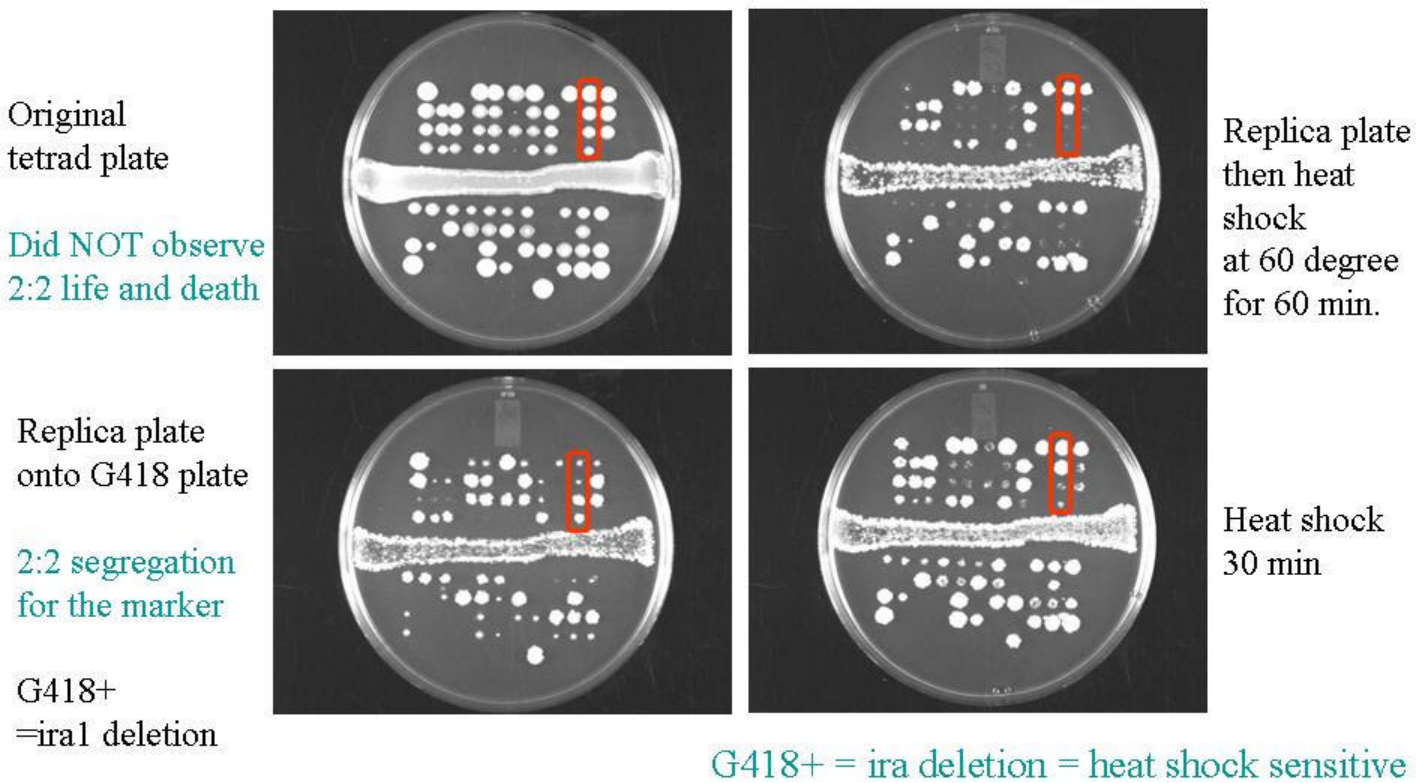
- a. PCR based one-step gene disruption using G418 marker and verify the strains by PCR

IRA1 gene specific primers were designed to obtain a PCR product that contains 45 nt of *IRA1* gene at the ends and G418 marker in the middle. Standard yeast transformation was performed and diploid strains containing one *IRA1* gene deletion was obtained.

- b. Perform tetrad analysis to find out from which strain background, the haploid offspring depend on *IRA1* for survival.

Several heterozygous diploid strains were subjected to tetrad analysis and viability of the haploid offspring will be scored. A 2:2 life to death segregation should be observed if *IRA1* is essential for yeast. However, many tetrads yielded 4 viable offspring, indicating that deletion of *IRA1* is not lethal. Furthermore, upon heat shock treatment, there is a 2:2 heat shock resistant: sensitive segregation. Heat shock sensitivity is a documented phenotype for *IRA1* deletion. Also all the heat shock sensitive offspring carry the G418 marker which is used to disrupt *IRA1*.

Tetrad analysis for *ira1* deletion



- c. Obtain or construct a plasmid carrying full length *IRA1* and *URA3* marker
 We obtained the plasmid carrying full length *IRA1* that was used to in the original screening to identify the gene. We also constructed a plasmid carrying an N-terminal HA tag in frame with the *IRA1* gene. Both of them are plasmids with *URA3* marker.

The following tasks were not initiated due to the result from 1-b, except that we have obtained the yeast library.

- d. Transform one of the diploid *ira1D* strains from a/b, with the plasmid made in c. Perform tetrad analysis to obtain a haploid strain only had *IRA1* supplied from the plasmid (*IRA1**).
- e. Construct the first set of N-terminal fragments of Ira/neurofibromin in yeast expression plasmids with a different marker.
- f. Test whether any N-terminal fragments of Ira/neurofibromin can rescue the lethal phenotype of *ira1D*
 Make further serial deletions of Ira/neurofibromin N-terminal fragment to identify minimal regions of complementation

Task 2. Isolate novel genes that can complement the *ira1D* phenotype through library screening.

- a. Transform a yeast library into the haploid *IRA1** strain obtained in 1d and score for survival on 5-FOA.

- b. Secondary screen to confirm the positive transformants
- c. Recover plasmids from these positive transformants and retest their ability to rescue the *ira1D*
- d. Sequencing of plasmids remained to be positive for rescuing *ira1D*
- e. Literature searches, database-mining to get some clues of how these new identities could rescue *ira1D*

KEY RESEARCH ACCOMPLISHMENTS:

- Constructed heterozygous diploid strains that has one copy of the IRA1 gene deleted.
- Performed tetrad analysis and found that IRA1 gene is not essential for yeast in several strains.
- Obtained and constructed IRA1 expression plasmids

REPORTABLE OUTCOMES:

Antonio Luna, a shared laboratory assistant who participated in this project is now a graduate student in San Francisco State University.

CONCLUSIONS:

We successfully generated diploid strains that have only one copy of IRA1 gene in several strains and also obtained the strains from the Saccharomyces genome deletion consortium. However, when we performed tetrad analysis to confirm that *ira1* deletion is lethal to yeast, we did not observe the same result. We could also generate double *ira1* and *ira2* deletion (complete open reading frame deletion) which suggest that Ira2 was not providing a compensatory function for Ira1. Supporting our result, among the Tet-promoter yeast collection generated by the Hughes laboratory, University of Toronto, the Tet-IRA1 strain is not sensitive to addition of Doxycycline which should have switched off the expression of the gene [6].

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