VRP1 deficiency in Saccharomyces Cerevisiae

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Abstract

Wiskott-Aldrich syndrome protein (WASP) is a vital component in cytoskeleton control and its absence causes complications such as eczema and immune deficiency. These symptoms of its namesake are most likely to be caused through an absence of correct cellular motility and an inability to withstand bodily temperatures due to cytoskeleton obstruction. The Saccharomyces Cerevisiae (baker's yeast) analogue of WASP is VRP1. The aim of this experiment was to analyze the effects on morphology and proliferation of yeast that were VRP1 deficient or had VRP1 transformed into its genome.

This process was achieved through analyzing the morphology and proliferation of wild-type and VRP1 deficient mutant yeast on a growth medium at both 25°C and 37°C. Through the use of restriction enzymes and plasmid DNA the VRP1 gene was reinserted into the yeast genome. The growth of this yeast at both standard temperatures on a growth medium was documented. The similar growth shown by the restored yeast and wild type yeast (prolific at both set temperatures) indicated a successful reinsertion.

The significant findings were that the successful reinsertion of VRP1 into VRP1-lacking yeast resolved its cellular motility deficiencies. This indicates to a potential treatment to WASP deficiency, as successful transformation would allow correct cellular motility, ceasing the development of its implications.

Introduction

Saccharomyces cerevisiae (bakers yeast) has many analogous traits to mammalian cells and as its genome has been completely sequenced the effects of mutating specific genes can be correlated to those in the human genome. Such a trait between mammalian and yeast cells is an actin-based cytoskeleton. Obstruction in the regulation of the cytoskeleton will lead to impaired and unfunctional cellular motility, endocytosis and replication leading to cellular necrosis.

A key process in cytoskeleton control is the regulation of cortical-actin polymerization by the Wiskott - Aldrich syndrome Protein (WASP) binding to an ARP2/3 complex in cells. The absence of WASP causes cellular deficiency in immunity and motility causing defecting cell morphology and proliferation. The yeast equivalent to the mammalian WASP is the VRP1 protein which when deficient in the cytosol causes similar implications to the lack of WASPs. Previous studies have suggested further implications to VRP1 deficiency decreases its host cells ability to cope with and proliferate at bodily temperatures.

By studying the phenotype, morphology and proliferation of wild type (control), mutated VRP1 yeast (mVRP1) and restored mVRP1 at both standard laboratory temperature and bodily temperatures the role that VRP1 and its mammalian analogue WASP plays in the cytoskeleton dependent cellular motility, immunity and temperature resistance can be determined. The effectiveness of VRP1 restoration into mVRP1 can be found through fragment size determination from comparison of electrophoresis of a 1kB DNA ladder and respective PCR products.

Methods

Morphology and Proliferation of WT and mVRP1 yeast: The morphology and proliferation of both wild type (wt) and mutant VRP1 (mVRP1) yeast were recorded from storing both samples on a YPD plate at both room temperature (25°C) and body temperature (37°C). Both samples were studied through a microscope at a phase contrast of x40, and corresponding morphology and proliferation were recorded.

Reinsertion of VRP1 into mVRP1: The mVRP1 was 'rescued' by pelleting the cells and removing the supernatant and then adding them to a transformation mix (containing lithium acetate, PEG3350 and either salmon sperm or plasmid DNA.) Both samples were transferred to an YPD plate in duplicate and stored at either 25°C or 37°C.

Morphology and Proliferation of restored mVRP1 and control (salmon sperm) yeast: A 1μL sample of restored VRP1 and control yeast were pipetted onto two YPD plates, which were stored at either 25°C or 37°C for 24 hours. Subsequently the proliferation and morphology were documented using a microscope at a phase contrast of x40.

DNA fragment determination: Genomic DNA from wt and mVRP1 yeast cells were isolated through a repeated process of micro -centrifuging, removing of supernatant, suspension of solution, addition of a dissolver (chloroform, ethanol or acid washed glass beads) concluding in a DNA sample being dissolved with a TENTS buffer. Both DNA samples underwent a PCR reaction by adding the template DNA to a PCR reaction buffer.

The DNA /PCR reaction buffer solution was then put through a thermo-cycler, initiating the PCR. An agarose gel solution was created and poured into an electrophoresis stand and a well comb was inserted to create wells. A 20μL sample of each PCR product into the gel wells, as well as a 10 μL of 1kB DNA ladder. The gel was then put under a voltage (200V) for 25 minutes. Under UV light, the gel was photographed and printed. The PCR product fragment sizes were found through relating their position on the gel to the corresponding DNA ladder fragment size.

Results

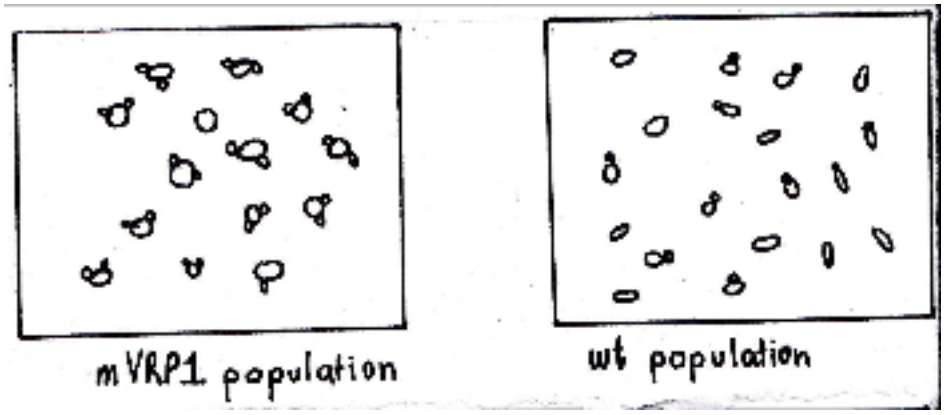

Figure 1: A microscopic representation of the morphology and proliferation of wt and mVRP1 yeast at a phase contrast of x40. The mVRP1 yeast is seen to replicate in clumps of 2 or 3 whilst the wt yeast bar those "budding" grew independently. The average cross section of wt was smaller (3.81+/-0.94µm) compared to that of mVRP1 (5.78+/- 1.63µm) respectively.

Temperature / Yeast Type	wt.	mVRP1
25°C	++	+
37°C	+++	0

Table 1.0: Proliferation of yeast over a 48 hour incubation at 25°C or 37°C. The colony sizes are displayed by relative proportions where 0 indicates null growth, and + represents relative growth. At 25°C wt had about twice the growth of the mVRP1 samples. At 37°C there was significant growth by the wild type sample, whilst the mVRP1 sample had null growth.

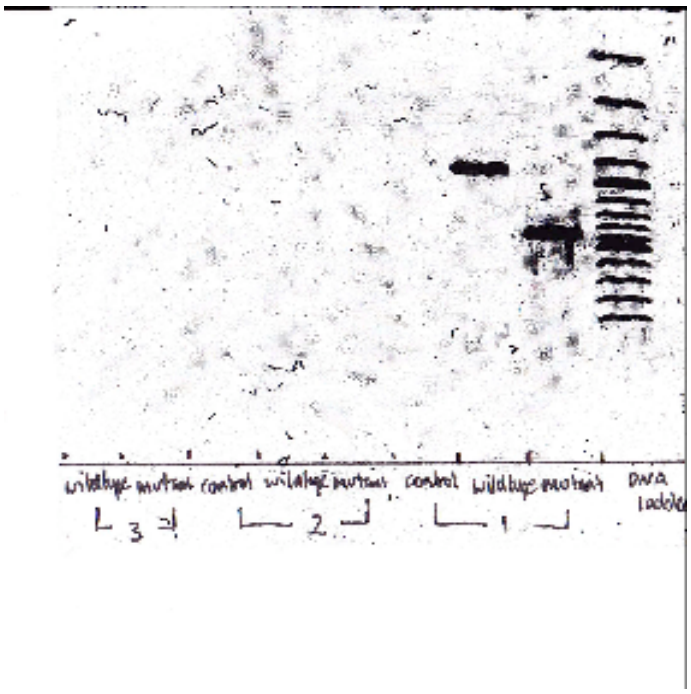

Figure 2.0: Result of electrophoresis on control, mVRP1 and wt PCR products as seen under UV light. The fragment size is found through comparison to the known fragment sizes of the1kB DNA ladder. Trials 1 and 2 appear to have dissolved, whilst trial 1 indicates a fragment size (2000bp (mVRP1) and 1000bp (wt)) about 2.5 times larger than its theoretical fragment size (473bp (mVRP1) and 850bp (wt)).

Condition / Temperature	Plasmid	Control
25°C	+	+
37°C	+	+

Table 2.0: Results of VRP1 insert on growth of yeast colonies on agar plates at 25°C and 37°C. The growth relative to separate conditions is indicated by +, where null growth is indicated as 0. Both plasmid and controlled showed similar growth at each temperature.

Discussion

The larger size and variation of the unregulated cell growth of mVRP1 followed expectations by producing an average cross section and larger standard deviation of size when compared to the wt yeast (Figure 1.0). Unregulated cell replication also was seen in the mVRP1 yeast as it formed in clumps of 2-3 whilst with odd exception of budding, wt yeast formed individually. This was an early but strong indication of the importance of VRP1 and therefore WASP cytoskeletal regulation of correct replication and motility.

Table 1.0 isolated the effect of temperature at both a laboratory standard of 25°C and a bodily temperature of 37°C. Whist wt yeast had significant growth at 25°C and even more at 37°C, the mVRP1 had little growth at 25°C and null growth at 37°C. This was a very strong indication of the need of cytosolic VRP1 in temperature resistance. This result made it clear why WASP deficiency had such devastating effects in the human body which maintains a temperature of about 37°C, as any cell lacking the WASP would undergo necrosis.

The electrophoresis of the PCR products trial 2 and trial 3 did not yield any discerning fragments. This result suggested that restriction enzymes had dissolved and the electrophoresis dispersed the PCR products throughout the gel.

Trial 1 indicated an approximate fragment size of approximately 2000bp (mVRP1) and 1000bp (wild type). From analyzing where the PCR primers were placed on the yeast genome sequence the theoretical fragment sizes were 473bp for mVRP1 and 850bp for the wild type. This indicated that the only resulting data was incorrect and did not conform to expectation.

The most plausible reason for this outcome was cross contamination in sample 2 and 3 and to a lesser extent sample 1. This could of lead to a complete degradation of the PCR products ensuring that there was no constant migration or a decreased migration through the agar gel. A second plausible failure could have occurred during the thermo-cycling process. Incorrect temperature duration could have caused a decreased primer affinity or inconsistent DNA denaturing, leading to discontinuous fragment production. Problems during PCR such as incorrect primer placement may have caused a backward mutation explaining the outcome in sample 1.

In table 2.0, the significant growth of the plasmid at both 25°C and 37°C indicated correct incorporation of the VRP1 gene into the plasmid. However unexpected growth of the salmon-sperm control at both temperatures indicated that another factor enabled both samples to survive at the increased temperature. A possible explanation could include cross contamination through the addition of the plasmid or a product that incorporated VRP1 into to the control sample. However a more likely cause would be not having the 37°C samples incubated at a high enough temperature. Because of this although it is likely there was correct transformation of VRP1 into mVRP1 it cannot be confirmed.

Conclusion

These results have indicated that the morphology and proliferation of yeast that are lacking VRP1 analogous to mammalian WASP are defective. The lacking of a sufficient cytoskeleton was also proven to significantly defect the cells ability to undertake temperature increases. This suggested that WASP/ VRP1 deficient cells at human body temperatures are completely defective. Although unconfirmed correct VRP1 transformation into mVRP1 cells was suggested.

This indicates a possible method to revive any WASP or VRP1 deficient cells. Future research efforts could focus the most effective method of insertion of the WASP gene into those deficient cells of Wiskott- Aldrich Sufferers. Such methods could include how to identify on a large scale which cells or tissue are WASP defective, as the effects of increased cytoplasm WASP concentrations are not documented.