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THE ROLE OF DNA POLYMERASE ALPHA IN THE CONTROL OF MUTAGENESIS IN *SACCHAROMYCES CEREVISIAE* CELLS STARVED FOR NUTRIENTS

❀ **In nature, microorganisms experience numerous environmental stresses and generally grow poorly most of the time. In the last two decades it has become evident that mutations arise not only in actively dividing cells but also in non-replicating or slowly replicating cells starved for nutrients. In yeast, precise base selection and proofreading by replicative DNA polymerases δ and ε keep starvation-associated mutagenesis (SAM) at basal levels. Less is known about the role of replicative DNA polymerase α (Pol α). Here we provide evidence that Pol α is involved in the control of SAM in yeast cells starved for adenine by participation in sporadic replication and/or DNA repair under these conditions.**

❀ **Key words**: *Saccharomyces cerevisiae*; DNA Polymerase α; starvation-associated mutagenesis.

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1. INTRODUCTION

In natural environments, microorganisms are frequently challenged by adverse conditions such as nutrient depletion. The evolutionary success of populations in a stressful environment is strictly dependent upon the existence of variant, adapted cells. Variants can be either pre-existent, formed before cells encounter the environment selecting against most of the cells, or arise under a selective condition. Genetically it translates to mutations that can be randomly generated either during the fast logarithmic growth phase or to mutations arising in cells, which divide very slowly or do not grow at all because of adverse conditions. The latter mode of generating variants has been named adaptive mutagenesis or starvation-associated mutagenesis **(SAM)** (Cairns, 1993; Babudri, 2006; Foster, 2007; Heidenreich, 2007). SAM has been demonstrated in bacteria and yeast. The experimental setup to study SAM in *Saccharomyces cerevisiae* is based on the reversion from auxotrophy to prototrophy. In the papillae test (Hall, 1992; Babudri, 2006; Heidenreich, 2007) auxotrophic cells are plated at low density on minimal medium with a limited amount of the required nutrient. Cells replicate up to the exhaustion of the limiting nutrient forming small colonies. Prototrophic revertant cells arisen either during the growth on selective medium or after cells ceased multiplications form papillae outgrowing colonies (Figure 1). Many cells are plated on minimal medium without the required nutrient in the colony test (Steele, 1992; Babudri, 2006; Heidenreich, 2007). Cells replicate during the first 24 hours because of the internal reserve of the nutrients and then stop multiplying. Prototrophic revertant cells form colonies clearly distinguishable on the slight background growth. In the yeast *S. cerevisiae* several mechanisms are able to generate starvation-associated mutations and the activities of several genes keep SAM at the wild type level (Babudri, 2006; Heidenreich, 2007); in this respect, DNA polymerases are of outmost importance. Proofreading exonucleases of Pol δ and Pol ε have been shown to prevent SAM in non-replicating yeast cells under nutritional stress (Babudri, 2001; Achilli, 2004; Heidenreich, 2007).

In the present work we investigated the possible involvement of Pol α in SAM by studying the effect of the pol1-L868M allele in yeast cells starved for adenine. Chromosomal DNA in eukaryotes is replicated by a concerted action of three DNA polymerases, Pol α, Pol δ, and Pol ε (Garg, 2005; Johnson, 2005; Pavlov, 2006-a; Kunkel, 2008; Pavlov, 2010). Defects in the catalytic sites of any of these polymerases preclude replication. Pol α is not processive and lacks an intrinsic proofreading exonuclease. It has a tightly associated activity for the synthesis of RNA primers at replication origins and during further replication on the lagging DNA strand and occasionally on the leading strand (for example, during replication restart when the synthesis of leading DNA strand is blocked by damage). Pol α extends these RNA primers by synthesizing short stretches of DNA. It is believed that the size of these patches is around 20–40 nucleotides (Bullock, 1991; Waga, 1998; Liu, 2004). A switch then occurs to start the processive synthesis by Pol δ or Pol ε. It is generally accepted that Pol α synthesizes a

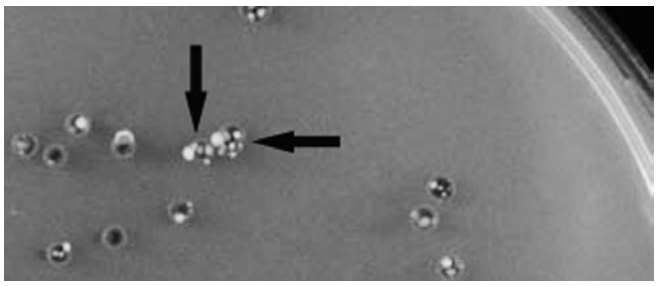

Fig. 1. Colonies of the strain Δ(−2) ψ⁻ *pol1-L868M* with papillae (white in the picture). The arrows point to two colonies with many papillae

small amount of DNA in comparison to other replicative DNA polymerases; however, mutations in the Pol α gene can lead to mutator phenotypes (Liu, 1999; Rogozin, 2001; Gutierrez, 2003; Ogawa, 2003; Suzuki, 2009). Conservatively, if Pol α was to synthesize even five nucleotides of each ~250-nucleotide Okazaki fragment on the lagging strand it could introduce 6,000 potentially mutagenic mismatches into the human genome during each replication cycle (Pavlov, 2006-b). In addition to its fundamental role in DNA replication, Pol α participates in the repair of Double Strand Breaks (DSBs) in the yeast *S. cerevisiae* (Lydeard, 2007; Deem, 2008). Niimi and co-workers (Niimi, 2004) isolated low-fidelity yeast mutants of DNA Pol α with amino acid substitutions of the conserved palm residue. Among them, the pol1-L868M allele encodes for a protein with a Leu868Met substitution. L868M Pol α copies DNA *in vitro* with normal activity and processivity but with strongly reduced fidelity. The pol1-L868M allele confers a weak mutator phenotype (1.5-fold over the wild type) for base substitution reversions at some loci in replicating yeast cells (Nick McElhinny, 2006; Pavlov, 2006-b). This mutator phenotype is dramatically increased upon inactivation of MMR and of the 3' exonuclease activity of Pol δ but not that of Pol ε. Pavlov (2006-b) suggested that the 3' exonuclease of Pol δ proofreads most errors generated by Pol α during initiation of Okazaki fragments. The rest of the mistakes are removed by MMR. In the present paper we provide evidence that the pol1-L868M allele becomes critical for mutagenesis in yeast populations starved for adenine and discuss the possible role of Pol α in yeast cell populations starved for nutrients.

2. MATERIALS AND METHODS

2.1. Strains and media

Prion-free derivatives of *S. cerevisiae* strain Δl(−2)l-7-B-YUNI300 (Pavlov, 2002): Δ7(−2) ψ⁻ [MAT *α ade2-1 his7−2 lys2-Δ(−2) trp1−289 leu2-3,112 ura3-*Δ] and Δ7(−2) ψ⁻ pol1-L868M [same, but *pol1-L868M*] were used in this study. We used yeast strains free from [PSI+] elements to avoid the suppression of the nonsense alleles tested in this work by self-propagating aggregates of the omnipotent suppressor SUP35 (Serio, 1999; Inge-Vechtomov, 2007). SD medium, YPD, amino acids and vitamins concentrations are described in Amberg et al. (2005). SD, with all the required nutrients except adenine/tryptophan, is named SD-ade/SD-trp throughout the paper.

2.2. Estimation of spontaneous mutant frequencies, cellular divisions and surviving fractions

2.2.1 Colony test

To estimate the frequency of Ade⁺ or Trp⁺ revertants in the strains Δ7(−2) ψ⁻ and Δ7(−2) ψ⁻ *pol1-L868M*, which are auxotrophic for adenine and tryptophan because of the nonsense alleles *ade2-1* (ochre) and *trp1-289* (amber), respectively, we plated 1.5×10^7 late exponential cells on 20 to 30 SD-ade/SD-trp dishes. Revertant colonies were detected at the stereomicroscope (20×). The reversion frequency in starved cells is given as the number of revertant colonies per plated cells. To estimate the total number of post-plating cellular divisions, the number of cells per plate was counted in a haemocytometer after washing dishes. The surviving fraction was determined by plating appropriate dilutions of the suspensions on YPD. The reversion rates during the logarithmic growth phase were estimated by the fluctuation test using the P_0 method (Rosche, 2000).

2.2.2 Papillae test

To estimate the frequency of revertants in the papillae test we plated between 70 and 80 cells on SD containing the required nutrients but with adenine (or tryptophan) in a limited amount (0.6 mg/L instead of 20 mg/L — final concentration) abbreviated as SD lim.ade./SD lim.trp. On those media revertant papillae are expected to grow out from the colony. Revertant papillae were detected by the stereomicroscope. The reversion frequency was calculated as: $\ln P_0/N$ where P_0 is the fraction of colonies without papillae and N is the number of cells per colony at the end of growth on SD.lim. We did not estimate the mutation frequency as the number of papillae divided by the number of cells per plate because we could not exclude that one revertant cell could give origin to more than one papilla. The number of divisions per plate and the surviving fractions were determined as described previously for the colony test.

2.2.3. Reconstruction experiments

Reconstruction experiments are routinely used to test the time span required by a single revertant cell to form a visible papilla (Hall, 1992; Heidenreich, 2007). We isolated Δ7(−2) ψ⁻ pol1-L868M Ade⁺ revertant clones by transfer on SD-ade. All isolates were tested on YPD for the white phenotype, typical of true Ade⁺ revertants revertants (Rojas Gil, 1999).

To determine the time span required by a single Ade⁺ revertant cell to give a visible colony in the colony assay we plated approximately 50 cells of Ade⁺ revertant clones with 1.5×10^7 Δ7(−2) ψ⁻ late exponential cells on SD-ade. Δ7(−2) ψ⁻ cell suspensions, either with or without few pre-existing revertants, were used.

To determine the time span required by a single Ade⁺ revertant cell to give a visible colony in the papillae test we plated from 70 to 80 cells of the strain Ade⁻Δ7 (−2) ψ⁻ on

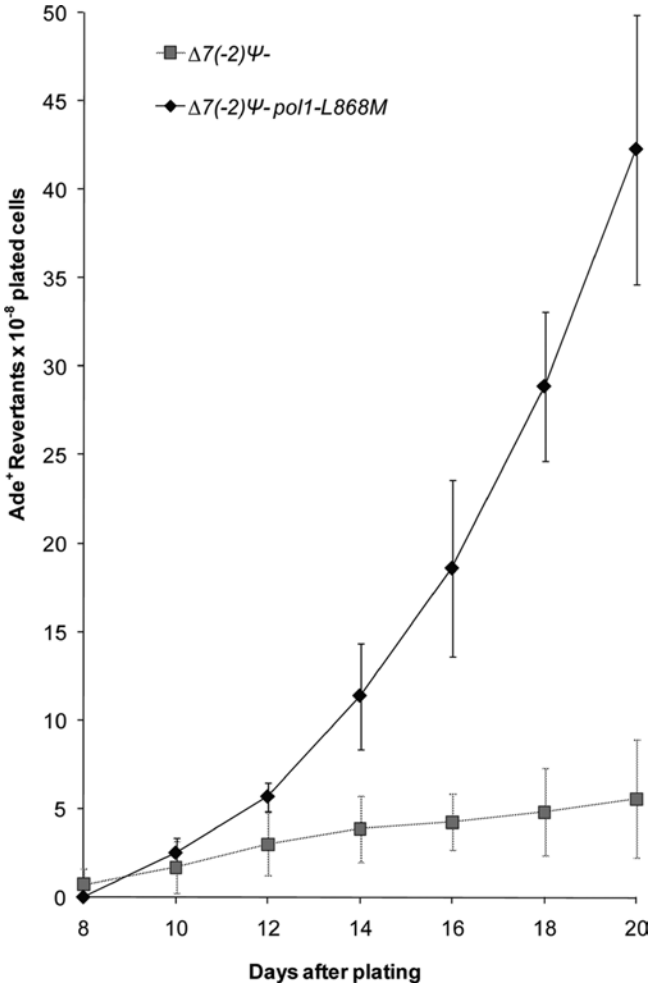

Fig. 2. Effect of the *pol1-L868M* allele on mutagenesis in the strains Δ7(−2)Ψ⁻ and Δ7(−2)Ψ⁻ *pol1-L868M* (colony test). The frequency of Ade⁺ revertant colonies against the number of days on SD-ade is reported. The mean values of three independent cultures are given. Vertical bars represent the standard errors of the mean

SD lim.ade. Dishes were incubated either for two or four days to allow for the formation of small colonies, which were expected to bear zero or few papillae at the most (see Table 2). At that point, water suspensions containing 5−10 cells per microliter, either of the Δ7(−2) ψ⁻pol1-L868M Ade⁺ revertants or of the Ade⁻ strain Δ7(−2) ψ⁻ *pol1-L868M*, were prepared and one microliter drops were placed onto at least ten Δ7(−2) ψ⁻ colonies grown on SD lim.ade. Dishes were incubated at 30°C and checked at the stereomicroscope for the appearance of papillae.

2.2.4 Molecular analysis of Ade⁺ revertants

The *ade2-1* allele carries a specific G to T transversion at nucleotide position (np) 190 (amino acid position 64). This introduced the TAA ochre codon instead of the GAA codon (glu), generating a nonsense mutation. Thus, the analysis of Δ7 (−2) ψ⁻ *pol1-L868M* Ade⁺ revertants and of the control strain Δ7 (−2) ψ⁻ *pol1-L868M* Ade⁻ on the molecular level has been performed by direct sequencing of a region of 910 base pairs (bps) surrounding the np 189. Primers used for amplification were created ad hoc — 41For (TGGGACGTATGATTGTTGAGG) and 950Rev (ATGGCGTTCGTTGTAATGGT) — and the PCR products were purified using the EXOSAP enzymatic system (GE Healthcare). Sequencing reactions have been performed by an external service (BMR-Genomics) with Big Dye terminators (v3.1, Applied Biosystem) using the specific primer 68For (ACAGGCTCAACATTAAGACG). An ABI 3730xl 96 capillaries sequencer is used to obtain electrophoretic profiles that were analyzed using the program Sequencher 4.7 (Gene Codes). Since the traces were of excellent quality and unambiguous, we sequenced only one strand.

3. RESULTS

3.1 Spontaneous mutation frequencies in cells starved for adenine

The frequency of Ade⁺ revertant colonies on SD-ade plates in the strains Δ7(−2)ψ⁻ and Δ7(−2) ψ⁻ pol1-L868M, respectively, is shown in Figure 2 which summarizes the results obtained from three independent experiments for each strain. The revertant frequency was about the same up to day 10 in the strain Δ7(−2) ψ⁻ and Δ7(−2) ψ⁻ *pol1-L868M*, respectively. Thereafter, it increased only slightly in the strain Δ7(−2) ψ⁻, but markedly in the strain Δ7(−2) ψ⁻ *pol1-L868M*. At the end of the experiment (day 20) the frequency of revertants in the strains Δ7(−2) ψ⁻ *pol1-L868M* and Δ7(−2) ψ⁻ were 42.3×10^{-8} and 5.59×10^{-8}, respectively. The strain Δ7(−2) ψ⁻ *pol1-L868M* accumulated 7.6-fold more revertants than the strain Δ7(−2) ψ⁻. We present the data by day 8 on to exclude early arising Ade⁺ revertant colonies originated solely from events which occurred in replicating cells during pre-culture in rich medium (see below). A fundamental issue in SAM is establishing if starved cells undergo divisions on the selective medium. We checked the number of divisions on SD-ade and, as expected (Heidenreich, 2007), the number of cells per plate doubled in the first 24 hours after plating, thereafter it remained constant. Therefore, we are confident that cells did not proliferate after day 2. As documented in Figure 3, the appearance of Δ7(−2) ψ⁻ *pol1-L868M* cells was very similar on day 3 and 17 after plating, respectively. However, cells on day 17 showed some evidence of degeneration. Cell degeneration and death could have caused cannibalism, a phenomenon by which dead cells provide alive cells with nutrients and allow their replication (Büttner, 2006; Heidenreich, 2006). However, in our hands, there was no evidence of cell replication, as documented by the absence of proliferating budding cells up to the end of the experiment. The surviving fractions are reported in Figure 4; they declined with time of adenine starvation and were similar in the strains Δ7(−2) ψ⁻ and Δ7(−2) ψ⁻ *pol1-L868M*. We estimated the reversion rates in the logarithmic growth phase; they were

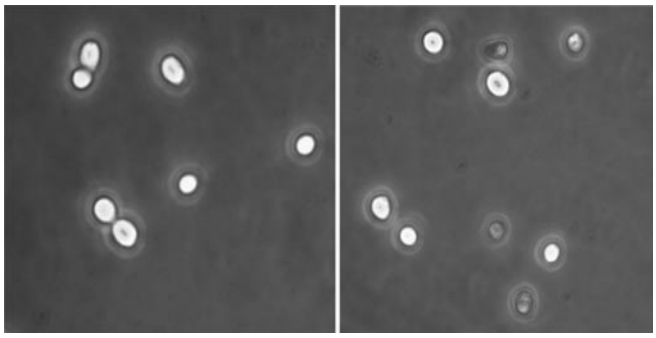

Fig. 3. Δ7(−2)Ψ⁻ *pol1-L868M* cells starved for adenine on day 3 (left) and 17 (right) after plating (40×)

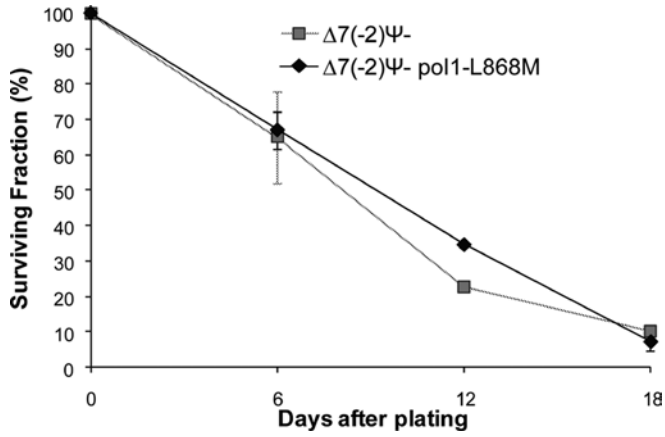

Fig. 4. Effect of the *pol1-L868M* mutation on survival in Δ7(−2)Ψ⁻ and Δ7(−2)Ψ⁻ *pol1-L868M* cells starved for adenine. The mean values of two independent experiments are given. Vertical bars represent the standard errors of the mean

0.16×10^{-8} and 0.18×10^{-8} for the strains Δ7(−2)ψ⁻ and Δ7(−2) ψ⁻ *pol1-L868M*, respectively, so the *L868M* allele did not influence Ade⁺ reversion rates in logarithmic growth phase cells. This result is consistent with earlier findings that the *pol1-L868M* allele is a weak mutator in dividing cells (Pavlov, 2006-b).

We tested 28 Δ7(−2) ψ⁻ pol1-L868M Ade⁺ revertants isolated from the experiments reported in Figure 2 to determine how much time was required by a revertant cell to form a colony visible at the stereomicroscope (see Material and Methods). Most of them (67.8 %) took three days at maximum to give visible colonies; the slowest growing revertant took seven days to form visible colonies. In Table 1 the molecular nature of revertants is also reported. Up to day 4, four locus revertants were detected out of six Ade⁺ clones tested; after that time, no locus revertant was found on 22 Ade⁺ clones tested.

In Table 2 we show the frequency of Ade⁺ revertants in the papillae test. By day 8 the frequency of colonies with at least one Ade⁺ papilla increased dramatically in the strain Δ7(−2) ψ⁻ *pol1-L868M*, while it remained low in the strain Δ7(−2)ψ⁻. On day 13 all colonies had papillae, and some more than one, in the strain Δ7(−2) ψ⁻ *pol1-L868M*, making a reliable estimation of the mutation rate impossible (Figure 1). Table 3 shows the number of divisions, given as the number of cells per colony, and surviving fractions (%) of cells starved for adenine in different days. The number of cells did not increase by day 6, suggesting that most replication occurred within day 6 and did not resume in the next days. However, the high percentage of colonies with papillae in the strain Δ7(−2) ψ⁻ *pol1-L868M* did not allow us to estimate the number of divisions by day 8 and onward (i. e. it did not allow to test whether cells resumed replication). The surviving fractions decreased with time of starvation and they were lower for the strain Δ7(−2) ψ⁻ *pol1-L868M*.

We tested 5 Δ7(−2) ψ⁻ *pol1-L868M* Ade⁺ revertants isolated from the papillae assay to find the time span required by a single revertant cell to form a papilla visible at the stereomicroscope. The time span expressed in days required by the Ade⁺ revertants tested to form visible papillae are as follows: two days for one locus revertant isolated on day 7; four to five days for two suppressors isolated on day 8 and 9, respectively; and seven days for two suppressors isolated on day 12 (Figure 5). Then the highest time span required by Ade⁺ revertants to form a visible colony/papilla was seven days. The molecular analysis was performed on 43 Ade⁺ revertant papillae isolated throughout the experiments. Up to day 8 we detected eight locus revertants out of 27 Ade⁺ revertants tested (29,6 %). From day 8 and onward, we found only one locus revertant out of 16 Ade⁺ revertants tested (three to four per day). In the colony test we observed a lack of Ade⁺ locus revertants by day 4 and onward (see above). A lack of Ade⁺ locus revertants was reported by Achilli (2004) at the ochre codon of the ade5−1 allele in adenine starved cells of the strain de3-01 defective in the proofreading activity of Pol δ. We did not investigate the mechanisms of suppression of adenine auxotrophy in adenine starved cells which might occur via different mechanisms (Prelich, 1999; Rojas Gil, 1999; Forsburg, 2001).

3.2 Spontaneous mutation frequencies in tryptophan starved cells

To learn if starvation of amino acid affects reversion frequencies in the strain Δ7(−2) ψ⁻ *pol1-L868M* as starvation of adenine, we tested SAM in conditions of tryptophan deprivation. The strains used in the presented research are auxotrophic for tryptophan because of a nonsense allele, which, as the allele *ade2-1*, can revert either by true reversions or suppression. Tryptophan starvation is not recommended in studying SAM by the colony test because Trp⁺ revertants can secrete tryptophan in the selective medium allowing starved cells to resume growth (Heidenreich, 2007). Importantly, Trp⁺ revertant cells are likely to form in the pre-growth in liquid YPD medium and

Table 1

Reconstruction tests and molecular analysis of revertants

Day of appearance of Ade$^+$ revertant colonies in the colony test [a]	Isolate #	Day of appearance of Ade$^+$ revertant colonies in reconstruction experiments	Molecular analysis of Ade$^+$ revertants [b]
2	2–1	2	TAA
	2–2	2	CAA
	2–3	3	TAC
4	4–1	3	TTA
	4–2	4	TAA
	4–3	2	TAC
6[c]	6–1	3	TAA
8	8–1	4	TAA
	8–2	3	TAA
	8–3	3	TAA
10	10–1	3	TAA
	10–2	3	TAA
	10–3	3	TAA
12	12–1	3	TAA
	12–2	3	TAA
	12–3	3	TAA
14	14–1	5	TAA
	14–2	6	TAA
	14–3	3	TAA
16	16–1	3	TAA
	16–2	4	TAA
	16–3	7	TAA
18	18–1	5	TAA
	18–2	3	TAA
	18–3	3	TAA
20	20–1	5	TAA
	20–2	3	TAA
	20–3	4	TAA

[a] Colonies were isolated in the colony test reported in Figure 2. Plates were inspected every three days; therefore, on a given day, we isolated Ade$^+$ revertant colonies which became visible either on that day or the day before. Only one revertant per experiment was taken at a given time to avoid isolation of siblings in the first days after plating. All colonies were white on YPD as expected for Ade$^+$ revertants of Ade$^-$ auxotrophic cells.
[b] TAA: suppressors; CAA, TAC, TTA: locus revertants.
[c] On days 5–6 only one Ade$^+$ revertant colony formed.

Table 2

The effect of the *pol1-L868M* mutation on mutagenesis in the papillae assay. The Ade$^+$ reversion rate was calculated as: ln P_0/N where P_0 is the fraction of colonies without papillae and N is the number of cells per colony at the end of growth on SD.lim.ade. The mean values of two independent experiments are reported

Strain	Day after plating	Percentage of colonies with papillae	Reversion rate
Δ7(–2) ψ$^-$	8	0.0	not determined
	11	0.0	not determined
	13	3.4	2.0×10^{-8}
Δ7(–2) ψ$^-$ *pol1-L868M*	8	9.0	6.9×10^{-8}
	11	64.0	7.5×10^{-7}
	13	>100	not determined

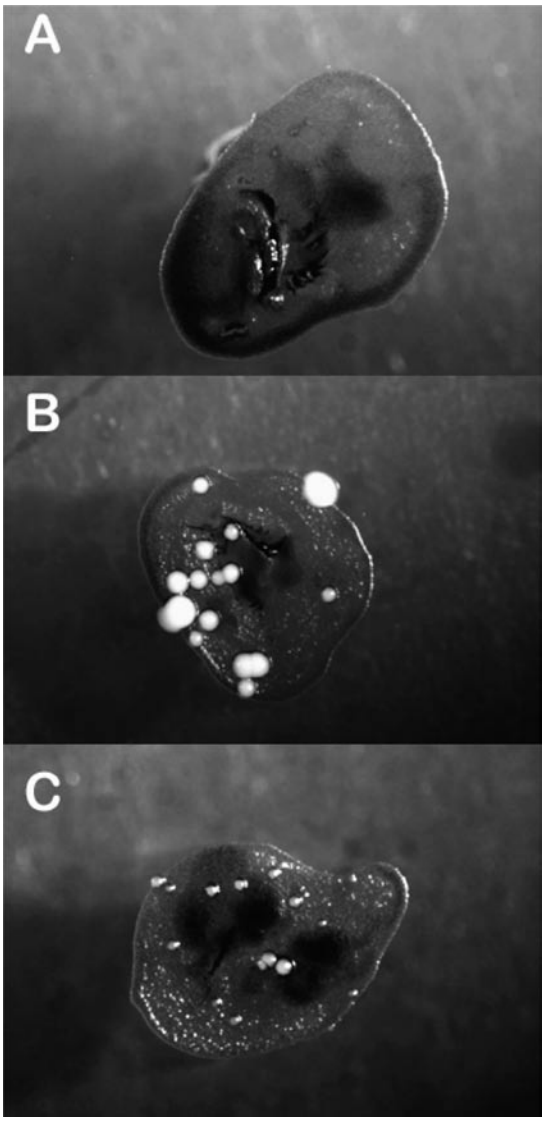

Fig. 5. Papillae assay: reconstruction tests. Water suspensions containing 5–10 cells per microliter either of the Δ7(−2) ψ⁻ *pol1-L868M* Ade⁺ revertants or of the Ade⁻ control strain Δ7(−2) ψ⁻ pol1-L868M were prepared and one microliter drops were placed onto Δ7(−2)Ψ-colonies grown on SD lim.ade. Dishes were incubated for seven days and checked at the stereomicroscope for the appearance of papillae. (A) A colony inoculated with the control strain Δ7(−2) ψ⁻*pol1-L868M* Ade⁻; (B) a colony inoculated with an Ade⁺ revertant isolated on day 8; (C) a colony inoculated with an Ade⁺ revertant isolated on day 12. Magnification: 75×.

they can give origin to Trp⁺ revertant colonies secreting tryptophan in the first days after plating on the selective medium. Notwithstanding this drawback, the test has been successfully used by Halas (2009) in a recent research to investigate the role of Non-Homologous End Joining (NHEJ) and Pol ζ in SAM. In our hands, a heavy growth was evident around Δ7(−2) ψ⁻ *pol1-L868M* Trp⁺ revertant colonies by day 7 and onward. Then we decided to compare the strains Δ7(−2) ψ⁻ *pol1-L868M* and Δ7(−2) ψ⁻ by the papillae test where a colony with a Trp⁺ papillae was not expected to have such a profound effect on the growth of other colonies on the plate. Indeed colonies were relatively sparse in the dish (80–90 per plate) and pre-existing revertant cells were unlikely to be present in such a small inoculum. In two small experiments (10 dishes each) protracted for 14 days we observed a slight increase (1.5 to 2-folds) in the frequency of Trp⁺ revertants in the strain Δ7(−2) ψ⁻ *pol1-L868M* with respect to the strain Δ7(−2) ψ⁻ (data not shown). Although the small size of the experiments do not allow statistically meaningful conclusions, it was evident that tryptophan starvation was not as mutagenic as adenine starvation in the strain Δ7(−2) ψ⁻ *pol1-L868M*.

4. DISCUSSION

Our data extend the previous observations about the role of DNA polymerases in yeast starvation-associated mutagenesis and demonstrate an unappreciated role of Pol α in yeast cell populations starved for nutrients. The results obtained in the colony and papillae tests, respectively, show a dramatic mutator effect of the *L868M* allele in yeast cells starved for adenine, while the same allele confers a slight (if any) mutator phenotype either to log phase cells or to cells starved for tryptophan (Pavlov, 2006-b; this work). The possible role of Pol α in cells starved for adenine is discussed below. In the colony test cells multiplied only in the first 24 hours after plating. Then, taking into account the results of reconstruction tests, Ade⁺ revertants colonies formed by day 8 and onward should have arisen in non-replicating cells. Indeed, the slowest growing revertants took seven days to form a visible colony. As shown in Figure 4, the surviving fractions declined with time of adenine starvation in both strains. Therefore, we cannot exclude that some divisions and/or abortive replications occurred because of the adenine released by dead cells. In the papillae test, the number of cells per colony did not change by day 6 and onward, suggesting that cells stopped dividing. However, as for the colony assay, a restart of replication would have been possible, and even more likely, because of the condition inside the colony. Indeed, in recent years, it has been increasingly appreciated the complex organization of yeast colonies, which behave more like primitive organisms than a simple aggregate of unicellular entities. Apoptosis has been documented in yeast and it has been proposed to confer a selective advantage to yeast colony populations allowing replication of alive cells at the expense of dead ones (Büttner, 2006). In starving cells in colonies, Pol α could be involved either in genome replications or in its repair. Because of the shortage of adenine, both processes would occur under nucleotide pool imbalance, which is known to be muta-

Table 3

Number of cells per colony and survival in the papillae test on SD lim.ade. in the strains Δ7(−2)Ψ⁻ and Δ7(−2)Ψ⁻ *pol1-L868M*, respectively. The mean values of two experiments are reported

Strain	Day after plating	N° of cells per colony	Surviving fraction (%)
Δ7(−2) ψ⁻	4	1.0×10^6	88.5
	6	1.6×10^7	58.4
	8	1.6×10^7	47.9
	11	1.5×10^7	not determined
Δ7(−2) ψ− *pol1-L868M*	4	0.7×10^7	69.8
	6	1.3×10^7	33.0
	8	1.3×10^7	23.7
	11	not determined	not determined

genic per se (Korogodin, 1991). Pol α is known to be a key polymerase in DNA replication (Garg, 2005; Johnson, 2005; Pavlov, 2006-a; Kunkel, 2008; Pavlov, 2010) and it is also known to participate in break-induced replication (BIR). BIR is suggested to be the primary repair pathway of one-ended breaks that can be formed as a result of replication forks collapse but it also functions to repair DSBs created in other ways (Malkova, 1996). BIR is carried out by several proteins which accomplish recombination and replication processes. The last step of BIR is DNA synthesis. It is known that in yeast it is performed by Pol α, Pol δ and Pol ε, but the exact role of the different DNA polymerases in BIR-related synthesis are still elusive (Deem, 2008; Lydeard, 2010). In adenine auxotrophic cells deprived of adenine, DSB are likely to form either because of the collapse of replication forks or because of other insults to the genome. Indeed, DSBs are considered to be a potential source of adaptive mutation (Heidenreich, 2007). Starvation-associated base substitutions and frameshifts require NHEJ to occur (Heidenreich, 2003; Halas, 2009) and it is known that DSBs are the substrate of NHEJ. In adenine starved cells BIR could attempt to restore collapsed replications forks and to repair DSBs formed via other mechanisms in competition with NHEJ. As a consequence of nucleotide pool imbalance, pol α could misincorporate nucleotides at higher rates than in actively dividing cells. In the strain Δ7(−2)ψ⁻ *pol1-L868M* they might be repaired inefficiently. Indeed, errors made by pol α, which is devoid of a 3' exonuclease activity, can be corrected by Pol δ proofreading activity and MMR in growing cells (Gutiérrez, 2003; Pavlov, 2006-b; NickMcElhinny, 2006). Pol δ could correct mistakes made by Pol α during BIR-associated DNA synthesis in adenine starved cells but, because of the nucleotide pool imbalance, the proofreading activity of pol δ might be saturated.

In conclusion, in the presented paper we show that adenine starvation and the consequent nucleotide pool imbalance exacerbate the mutator phenotype of the strain Δ7(−2) ψ⁻ *pol1-L868M*, thus revealing the fundamental role of Pol α in yeast cells populations subjected to nutrient deprivation. We put forward the hypothesis that Pol α is involved in the repair of DSBs via BIR. Although challenging, it would be interesting to verify if BIR is involved in controlling mutagenesis in cells starved for nutrients, a condition which microorganisms experience for most of the time in nature.

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РОЛЬ ДНК-ПОЛИМЕРАЗЫ АЛЬФА В КОНТРОЛЕ МУГАГЕНЕЗА ИНДУЦИРОВАННОГО ГОЛОДАНИЕМ У *SACCHAROMYCES CEREVISIAE*

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❀ **SUMMARY: Микроогранизмы в естественных условиях большую часть времени растут медленно из-за стрессовых условий. За последние две декады стало ясно, что мутации возникают не только в активно делящихся клетках, но и в нереплицирующихся или медленно реплицирующихся клетках при голодании (мугагенез индуцированный голоданием, МИГ). Основными факторами, поддерживающими низкий базальный уровень МИГ являются способность ДНК-полимераз δ и ε точно подставлять нуклеотиды в ходе репликации и эндонуклеазная корректирующая активность этих ферментов. Гораздо меньше исследована роль репликативной ДНК-полимеразы α (Polα). В статье представлены аргументация в пользу того, что Polα вовлечена в контроль МИГ при голодании по аденину. Мы предполагаем, что в этих условиях Polα участвует в эпизодических актах репликации/репарации.**

❀ **KEY WORDS:** *Saccharomyces cerevisiae*; ДНК-полимераза α; мугагенез индуцированный голоданием.

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