Investigating translational fidelity in *Schizosaccharomyces pombe* mutants

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Proteostasis is increasingly recognised as a key process disrupted during ageing and disease. While the degradation of damaged proteins via pathways like the ubiquitin-proteasome system is well-studied, the decline in translational fidelity with age has received less attention. Translational errors can accumulate over time, potentially contributing to proteotoxic stress. This project aims to explore the impact of translational fidelity on ageing by assessing the lifespan of *Schizosaccharomyces pombe* mutants with PKA1 deletions, considering differences when grown in nutrient-rich Yeast Extract Supplemented (YES) versus nutrient-limited Edinburgh Minimal Medium (EMM). As yeast grow slower in EMM (Petersen & Russell, 2016), lifespan differences between wildtype and PKA strains may be exaggerated, aiding our investigation and providing insight into the role of translation fidelity in ageing. PKA1 deletions are reported to increase lifespan in *S. pombe*. Here, I seek to further validate this in both YES and EMM media.

For this project, I assessed the lifespan of six different *S. pombe* strains, including wild type (WT) and PKA1 deletion mutants, with and without luciferase cassettes for future luciferase assays. Each strain had a duplicate, totalling 12 samples (Table 1).

Genotype	**Flasks**	**Cassettes**
WT	1, 7	Strain used to create all strains below
ΔPKA1	2, 8	PKA deletion
WT 688	3, 9	Luciferase Cassette: Renilla + Firefly
WT 868	4, 10	Luciferase Cassette: Renilla + mutant Firefly
PKA 688	5, 11	PKA Deletion Luciferase Cassette: Renilla + Firefly
PKA 868	6, 12	PKA Deletion Luciferase Cassette: Renilla + mutant Firefly

Table 1 *Genotypes and Properties of Samples used throughout the experiment*

To set up the lifespans, I synchronized the cultures in stationary phase using a specialized Rallis lab calculator, which accounts for generation times and optical density (OD) of the pre-culture, providing precise volumes required to generate synchronised cultures. Using this calculator, I tried multiple generation times to perfect synchronisation (Table 2).

WT- YES	PKA- YES	WT- EMM	PKA- EMM
2	2.6	2.5	3.9
2.1	2.7	2.7	4.2
2.2	2.8	2.8	4.5
	2.9	3	
	3	2.9	
	3.2	3.1	
	3.3		
	3.4		
	3.5		

Table 2 *Doubling times in hours trialled for WT and PKA strains in YES and EMM. Highlighted in green are those times which resulted in synchronised cultures.*

Subsequently, I measured colony-forming units (CFUs) found daily in all 12 samples, in both YES and EMM. This lifespan assay revealed that in YES, PKA1 deletion mutants lived approximately three times longer than wild type strains, confirming prior reports of their extended longevity (Figure 1). In EMM, the lifespan difference was less pronounced, potentially due to technical issues, including inconsistent incubator temperatures and contamination observed on the plates. These results may suggest that nutrient availability (as in EMM) may diminish the effect of PKA1 deletion on lifespan, although further replicates are needed to confirm this observation. Moving forward, I will combine these results with luciferase assays to evaluate translational fidelity in these strains, focusing on the impact of translation errors on aging. I will focus on ribosomal mutants. A Lysine to Arginine mutation (K60R) in RPS23, a ribosomal protein, has been reported to improve the accuracy of protein synthesis with age (Martinez-Miguel et al., 2021). My future work will explore other, similar mutations and assess their impact on translational fidelity.

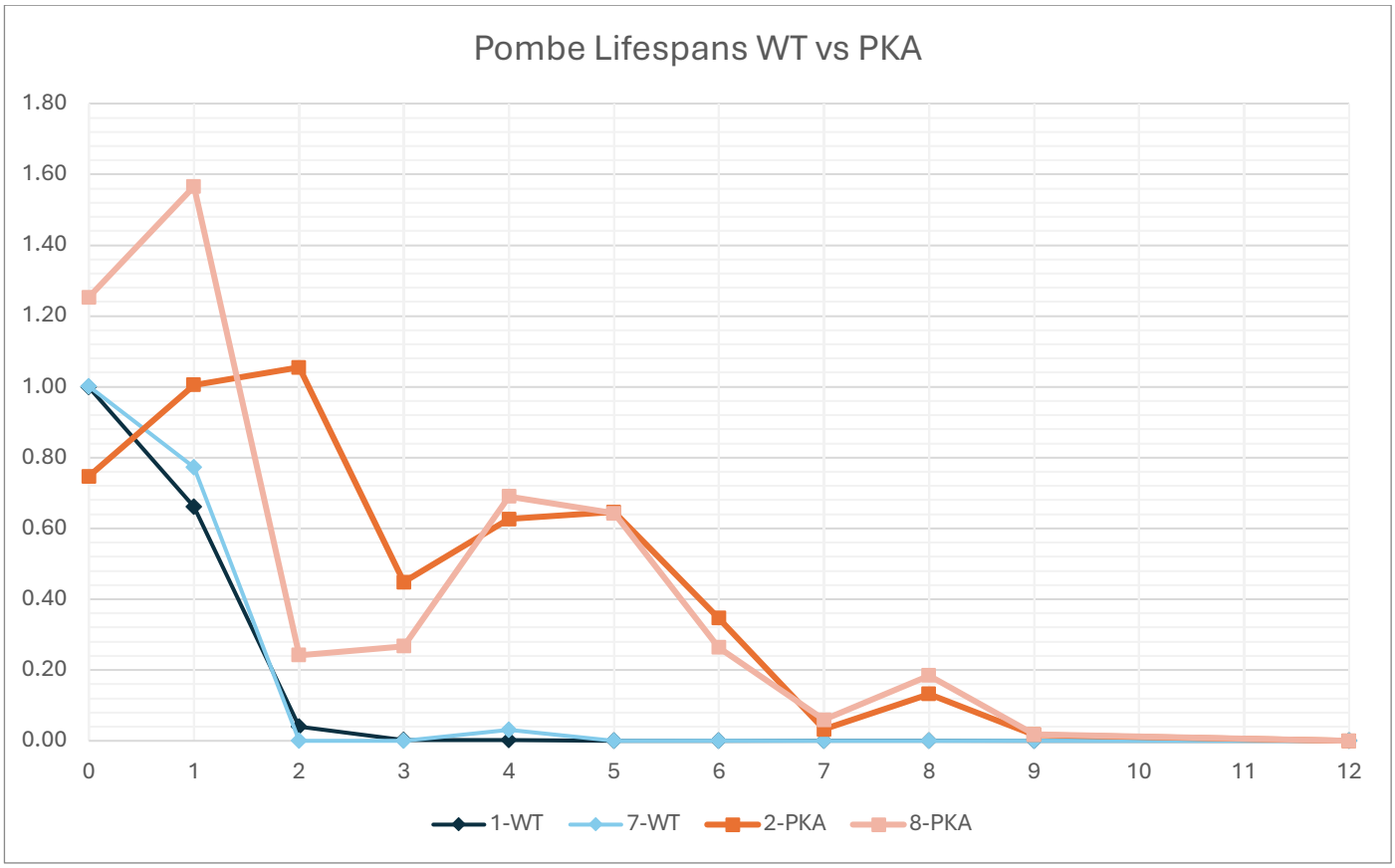

Figure 1 *Lifespans of WT vs PKA1 mutants in YES*

This project highlights a relatively neglected area of aging research—translational fidelity and its role in the maintenance of proteostasis. The findings of my work, and that of the Bjedov Group, could have broad implications, not only in understanding the aging process but also in addressing diseases associated with protein misfolding and aggregation, such as cancer and neurodegenerative conditions like Alzheimer's and Parkinson's disease. By providing tools and assays to measure translation errors, this research can stimulate further investigation into therapeutic strategies that enhance translational fidelity.

Additionally, this work aligns with the Biochemical Society's strategy by fostering innovation in the molecular biosciences. It promotes interdisciplinary approaches to understanding the molecular

mechanisms underlying aging and disease, integrating genetics, molecular biology, and aging research to explore translational fidelity's role in age-related diseases.

I have developed a range of skills critical to success in a research environment. A key aspect of my training has been the meticulous documentation of experimental work, ensuring that results are clearly presented and methods comprehensively detailed, and is essential for replication of experiments. Additionally, I have gained valuable experience in experimental design, including the use of both positive and negative controls, and in the application of appropriate statistical techniques for data analysis.

My practical molecular biology skills have also expanded significantly, including tasks such as plate pouring, streaking *S. pombe* cultures, and acquiring foundational knowledge in yeast genetics. Furthermore, I assisted in luciferase assays, yeast transformations, and PCR-based verification of cassette insertion. I independently conducted two lifespan experiments, troubleshooting challenges as they arose and seeking assistance when necessary. Participation in lab meetings has honed my presentation skills, and I will soon present my findings—a crucial capability for scientific communication. These experiences have equipped me with both technical and transferable skills essential for a career in molecular bioscience.

This studentship has been an invaluable learning experience. It aided in making connections with key members of the UCL Cancer Institute and secured my master's project. Moreover, it has shown me that a career in research is what I want and confirmed my choice in specialising in Cancer Genetics.

References

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