



Can yeast harness the adaptive potential of extrachromosomal circular DNA?

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1. Introduction 4. Results: Circles and Adaptation eccDNAs are a source of genetic heterogenity: CUP1 eccDNA allows robust adaptation to copper stress: Asymmetric inheritance Survival on 2 mM CuSO4 B Α Detected in a wide range prior to stress 0.8 p = 0.99 p = 0.0036 of Eukaryotes CuSC Produced by a variety of 0.6 Only aged, CUP1 Southern: kB. FLP+ survive plating mechanisms 5 0.4 Non-Stress Media Carry and express genes မ္ပိ Y = Young (Log) 0.2 symmetri A = Aged 24 hrs 0.0 1 FLP Young Aged Growth in liquid culture Detection of CUP1 circles С Non-Mendelian inheritance: 2 by Southern Blot Voung, FLP-Aged, FLP-Parental 2 mM CuSO eccDNAs do not have centromeres. (No resistance) resistant and do not segregate 1:1 Many generation 3 R R E 10 eccDNA accumulation has **OD**600 been linked to adaptive Majority Oldest cells phenotypes¹ retain circles All strains grow without copper 24 48 2. Aims chromosoma 10 Our Question: OD600 Only aged, FLP+ grow in 2 mM CuSO₄ If eccDNA is asymmetrically inherited, how mechanistically CUF can circles support long-term adaptive phenotypes STRESS within a cell population? Our Model: 24 48 Time (hrs) As shown centrally, S. cerevisiae retain circles in the Do circles Mother cell². By using the gene CUP1 and the enable 5. Results: Circle Stability stressor copper, we aim to dissect the emergence of survival? resistance via eccDNA accumulation and track CUP1 eccDNA is stable once acquired: whether inheritance changes under stress, linking a single circle to a specific adaptive phenotype. Growth rate in 2 mM CuSO₄ Southern Blot Α Inheritance 6 weeks without change CuSO₄ culture: under stress? 3. Methods 2 mM CuSO₄ resistant **OD**600 R E After 6 weeks continuous culture D GFP-Tagged CUP1 eccDNA: o change, despite lacl Grown without CuSO. selection pressure 24 48 Time (Hrs) В CUP1 eccDNA vs. Centromeric plasmid Age Population 100 distributes with age only Copper resistar CEN pl testeo -URA Redacted Marker 10 CUP1 eccDN/ 2mM Ci Åge Circles stil rcles more stable alle ba the most circles Genomic GEP 6. Future Directions Our data shows that circles can indeed support long-term adaptation to stress at the population level. Future work will investigate why CUP1 circles become so unusually stable in our experimental system.

Key References: 1. Nguyen, D.D. The Interplay of mutagenesis and ecDNA shapes urothelial cancer evolution. Nature 635, 219–228 (2024). 2. Denoth-Lippuner A: Role of SAGA in the asymmetric segregation of DNA circles during yeast ageing. Elife, 2014 Nov 17.

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