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Investigating the consequences of asymmetric endoplasmic reticulum inheritance in *Saccharomyces cerevisiae* under stress using a combination of single cell measurements and mathematical modelling

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ABSTRACT

Adaptation allows organisms to maintain a constant internal environment, which is optimised for growth. The unfolded protein response (UPR) is an example of a feedback loop that maintains endoplasmic reticulum (ER) homeostasis, and is characteristic of how adaptation is often mediated by transcriptional networks. The more recent discovery of asymmetric division in maintaining ER homeostasis, however, is an example of how alternative non-transcriptional pathways can exist, but are overlooked by gold standard transcriptomic or proteomic population-based assays. In this study, we have used a combination of fluorescent reporters, flow cytometry and mathematical modelling to explore the relative roles of asymmetric cell division and the UPR in maintaining ER homeostasis. Under low ER stress, asymmetric division leaves daughter cells with an ER deficiency, necessitating activation of the UPR and prolonged cell cycle during which they can recover ER functionality before growth. Mathematical analysis of and simulation results from our mathematical model reinforce the experimental observations that low ER stress primarily impacts the growth rate of the daughter cells. These results demonstrate the interplay between homeostatic pathways and the importance of exploring sub-population dynamics to understand population adaptation to quantitatively different stresses.

1. Introduction

Adaptation is the basic mechanism that enables organisms to thrive in a changing, and often challenging, environment. Both single and multicellular organisms have evolved a set of internal conditions that allow them to fully exploit an ecological niche. Organisms maintain this homeostasis by adapting: different stresses are detected by specific sensors, which trigger bespoke transcriptional responses [1]. This regulation of gene networks collectively acts to reinstate homeostasis, be it through an adjustment of metabolism [2], cellular transport [3], or motility [4]. One example of adaptation is the maintenance of endoplasmic reticulum (ER) homeostasis. The ER, a large organelle comprising a single lipid bilayer and enclosed lumen, extends as a network throughout cell and is responsible for a diverse range of functionalities including: (i) protein synthesis, folding and quality control, (ii) calcium storage and (iii) lipid metabolism [5–7].

Focusing on protein synthesis, when nascent polypeptides enter the ER they interact with a range of chaperones and foldases that direct protein folding, and ensure only those with the native conformation progress through the secretory pathway. During high protein expression or in sub-optimal environments, however, these pathways are overloaded and the ER becomes crowded with unfolded and misfolded proteins, activating the unfolded protein response (UPR, Fig. 1). The stress sensor in this case is the transmembrane protein Ire1, and in combination with the chaperone BiP (Kar2 in yeast) and the transcription factor Hac1p, constitute the highly conserved components of the eukaryotic unfolded protein

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Abbreviations

ACT1	Actin 1
ConA	TRITC-labelled concanavalin A
DTT	Dithiothreitol
ER	Endoplasmic reticulum
ERAD	ER associated degradation
ERSU	ER stress surveillance pathway
ERO1	Endoplasmic Reticulum Oxidoreductin 1
eroGFP	ER-targeted redox-sensitive GFP
GFP	Green fluorescent protein
OD_{600}	optical density at 600 nm
ODE	Ordinary Differential Equation
Tm	Tunicamycin
UPR	Unfolded protein response
YPD	Yeast Peptone Dextrose medium

response (UPR) [8,9]. The excess unfolded protein sequesters BiP, causing dissociation of the lumenal Ire1-BiP complex. This allows Ire1 to oligomerise, activating its cytoplasmic RNAse domain, which in turn cleaves an intron from the *HAC1* mRNA permitting translation [10,11]. The Hac1p transcription factor retrotranslocates to the nucleus where it regulates the transcription of around 400 genes, associated with protein trafficking and quality control, metabolism, and cell wall biosynthesis, which collectively restore ER homeostasis [12].

The importance of adaptation mechanisms to these deviations can be inferred through the prevalence of biological redundancy, conferring robustness. In this instance, the role of the UPR in maintaining ER homeostasis is so critical that additional branches have evolved in higher eukaryotes [13], and the network is often implicated in diseases such as neurodegeneration [14], viral infection [15], and cancer [16]. This redundancy does, however, complicate our understanding of the system and increases the importance of knowing not only the identity of the pathways, but in determining their relative roles and interactions [17]. Although research into the UPR has elucidated the molecular interactions of Ire1 and BiP [18–20], links to other regulatory pathways and the presence of additional mechanisms are to be expected. This is particularly pertinent in low and medium stress conditions: most environmental changes are not binary in nature but continuous, and therefore, cells may use a variety of different mechanisms including those that operate without the need to activate changes in gene expression.

One such adaptation mechanism which is becoming increasingly apparent is to trigger asymmetric division of organelles [21]. Research in this field has focused on understanding how these complex structures, including the mitochondria and vacuole, are divided between mother and daughter yeast cells as *de novo* generation is often slow – and in cases such as the ER, impossible [22]. There is now an increasing precedent for asymmetric division of ER under stress, particularly with the discovery that mother cells can retain a greater majority of damaged components during budding through the ER surveillance (ERSU) pathway [23]. This mechanism is independent of the UPR and operates through the MAP kinase Slt2, along with components of the cell wall integrity pathway, to delay the passage of damaged ER to daughter cells [24] through the formation of a lipid barrier at the bud neck [25]. This delay extends cytokinesis until a minimal threshold of ER functional capacity is reached, ensuring mother cell viability [23].

Here, we sought to understand the roles of asymmetric division and UPR activation in population adaptation to low ER stress. In research scenarios, ER stress is frequently induced with high (mM) concentrations of chemical inhibitors, such as DTT or tunicamycin, to ensure strong activation in all cells [26]. This has been vital for understanding the molecular basis of these pathways, but reveals the mechanisms under extreme conditions. Here, we decreased the concentration of tunicamycin from the typical (2 μg/mL) to a more physiologically relevant value (100 ng/mL) based on the IC_{50} value of its target, *ALG7* [27,28]. Furthermore, in place of more traditional population-wide readouts such as RNA splicing, qRT-PCR, and Western blotting for phosphorylation changes [26], we used a combination of fluorescent reporters and flow cytometry to obtain single cell data. We coupled this data to mathematical modelling to subsequently explore the consequences of asymmetric ER inheritance for population growth rate changes, and therefore, adaptation.

2. Material and methods

2.1. Yeast strains and plasmids

All yeast strains and plasmids used in this study are summarized in Appendix A. The reporter plasmids for UPR activation and ER content were derived from pRS403 [29], pPM28 (Addgene #20131), pPM47 (Addgene #20132) [30] and pMaM175 [31] via standard molecular biology techniques (overlap extension PCR and

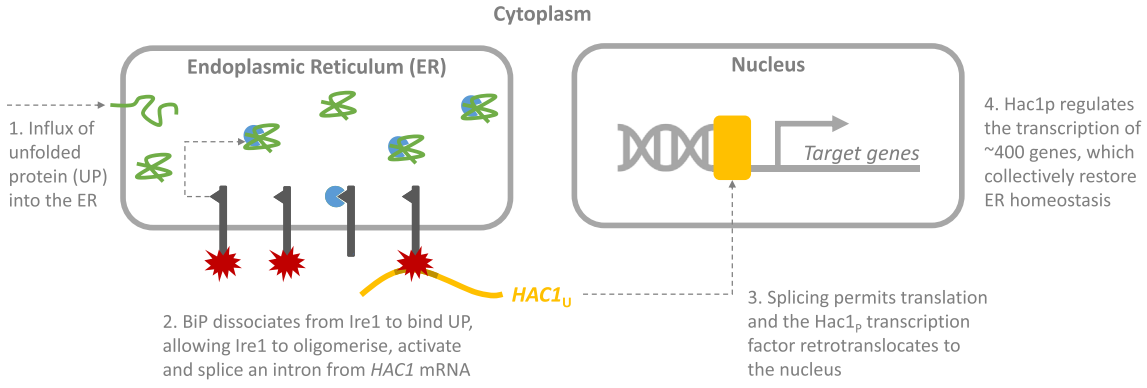

Fig. 1. Unfolded protein response (UPR) signalling. The UPR is a transcriptional response to deviations in endoplasmic reticulum (ER) homeostasis. For instance, an influx of unfolded protein (green) to the ER causes the chaperone BiP (blue) to dissociate from the transmembrane stress sensor Ire1 (dark grey) to help protein folding. Ire1 subsequently oligomerises and activates (red), allowing its cytoplasmic RNAse domain to cleave an intron (brown) from the *HAC1* mRNA (yellow) permitting translation. The Hac1p transcription factor retrotranslocates to the nucleus where it regulates the transcription of around 400 genes, which act collectively to restore ER homeostasis.