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Original Research

Xrn1 Exoribonuclease—An Intrinsic Marker of Yeast Population Growth

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Abstract

Background: Xrn1 exoribonuclease is the major mRNA degradation enzyme in *Saccharomyces cerevisiae.* In exponentially growing cells, Xrn1 is localised in the yeast cells and directs the degradation of mRNA molecules. Xrn1 is gradually deposited and presumably inactivated in the processing bodies (P-bodies) as the yeast population ages. Xrn1 can also localise to the membrane compartment of the arginine permease Can1/eisosome compartment at the yeast plasma membrane. This localisation correlates with the metabolic (diauxic) shift from glucose fermentation to respiration, although the relevance of this Xrn1 localisation remains unknown. **Methods**: We monitored the growth rates and morphology of Xrn1-green fluorescent protein (GFP) cells compared to wild-type and Δ*xrn1* cells and observed the Xrn1-GFP localisation pattern in different media types for up to 72 hours using fluorescence microscopy. **Results**: We present the dynamic changes in the localisation of Xrn1 as a versatile tool for monitoring the growth of yeast populations at the single-cell level using fluorescence microscopy. **Conclusions**: The dynamic changes in the localisation of Xrn1 can be a versatile tool for monitoring the growth of yeast populations at the single-cell level. Simultaneously, Xrn1 localisation outside of P-bodies in post-diauxic cells supports its storage and cytoprotective function, yet the role of P-bodies in cell metabolism has still not yet been entirely elucidated.

Keywords: Xrn1; P-bodies; eisosomes; diauxic shift; yeast

1. Introduction

Xrn1, a 5′–3′ exoribonuclease, is an evolutionarily conserved protein and the major mRNA degradation enzyme in *Saccharomyces cerevisiae*; it also participates in several steps in RNA metabolism [1]. Although not essential, the deletion mutant displays severe phenotypes [2]. Additionally, Xrn1 is gradually deposited and presumably inactivated in processing bodies (P-bodies) as the yeast population ages or during mild stress conditions (e.g., heat shock at 42 °C) [3,4].

P-bodies are messenger ribonucleoprotein assemblies found in unstressed cells that propagate when subjected to stress. These non-membrane organelles are formed by the phase separation process and can accumulate translationally repressed mRNA molecules, including mRNA decay machinery components, such as Xrn1 exonuclease, decapping enzymes, such as decapping protein 1 (Dcp1) and Dcp2, and decapping activators, such as the DEAD-box RNA helicase Dhh1 [5]. Due to their composition, it was generally believed that P-bodies play an active role in the degradation of mRNA molecules [6,7]. However, the most recent data indicate that mRNA degradation occurs independently of the presence of P-bodies [5,8]. Thus, P-bodies are currently recognised as transient accumulations of translationally repressed mRNA molecules and associated proteins, which influence the translational response by the cell to changing environmental conditions rather than actual sites of mRNA degradation [8,9].

Previously, we showed that Xrn1 also localises to the membrane compartment of arginine permease Can1 (MCC)/eisosome compartment in the plasma membrane of post-diauxic yeast cells [7]. The MCC possesses characteristic protein and lipid compositions and forms a microdomain in the yeast plasma membrane [10]. The MCC compartment is organised by cytosolic, membrane-attached complexes called eisosomes [11]. The eisosomal core components, Pil1 and Lsp1, shape the plasma membrane to characteristic furrow-like invaginations, which are conserved in yeast, plants, and bacteria [12]. The MCC/eisosomes compartment regulates the internalisation of membrane proteins, lipid homeostasis, and cellular signalling and promotes stress resistance [13,14]. The physiological relevance of the association between Xrn1 and MCC/eisosomes microdomains remains unknown. However, it has been proposed that the localisation serves to temporally separate the Xrn1 enzyme from the rest of the mRNA degradation machinery in the cytosol and P-bodies to inhibit any unwanted mRNA decay [15].

Here, we present the dynamic changes in Xrn1 localisation as a versatile tool for monitoring the growth of yeast populations at the single-cell level using fluorescence microscopy. We monitored the growth rates and morphology of Xrn1- green fluorescent protein (GFP) cells and compared them to wild-type and Δ*xrn1* cells. Additionally, we observed the Xrn1-GFP localisation pattern in different media types for up to 72 hours. We observed the gradual de-

position into P-bodies as cells reached a diauxic shift and the increasing association with the MCC/eisosomes plasma membrane compartment in post-diauxic shift cells. The depicted Xrn1 localisation pattern correlated with changes in cell metabolism, enabling the assessment of the growth status of individual cells within the yeast population.

2. Methods

2.1 Yeast Strains and Growth Conditions

S. cerevisiae strains were derived from the BY4741 background and are listed in **Supplementary Table 1**. Yeast cultures were grown at 28 °C, either in yeast extract peptone dextrose (YPD) medium (1% yeast extract, 2% peptone, 2% glucose) or synthetic defined (SD) medium (0.17% yeast nitrogen base (YNB) without amino acids and ammonium sulphate, 0.5% ammonium sulphate, 2% glucose, supplemented with a complete or appropriate mixture of amino acids). The corresponding solid media contained 2% agar. The respective amino acid was omitted from the dropout mix to select for auxotrophies.

Overnight cultures of tested strains were diluted in fresh YPD or SD media to an optical density at 600 nm (OD_{600}) of 0.2 and further cultivated at 28 °C under shaking. Samples for analyses were taken at the indicated time points. Standard spot tests and growth curve measurements were performed to compare the growth rates of the tested strains.

2.2 Wide-Field Microscopy

The cells were inspected immediately after being mounted onto coverslips and coated with a slice of 1.5% agarose in SD medium (0.17% YNB without amino acids and ammonium sulphate, 0.5% ammonium sulphate, supplemented with an appropriate mixture of amino acids) without glucose. Wide-field single-layer or z-stack images were acquired using an Olympus IX81 microscope (Olympus, Tokyo, Japan) equipped with a 100 × 1.58 oil objective and 95B camera (Photometrics Inc., Huntington Beach, CA, USA), and the z-stacks were further deconvolved (Wiener filter, cellSens 4.1; https://www.olympus-lifescience.com). Acquired images were further processed using the ImageJ/Fiji (ImageJ 1.53c, https://imagej.nih.gov/), General Image Manipulation Program (GIMP 2.10.36; https://www.gimp.org/) and Inkscape (Inkscape 1.1, https://inkscape.org/) software. The z-stacks were collapsed to a single image using ImageJ/Fiji for quantification.

2.3 mCardinal Integrative Cassette

The mCardinal fluorophore gene [16] was purchased from Eurofins Genomics (Louisville, KY, USA). The corresponding mCardinal DNA fragment was amplified using the following primer pair: mCardinal: Fwd (SalI) (5′–GCTGTCGACATGGTGAGCAAGGGCG–3′); mCardinal: Rev (BamHI) (5′–GCTGGATCCTTAGGCTCGAGCCTTG–3′). The PCR product was cloned into the pFM699 (pFA6a-mCherry-natNT2) vector [17] using SalI/BamHI restriction enzymes to replace the mCherry gene. The final integration cassette, pFM699-mCardinal (**Supplementary Table 2**), was confirmed by sequencing and is available upon request. The following pair of primers was designed to tag the Xrn1 C-terminal with an mCardinal fluorophore using the pFM699 integration cassette: Xrn1-mCardinal: Fwd (5′–GCAATGCTGCTGACCGTGATAATAAAAAAGACGAATCTACTCGTACGCTGCAGGTCGAC–3′); Xrn1-mCardinal: Rev (5′–GTAACCTCGAATATACTTCGTTTTTAGTCGTATGTTATCGATGAATTCGAGCTCG–3′).

3. Results

We verified that tagging the Xrn1 protein did not produce a novel phenotype by the yeast cells. We performed a standard spot test assay, growth curve measurement, and microscopy-based observation of the wild-type, Δ*xrn1*, and Xrn1-GFP cells. We observed that while the Δ*xrn1* strain displayed a slow growth phenotype on the spot test assay, the Xrn1-GFP cell growth was indistinguishable from that of the wild-type cells (Fig. 1A); data that is consistent with our previous results [7]. We achieved the same results for the growth curve measurement performed in liquid media (Fig. 1B). Finally, we compared the morphology of the strains using wide-field microscopy and observed that the Δ*xrn1* cells had an aberrant shape (i.e., enlarged cells with elongated buds), and the Xrn1-GFP cells presented a wild-type-like phenotype (Fig. 1C,D). These results suggest that Xrn1 tagging does not interfere with functionality and can be used to monitor genuine Xrn1 localisation in yeast cells.

We monitored the localisation pattern of the Xrn1-GFP protein in a growing yeast population cultivated in YDP or synthetic media for up to 72 hours. In both media types, we observed a gradual re-localisation of Xrn1-GFP as the yeast population aged, from a diffused cytosolic signal to the P-bodies (Fig. 2). As we previously reported [7], when cells reached a diauxic shift, Xrn1 becomes localised in the plasma membrane-located eisosomes (Fig. 2A,C). This localisation became more pronounced as the yeast population aged (Fig. 2B,D). The kinetics involved in Xrn1 re-localisation were media-dependent, probably owing to the different availabilities of glucose and additional nutrients in the respective media.

Since the GFP fluorophore (ex. 488 nm/em. 507 nm) is widely used in microscopy-based analyses and Xrn1 tagging could interfere with downstream applications, we designed an integrative cassette with a far-red, mCardinal fluorophore (ex. 604 nm/em. 659 nm) (Fig. 3). The cassette has nourseothricin resistance and is compatible with the S1–S4 tagging system [18]. Moreover, using a far-red fluorophore for Xrn1 tagging means other commonly used fluorescence channels are available for downstream analyses.

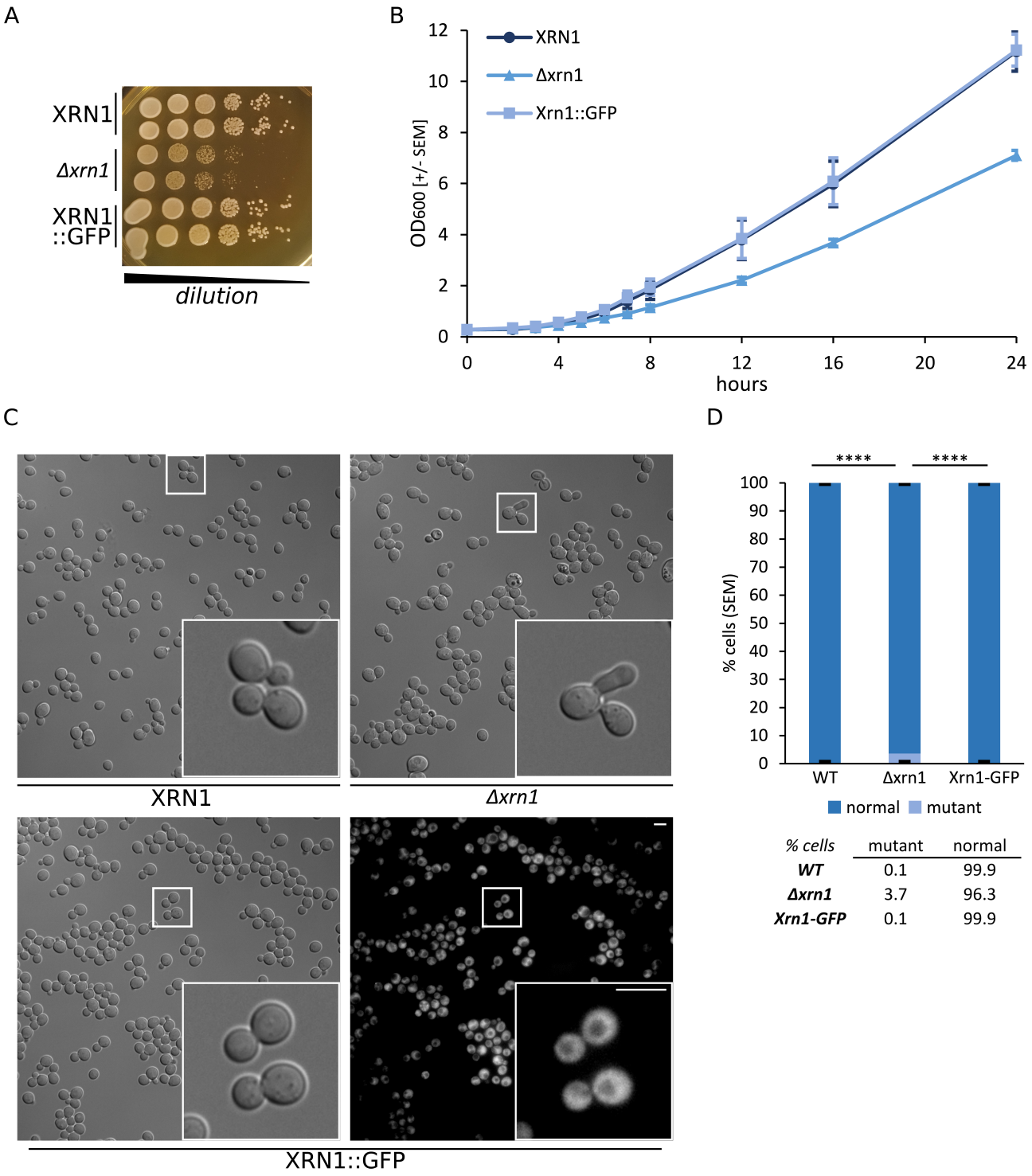

Fig. 1. Xrn1 tagging does not interfere with its functionality. (A) Spot test comparison of wild-type (XRN1), deletion mutant (Δ*xrn1*), and green fluorescent protein (GFP)-tagged (XRN1::GFP) cell growth rates. The dilution series of the respective strains is shown. (B) Growth curve, including the standard error of the mean, of the same strains as in (A) in liquid yeast extract peptone dextrose (YPD) media. The graph represents pooled data from three biological replicates. (C) Wide-field differential interference contrast (DIC) images of the same strains as in (A) and corresponding GFP images of the XRN1::GFP strains are shown. The insets display a higher magnification of the boxed area. Scale bar: 5 µm. (D) The graph displays the percentage of cells, including the standard error of the mean, with the wild-type ("normal") and mutant ("mutant") morphology of the same strains as in (A) ($n > 500$ cells per strain). The graph represents pooled data from three biological replicates. ****$p < 0.0001$ (two-sample *t*-test).

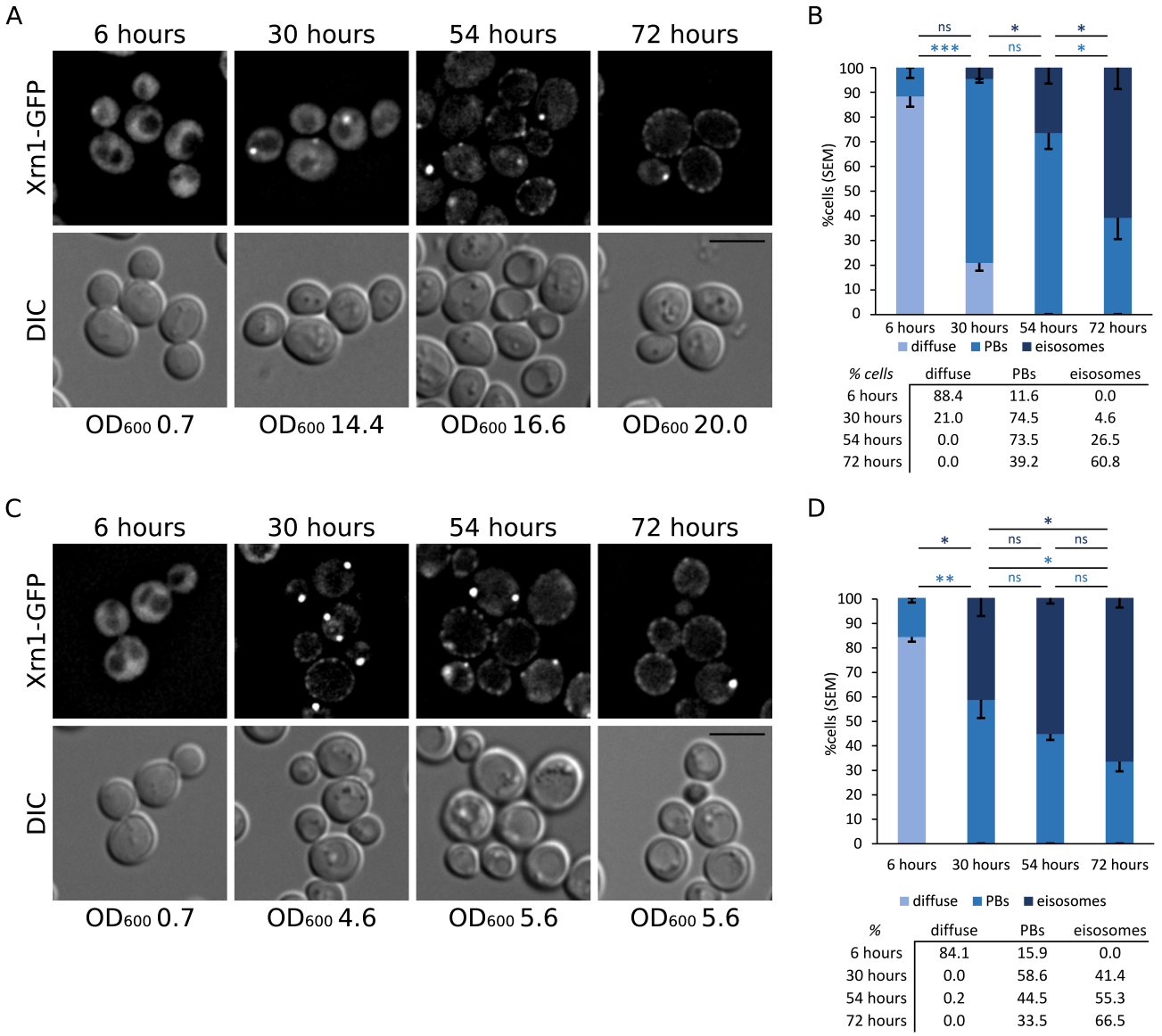

Fig. 2. Xrn1 is gradually deposited in P-bodies and eisosomes as the yeast population ages. Localisation patterns of Xrn1-GFP, the corresponding DIC images at the indicated time points, and optical density at 600 nm (OD_{600}) values in YPD (A) and synthetic defined (SD) (C) media are shown. Deconvolved wide-field single-layer images are presented. Scale bar: 5 µm. The percentage of cells, including the standard error of the mean, with cytoplasmic ("diffuse"), located P-bodies ("PBs"), and eisosomes-associated ("eisosomes"), Xrn1-GFP signal is shown at the indicated time points (YPD:B; SD:D). The graphs represent pooled data from three biological replicates ($n > 100$ cells per time point). Statistical analyses are colour-coded for clarity (i.e., light blue for P-bodies and dark blue for eisosomes). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (two-sample *t*-test).

4. Discussion

Yeast cells, especially *Saccharomyces cerevisiae*, are used in various biotechnological applications (e.g., bioethanol or vanillin production) [19,20]. Monitoring the physiological status of yeast cells is crucial for achieving the best yields possible. Here, we present the dynamic changes that occur in the localisation of Xrn1, which can be used as a versatile tool for monitoring the growth of yeast populations at the single-cell level through fluorescence microscopy. We previously showed that Xrn1 possesses a unique tripartite localisation pattern in yeast cells [7]. Subsequently, we have extended these results by monitoring the growth rates and morphologies of Xrn1-GFP cells compared to wild-type and Δ*xrn1* cells, in addition to observing the Xrn1-GFP localisation pattern in different media types for up to 72 hours.

Monitoring the Xrn1-GFP localisation pattern revealed that the protein is initially diffusively distributed in the cytoplasm, yet as the yeast population aged, it began to be gradually deposited in P-bodies, thus, confirming previously shown data [3]. When the glucose is used as the pri-

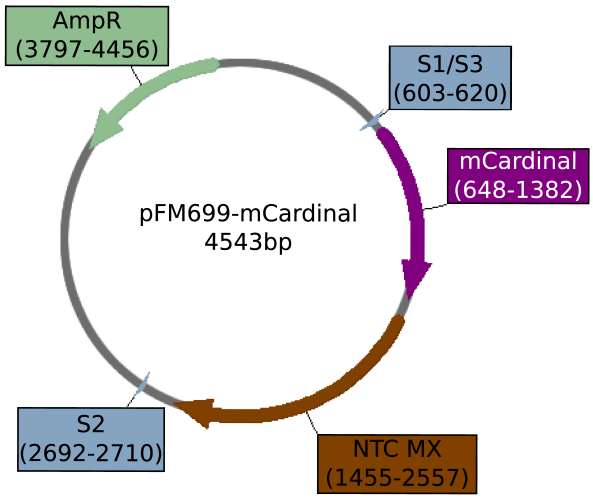

Fig. 3. mCardinal integration cassette. Graphical visualisation of the pFM699-mCardinal integration cassette (adapted from Serial Cloner (Serial Cloner 2.6.1, http://serialbasics.free.fr) software). NTC MX and *AmpR* denote the nourseothricin and ampicillin resistance genes, respectively.

mary carbon source for yeasts becomes limited, cells reach a diauxic shift, where they switch from fermentation to respiration metabolism, which is reflected by Xrn1 undergoing re-localisation to the plasma membrane-located eisosomes ([7,21] and this work). We showed that Xrn1 enrichment at the eisosomes also continues as the yeast population ages. The kinetics of Xrn1 re-localisation are media-dependent, thereby reflecting the different nutrient availabilities in rich YPD and synthetic media. Glucose, the primary carbon source for *Saccharomyces cerevisiae* cells, is the essential nutrient that regulates Xrn1 localisation. Therefore, when it becomes limited, Xrn1-containing P-bodies are formed and enlarged [22,23]. After the diauxic shift to conditions with no glucose, Xrn1 is gradually deposited into the MCC/eisosomes compartment ([7,19] and this work). However, the association of Xrn1 with the MCC/eisosomes compartment can be reversed by adding glucose to the cultivation media [7]. Furthermore, Xrn1 distribution within the cytosol, P-bodies, and the MCC/eisosomes compartment can be directly linked to the actual metabolic status of the cell. For example, in the model system using yeast giant colonies, Xrn1 was diffusely localised in the outer and most active cells, whereas in the less active subsurface cells, it is deposited in P-bodies; finally, in the most inner and resting cells, it was associated with the MCC/eisosomes compartment [15,24].

Standard analyses that assess yeast population growth and fitness rely on indirect measurements of optical density and nutrients (e.g., glucose concentration in medium or staining by chemical dyes), which are usually toxic to the cells (see, e.g., [25,26]). The limitation of these methods is that they only provide information regarding the entire population. Thus, using fluorescently labelled Xrn1 can avoid such limitations. Observing fluorescently labelled Xrn1 by microscopy enables the non-invasive monitoring of the growth status of individual cells with potential in many downstream applications. We confirmed using standard growth assays and microscopic observations that the functionality of Xrn1 is not disrupted by GFP tagging. Moreover, we designed a far-red fluorophore to encode an integration cassette for Xrn1 tagging with far-red mCardinal fluorophore to perform downstream analyses that could potentially use the GFP channel.

Simultaneously, observing Xrn1 localisation outside of the P-bodies in post-diauxic cells supports the notion of the P-bodies performing storage and cytoprotective functions during cellular metabolism. Due to P-bodies harbouring translationally inactive mRNA molecules and mRNA decay components, such as Xrn1 and decapping enzymes, it was originally believed that P-bodies participated in the Xrn1-mediated degradation of mRNA molecules [3]. However, recent data have shown that mRNA degradation occurs, with some exceptions, outside P-bodies [8,27,28]. Thus, the particular involvement of P-bodies in mRNA decay remains unresolved. Currently, P-bodies are recognised as cytoprotective non-membranous organelles that regulate mRNA metabolism and protein homeostasis, similar to stress granules and misfolded protein aggregates [29,30]. In connection, the formation of P-bodies is required for the long-term survival of yeast cells [31,32], thereby supporting the physiological relevance of Xrn1 sequestration into the MCC/eisosomes plasma membrane compartment in post-diauxic cells. Although the involvement of P-bodies in mRNA quality control, mRNA storage, and translation repression has been documented, their general function in cell metabolism remains unknown [27,33].

5. Conclusions

Here, we report, to a single-cell resolution, the unique tripartite localisation of the Xrn1 exoribonuclease, which can be used as an intrinsic marker of the yeast population growth status. We confirmed that tagging Xrn1 with a GFP fluorophore does not interfere with its enzymatic activity and, thus, can be used to monitor the status of Xrn1 in yeast cells. By following the Xrn1-GFP localisation pattern in complete YPD and synthetic SD media for up to 72 hours, we observed its gradual deposition into P-bodies as cells reach a diauxic shift and its increasing association with the MCC/eisosomes plasma membrane compartment in post-diauxic shift cells. The observed changes in Xrn1 localisation correlate with changes in cell metabolism and can be used to assess the growth status of individual cells within the yeast population, thereby providing research and biotechnological potential. The re-localisation of Xrn1 from the rest of the mRNA decay machinery deposited in the P-bodies to the MCC/eisosomes compartment in post-diauxic cells supports the storage and cytoprotective function of these RNA granules.

Availability of Data and Materials

Data will be made available on request.

Author Contributions

TG: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—original draft, Writing—review & editing, Visualization, Supervision, Project administration, Funding acquisition; TV: Validation, Formal analysis, Writing—original draft. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. Both authors read and approved the final manuscript. Both authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbe1601001.

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