The exonuclease Xrn1: a key regulator of gene expression under physiological and perturbed conditions

Leire de Campos Mata

TESI DOCTORAL UPF / 2019

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DEPARTAMENT DE CIÈNCIES EXPERIMENTALS I DE LA SALUT

A mi madre

ACKNOWLEDGEMENTS

Ha llegado el día… ¡Anda que no he soñado veces con este momento! Me imaginaba escribiendo los agradecimientos cinco minutos antes de llevar mi tesis a imprimir (es exactamente lo que estoy haciendo), sin haber dormido la noche anterior… En fin, Leire style. En cualquier caso, aquí estoy, súper contenta y orgullosa de haber recorrido este largo camino. Por supuesto, ha habido mucha gente maravillosa que me ha ayudado a recorrerlo, y por eso escribo las siguientes líneas para agradecerles su apoyo.

En primer lugar quiero darle las gracias a **Juana** por haberme guiado a través de esta experiencia. Hemos pasado por momentos de amor y odio, de cercanía y lejanía, pero al final hemos conseguido crear un proyecto interesante juntas y desde el respeto mutuo. Estoy muy contenta de haber hecho el doctorado contigo.

¡**Jennifeeeer**!. Primero de todo, muchas gracias por haber aceptado ser mi codirectora de tesis, ¡he aprendido de la mejor! Ahora solo te falta enseñarme todo lo que sabes de volley para acabar tus responsabilidades como thesis supervisor…¡¡ Al fin y al cabo sabes que el volley forma parte importante de mi tesis!! Me alegro de que nuestra amistad haya sido lo suficientemente fuerte como para superar correcciones y demás momentos delicados de la tesis. Te aprecio mucho ☺

René! You have been of great support during the last chapter of my thesis. Thank you very much for your advice and work on the paper and for your help during the construction of the thesis. Sadly, the endless summer is coming to its end… ☹ I also want to thank **Seisi** for his help in this project. Your support has been of great importance to go on with the project! I owe you both tons of beer! También quiero agradecer a **Mariona** y a **Jorge** del labo de Posas por fundar los orígenes de mi proyecto.

Mi querido **Marc**... Ha sido un calvario tenerte de compi de lab, y lo sabes. Todo el día criticando mis gustos musicales, liándome para ir a tomar cervezas, interrogándome sobre mi vida privada… Ai ama!! Aunque bueno, compartir labo

contigo también ha tenido sus cosas buenas… Como la destreza con la que compraste las entradas para el concierto de la Rusalia ;) No, ahora en serio. Gracias por haber reído conmigo en los días alegres, por haber respetado mis días tristes, por ser mi amigo. Ya tu sabes mi amoll que te quiero mucho. Aprofito per enviar-li un petonet molt fort també al "**Gibert**".

¡**Gemma**! ¡Si te digo a qué hora he estado saliendo estos días del lab me matas! La mamá del lab… Sin ti no habríamos sobrevivido ni una semana. ¡Pero cuidado que yo he aprendido a hacer comandas y soy imparable! Muchísimas gracias por tu apoyo Gemma, dentro y fuera del lab. Por tu tiramisú riquísimo, por contagiar a Jenni de vez en cuando supuestamente por culpa de Biel…. Te mando muchos besitos.

Por supuesto, también quiero agradecer a otros miembros del lab con los que he coincido más brevemente. **Andrew**! Thank you very much for discovering my love for amateur wrestling ☺ Hit him with the chair!!!! We will for sure share more 1€ beers while we cheer for Santiago Sangriento. I hope you become a ref!! ¡¡**María Eugenia**, fue muy divertido tenerte en el lab!! Gracias por tu positividad y por tu sonrisa. También quiero agradecer a **Andreas** Meyerhans y la gente de su lab por el feedback científico en seminarios y retreats, y por el buen rollito en actividades beyond science.

Jope, ¡me estoy dando cuenta de que hay muchísima gente a la que quiero agradecer! Y yo siempre decía que mis acknowledgements iban a ser cortos… Bueno, quiero mandar un besito y un "GRACIAS" enorme a los **Posas**! Se os echa mucho de menos… El pasillo de la tercera está muy soso sin vosotros por aquí. Gracias por los planazos que hemos hecho juntos, por los ánimos, por las strains que os he robado… En fin, erais los vecinos de lab perfectos ☺ And, I specially want to thank **Predrag!!** Lab ex-neighbor, flat ex-neighbor. You keep running away from me!! Hehehe. Now you cannot bring me strains anymore!! Drama! But most importantly, we did a lot of Cokes&Cigs sessions that were real fun! I am looking forward to having more of them.

También quiero mandar un besito a los **Pombe**!! Por las conversaciones de basket con Rodrigo, por compartir la fiebre pretésica con Luis, Cristina y Claudia. ¡Y por las beer sessions divertidas con todos vosotros! Incluido ex Pombes como Albertito. ¡¡Gracias también a los **Immuno** por los buenos ratos!! ¡**Berta y Héctor**, vosotros sois la interconnection entre labs! Como mi proteína (chiste malísimo). ¡Muchas gracias por ser cómo sois! Os deseo lo mejor. **Anna Almor**, ¡te aprecio mucho! Con ganas de que sigamos haciendo planes guays juntas.

Por supuestísimo, no me olvido de las **chicas de cocina**. La Mari, la Montse, Chelo, Enri, Juanita… ¡No sé ni qué deciros! Gracias por hacer las cosas tan fáciles, por ayudarnos poniéndonos autoclaves cuando no toca, por lavarme de urgencia los malditos matraces de dos litros, por vuestros chistes, vuestros ánimos, vuestros cotilleos, vuestro bailecito de la Rosalía. Y, cómo no, ¡mil gracias por la ronda de villancicos que cantáis cada Navidad y que se han vuelto ya súper tradicionales! Sois las mejores. También quiero agradecer a **Sonia** y a **Isabel**, que me odiarán porque siempre estoy pululando por el lab a partir de las 18:00 y no les dejo hacer su trabajo a gusto.

También agradecer enormemente a las chicas del labo de **anti-doping del IMIM** (Noe, Lidia, Indira) por dejarme su maravillosa cámara de quimioluminiscencia para revelar millones de membranas a lo largo del doctorado… Cómo hablamos el otro día, Noe, ¡al final marchas tú antes que yo! Y mira que yo llevo tiempo amenazando…

Quiero agradecer especialmente a toda la gente que pertenece a la **comunidad voleibolística** (suena un poco a secta, lo sé, pero me gusta). People think that I am addicted to volley, that I cannot stop playing… But the truth is that I just enjoy spending time with you all. Through volley I have met a lot of cool people that have become very important in my life!! **Moritz**! Our encounter was so random yet turned out so well! Is it mean to say that I am glad that Jackie broke her toe so we could meet each other (don't tell her)?? **Sonia**, me alegra muchísimo haber tenido la oportunidad de conocerte mejor durante esta última etapa de mi doctorado. Espero que esto solo sea el comienzo de

una larga amistad, ¡y de una balda llena de trofeos! Te aprecio mucho. Sonia, no leas lo que viene ahora. STOP. **Ángela**, ¿quieres ser mi nueva compi de 2x2? Necesito más pisapapeles para la tesis ;) **Marcos**, mi crush del vóley!! Qué divertido es jugar torneos contigo, que nos expliques los cotilleos de los PRO... ¡Algún día cumpliremos nuestro sueño de hacer podio! **Andreu rompeparejas**, tú deberías darme las gracias a mi por querer jugar contigo los torneos cuando Marcos no puede… **TOBAIAS**, thanks for always reminding me that I started too late to write the thesis. **Davide,** me alegro mucho de haberte conocido! Seguirás siendo mi amigo mientras me dejes ganarte en los torneos… Tú verás lo que haces. **Raquel**, ese torneíto que jugamos juntas, ¡lo petamos! Habrá más. Me alegro mucho de haberte conocido más este año. **Silvia, Anna Almor, Gerard, Andrea Tassinari, Spiking Neurons, Bye-bye Jenni, Sami, Ezequiel, Sandra, Jesse, JONÁS** y gente de los entrenos de Jonás…. Especialmente quiero agradecer a **Matteo** y **Vincenzo** por una diversidad de motivos que no voy a escribir ☺. Seguro que me dejo un millón de nombres. Espero que comprendáis que estoy escribiendo esto cinco minutos antes de imprimir y ando un poco nerviosa.

¡**Grupo Desperados**! ¡¡Me lo he pasado muy muy bien en el PRBB Weekends Festival 2019!! Sois la caña. Gracias por hacer de la escritura de la tesis una fase amena con vuestra presencia. No me echéis del grupo porfa, prometo seguir haciéndoos compañía los findes si no me echáis. Besitos.

¡**Mi cuadrilla de Vitoria**! ¿Sabéis que a los catalanes les hace muchísima gracia la palabra “cuadrilla”? Se parten de risa cada vez que lo digo. En fin chicas, que os quiero muchísimo. Muchas gracias por vuestro apoyo dentro y fuera del ámbito tesis. Maite, gracias por traer al mundo a Mia. Mia me ha recordado que esta vida no solo se compone de eventos tristes… Sois súper importantes para mí. **Álvaro**, ¿qué puedo decir? Que esta amistad que empezó en la guardería dure muchos años. Te quiero un montón. A mis **compis de la uni**… Iratxe, Iñigo, Sofía, Aida, Javi, Imanol. ¡Leile os quiere un montón, y Leitres también! **Andrea Zaragoza,** gracias por tus palabras de apoyo, por tus ánimos, por esas reuniones en mi casa, eres la caña. Y tranqui, que aunque

acabe la tesis no significa que te vayas a librar de mi… ¡Esta amistad no ha hecho más que empezar! **Maggie**, cari, que me lo pasé súper bien en el Congost de Montrebei, tenemos que hacer más escapadas como esta… A **mis compis de piso**, ¡gracias por aguantarme estos meses en los que he descansado poco, he dormido mal, he comido peor! Seguro que ha sido un suplicio aguantarme. Jose, gracias por esos puchos en el balcón. ¡Eres repiola!

Luis, no me voy a enrollar mucho porque la cosa se pone seria. Gracias por apoyarme en todos los formatos de relación por los que hemos pasado. Te quiero mucho.

Tati, llego a este párrafo con mucho dolor… Tanto que no sé ni qué decir… Te quiero muchísimo, te admiro mucho, y tengo muchas ganas de abrazarte. **Mamá,** todo lo que soy, lo que veo, lo que digo, lo que siento, es gracias a ti. Todavía recuerdo los sentimientos que me invadían al cogerte de la mano… Es la sensación más bonita que he tenido en mi vida. Te quiero.

ANTI-ACKNOWLEDGEMENTS

Debido a los acontecimientos del último mes y medio aproximadamente, me he visto obligada a escribir una sección de anti-acknowledgements en mi tesis… Y es que hay gente que se ha ganado su mención en este apartado a pulso. ¡Si no fuera por vosotros habría depositado un mes antes! Voy a poner una serie de ejemplos… Primero, Marcos preguntándome en pleno momento pre-tesis a ver si quiero jugar el Svat con él, ¡con lo que me gusta jugar torneos con Marcos! Then, Tobias keeps telling me the whole summer that I should focus on my thesis and right before submission he whatsapps me to play voleiboom together… Andreu, tú me mandaste una foto con tu calendario de verano que me deprimió muchísimo… Y yo todo el verano en el PRBB… ☹. Sonia, tú me has buscado un entrenador nuevo para que entrenemos más y ganemos de una vez por todas algún torneo juntas. ¿Y mi tesis cuándo la escribo? Jeroen, ¡no me he olvidado de ti! "Leireeeeee, busca ropa de deporte y baja a jugaaaaar, va a haber muy buen rollito!" Y luego está Manel… "Leire, ¿nos tomamos una cervezas y jugamos al futbolín?" Ha sido muy difícil resistirme a las tentaciones chic@s... Aunque he de reconocer que en el fondo, muy en el fondo, ha sido divertido teneros a mi alrededor pervirtiéndome. ☺

ABSTRACT

The highly conserved exonuclease Xrn1 plays a dual role in gene expression by degrading cellular mRNAs and promoting their transcription initiation and elongation. In this thesis we uncover an unanticipated role of Xrn1 in translational control under physiological conditions and in cell adaptation and survival under osmotic stress conditions.

Here we show that Xrn1 promotes translation of brome mosaic virus (BMV) RNAs in *Saccharomyces cerevisiae*. By integrating ribosome profiling analysis to functional and biochemical studies we report a broader role of Xrn1 in translation initiation of a subset of yeast mRNAs encoding membrane proteins. Xrn1-dependent yeast transcripts, as the viral ones, harbor highly structural traits around the translation initiation site (TIS) that confers a poor context for translation initiation. Interestingly, functional studies indicated that the unstructured C-terminal domain of Xrn1 interacts with components of the translation initiation machinery to facilitate protein synthesis and that Xrn1 mediates the correct localization of Xrn1-dependent mRNAs at the endoplasmic reticulum, the cellular translation compartment where membrane proteins are synthesized. Importantly, Xrn1 promotes transcription, translation and decay of the same group of mRNAs. Together, our results reveal a novel crosstalk between the three major steps of gene expression coordinated by Xrn1 to finely tune expression of membrane proteins. We surmise that this linkage has evolved to avoid toxic aggregations, as membrane proteins contain hydrophobic domains prone to aggregate.

Not only is gene expression an important cellular process under physiological conditions, but it also plays a key role in the adaptation and survival of cells to changing environmental conditions. Importantly, previous studies linked Xrn1 to the regulation of yeast mRNA homeostasis in response to glucose deprivation. In this thesis we show that Xrn1 modulates cellular transcriptional and translational responses upon hyper-osmotic shock by combining genome-wide RNA sequencing with functional and biochemical analyses. Microscopy imaging revealed that Xrn1 localizes to stress-induced aggregates shortly after osmotic

shock in a manner dependent on its unstructured C-terminal domain. This localization is mediated by the major signal integrator Snf1 adenosine monophosphate-activated protein kinase (AMPK). Under these conditions Xrn1 maintains a diminished exonuclease activity and assists in the transcriptional and translational activation of a subset of osmo-induced genes that are enriched for proteins interacting with Hog1, the main mitogen-activated protein kinase involved in osmoregulation. Based on the evidence provided we claim that the exonuclease Xrn1 links the Snf1 and Hog1 pathways to control gene expression upon osmotic stress.

Collectively, our results point to Xrn1 as a key regulator of gene expression under physiological and perturbed conditions.

RESUMEN

La exonucleasa altamente conservada Xrn1 desempeña un doble papel en la expresión génica al degradar los ARNm celulares y promover la iniciación y el alargamiento de la transcripción de los mismos. En esta tesis descubrimos un papel no anticipado de Xrn1 en el control de la traducción de ARNm bajo condiciones fisiológicas y en la adaptación y supervivencia celular bajo estrés osmótico.

Aquí mostramos que Xrn1 promueve la traducción de ARN del virus del mosaico del bromo (BMV) en *Saccharomyces cerevisiae*. Al integrar el análisis de perfiles de ribosomas a estudios funcionales y bioquímicos, confirmamos un papel más amplio de Xrn1 en el inicio de la traducción de un subconjunto de ARNm de levadura que codifica proteínas de membrana. Los tránscritos celulares dependientes de Xrn1, como los virales, albergan rasgos altamente estructurales alrededor del sitio de inicio de la traducción (TIS) que confieren un contexto pobre para el inicio de la traducción. Además, estudios funcionales indicaron que el dominio C-terminal no estructurado de Xrn1 interactúa con los componentes de la maquinaria de iniciación de la traducción para facilitar la síntesis de proteínas y que Xrn1 media la localización correcta de los ARNm dependientes de Xrn1 en el retículo endoplasmático, el compartimento de traducción celular donde las proteínas de membrana son sintetizadas. Es importante destacar que Xrn1 promueve la transcripción, traducción y degradación del mismo grupo de ARNm. Juntos, nuestros resultados revelan una nueva diafonía entre los tres pasos principales de la expresión génica coordinada por Xrn1 para regular la expresión de proteínas de membrana. Suponemos que este enlace ha evolucionado para evitar agregaciones tóxicas, ya que las proteínas de membrana contienen dominios hidrófobos propensos a agregarse.

La expresión génica no solo es un proceso celular importante en condiciones fisiológicas, sino que también juega un papel clave en la adaptación y supervivencia de las células a condiciones ambientales cambiantes. Es importante destacar que estudios previos vincularon Xrn1 a la regulación de la

homeostasis de ARNm de levadura en respuesta a la privación de glucosa. En esta tesis hemos combinado la secuenciación de ARN de todo el genoma con análisis funcionales y bioquímicos y hemos descubierto que Xrn1 modula la transcripción y traducción de ARNm tras un choque hiperosmótico. Las imágenes de microscopía revelaron que, poco después de añadir sal a las células, Xrn1 se localiza en agregados citosólicos inducidos por el estrés de una manera dependiente de su dominio C-terminal no estructurado. Esta localización está mediada por la proteína quinasa activada por AMP Snf1. En estas condiciones, Xrn1 mantiene una actividad exonucleasa disminuida y ayuda a la activación transcripcional y traduccional de un subconjunto de genes inducidos en condiciones de estrés que están enriquecidos en proteínas que interactúan con Hog1, la principal proteína quinasa activada por mitógeno involucrada en la osmoregulación. Con base en la evidencia proporcionada, afirmamos que la exonucleasa Xrn1 une las vías Snf1 y Hog1 para controlar la expresión génica bajo estrés osmótico.

En conjunto, nuestros resultados apuntan a Xrn1 como un regulador clave de la expresión génica en condiciones fisiológicas y cambiantes.

PREFACE

Gene expression is a fundamental process in cell cycle. Genes encode for proteins and other functional gene products that will determine the fate of a cell. Each step in the flow of information from DNA to RNA and from RNA to protein represents a layer of cell self-regulation of its functions. Although classical biology considered them as isolated steps, recent research has revealed that transcription, translation and degradation are interconnected and regulated by shared elements like the RNA polymerase II subunits Rpb4 and Rpb7 which shuttle between the nucleus and the cytoplasm to aid in all steps of gene expression. Similarly, the translation initiation factor eIF4G is imported to the nucleus to act in splicing thereby intertwining transcription and translation. Another example that bridges nuclear and cytoplasmic events is the exonuclease Xrn1 that has been recently reported to regulate mRNA homeostasis by promoting transcription and degradation of cellular mRNAs.

Precise tuning of gene expression is of major importance to ensure adaptation and survival of cells when faced with suboptimal environmental conditions. Under stress, cell-growth related genes are shut down and expression of stress-protective genes is favored by the interconnection of several stress-activated signaling pathways. Xrn1 has been shown to play an essential role under glucose deprivation by maintaining mRNA homeostasis of adenosine monophosphate-activated protein kinase Snf1-dependent genes.

In this thesis we characterize the role of the exonuclease Xrn1 as a major regulator of gene expression by coupling degradation, transcription and translation of a subset of mRNAs with shared features. In addition, we reveal that Xrn1 modulates transcriptional and translational responses upon hyper-osmotic shock in a yeast model system. These results place Xrn1 at the crossroads of transcription, translation and degradation, the three major stages of gene expression and furthermore show the importance of Xrn1 under perturbed environmental conditions.

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INTRODUCTION

1. Introduction to mRNA decay

The degradation of cellular mRNAs plays a crucial role in controlling the abundance of cellular transcripts under physiological and stress conditions. In addition, mRNA decay is in charge of checking the quality of cellular mRNAs along their life cycle to avoid accumulation of aberrant transcripts that could be translated into harmful proteins for the cell (Parker, 2012). Here I will review the current findings related to cellular mRNA degradation (Figure 1).

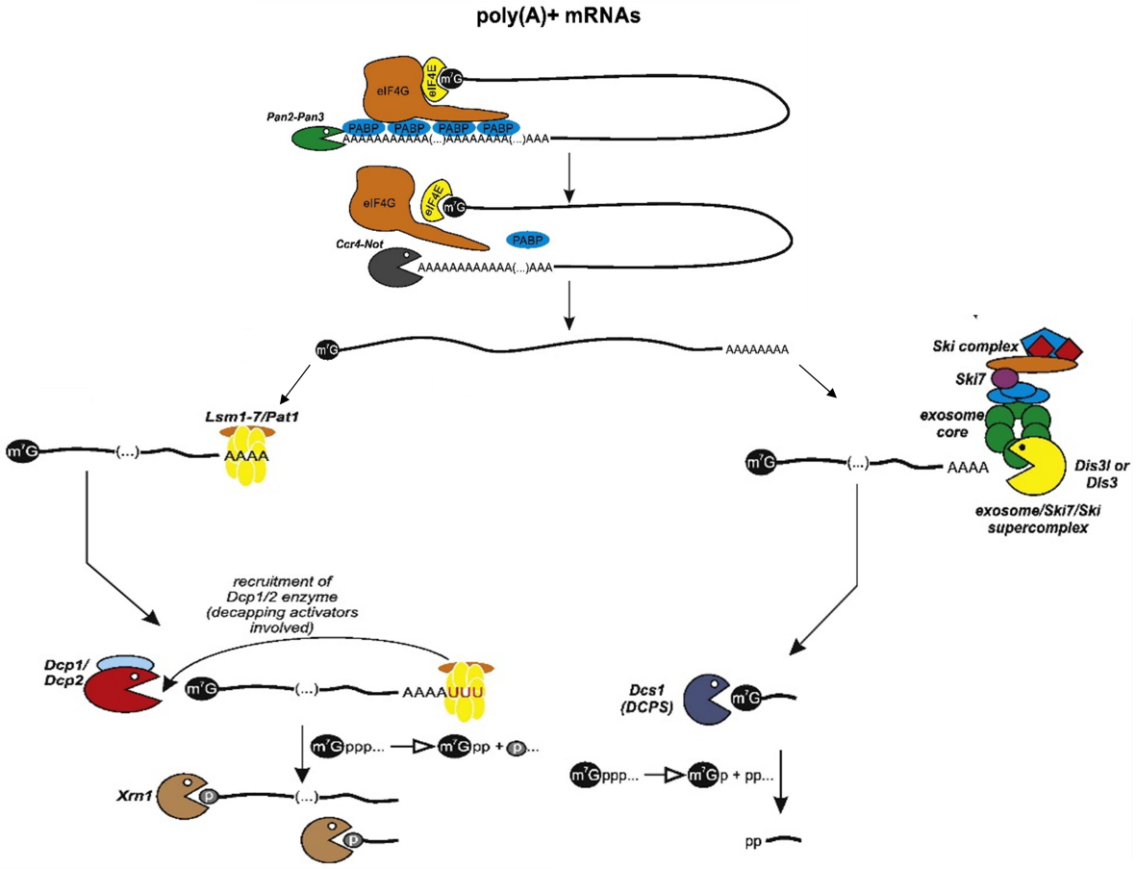

Figure 1: General mRNA decay pathways. General decay starts with the degradation of the poly(A) tail and consequent disruption of the circular state of the transcript. Further degradation of the mRNA can be performed in 3'-5' sense by the Ski-exosome complex activity (right) or following decapping and Xrn1 5'-3' exoribonuclease activity (left). Figure adapted from (Łabno et al., 2016).

General mRNA decay starts with the exposure of the transcript to the decay machinery. The ends of a mature, ready to be translated mRNA is protected in its ends by a poly(A) tail in the 3'UTR and a cap structure in the 5'UTR. In

specific cases, some endoribonucleases can overcome the transcripts' protected sequences and perform degradation of the mRNA (Tomecki and Dziembowski, 2010). However, general mRNA degradation begins with the shortening of the 3' poly(A) tail by a process called deadenylation. Deadenylation leads to the trimming of the poly(A) tail, release of the PABPs, binding of the Lsm1/Pat1 complex and ultimately translation repression (Cooke et al., 2010). Furthermore, binding of the Lsm1/Pat1 complex promotes cap hydrolysis and further decay by the main exonuclease Xrn1 in a 5'-3' sense. Alternatively, the RNA exosome complex can degrade a capped mRNA in a 3'-5' sense after further degradation of the 3' end (Łabno et al., 2016).

1.1. Deadenylation

Shortening of the poly(A) tail is a very dynamic process that is regulated by a high number of enzymes and regulatory factors. The poly(A) binding proteins or PABPs bind to the poly(A) tail and create a stable circular structure that promotes translation of the transcript through the interaction with the cap-binding proteins (Cooke et al., 2010). The main deadenylases in eukaryotes are the Ccr4-Not and the Pan2-Pan3 complexes. The Pan2-Pan3 complex is believed to perform the initial steps of poly(A) trimming, since this complex is not able to degrade the poly(A) tail completely (Wahle and Winkler, 2013). Pan3 promotes the recruitment of the complex to the mRNA, whereas Pan2 is the catalytic enzyme whose activity is stimulated by PABPs. When the poly(A) tail is shortened, the PABPs detach from the mRNA, which inhibits Pan2 activity and activates the Ccr4-Not complex for further degradation (Tucker et al., 2002). The Ccr4-Not complex has two subunits with catalytic activity, which are the Ccr4 and Caf1/Pop2 proteins. They have 3'-5' poly(A)-specific exonuclease activity. Not1 instead serves as a structural scaffold. Whereas the Pan2-Pan3 complex is not essential for poly(A) trimming, Ccr4/Pan2 double mutant yeast cells encounter a block of deadenylation and severe growth defects (Tucker et al., 2002). Pan2/Pan3 and Ccr4/Not complexes are known to be components of P-bodies. P-bodies are cytoplasmic foci formed by proteins involved in the

decay machinery and translationally-repressed mRNAs. Still, yeast mRNAs with trimmed poly(A) tails accumulate in polysomes, meaning that deadenylation occurs co-translationally (Hu et al., 2009).

1.2. Decapping-dependent 5'-3' degradation

The 5'-3' decay is the major degradation pathway in eukaryotes. It begins with the removal of the 5' cap or decapping. Following deadenylation, Lsm1-7 proteins bind to the transcript in the 3' end and recruit the decapping complex (Bouveret et al., 2000; Nissan et al., 2010). Dcp2 is the main decapping enzyme in yeast, which is activated by a conformational change promoted by Dcp1 activity. The decapping enzymes are aided by auxiliary decapping activators, such as Pat1, Edc3, Scd6 and Dhh1 (Bouveret et al., 2000; Coller and Parker, 2005; Fischer and Weis, 2002). The decapping auxiliary factors enhance decapping either by promoting the activity of the Dcp2/Dcp1 complex or by repressing translation. For example, Edc1-3, Scd6 and Pat1 bind directly to Dcp2 and enhance its catalytic activity in vitro (Fromm et al., 2012; Nissan et al., 2010). Besides, Dhh1 and Pat1 are known to repress translation initiation prior decapping (Coller and Parker, 2005). Overall, the dynamic competition between translation initiation factors and decapping activators in an mRNA determine the fate of the transcript that could either undergo translation or degradation. After activation of the decapping complex, the 7-methylguanylate cap is removed by hydrolysis (Steiger et al., 2003). The mRNA bearing a 5' monophosphate is now susceptible to degradation by the exoribonuclease Xrn1 (reviewed in Jones et al., 2012).

The canonical decapping pathway is preceded by the deadenylation of the transcript. However, there are some *in vivo* examples of transcripts that get decapped independently of deadenylation, such as the *EDC1* transcript in yeast cells (Muhlrad et al., 1995).

1.3. 3'-5' mRNA decay directed by the exosome and Ski complexes

When deadenylation occurs, the PABPs are removed from the mRNA and the transcript can be degraded by exoribonucleases in a 3'-5' sense. The major exoribonuclease that degrades mRNAs from the 3' to the 5' end is the RNA exosome complex, which is highly conserved among eukaryotes. Even if the 3'-5' decay is not the major degradation pathway in yeast, the exosome plays an important role in specialized decay surveillance pathways such as non-stop decay (NSD), nonsense-mediated decay (NMD) and no-go decay (NGD).

In yeast, the RNA exosome is the only essential exoribonuclease in the 3'-5' decay pathway. It consists of six subunits forming a barrel-like structure which lacks catalytic activity. The catalytic activity is obtained by the interaction with Dis3p, a protein that has both exo- and endonucleolytic activity (Chlebowski et al., 2013). After 3'-5' degradation is performed by Dis3p, the 5' cap is degraded by the scavenger decapping enzyme Dcs1p/Dcs2p (Milac et al., 2014). The Ski complex is the activator of the exosome in the cytoplasm. This complex is composed of Ski2p and Ski3p, which are ATP-dependent helicases, and two copies of Ski8p. In yeast, the Ski complex has another protein, the Ski7p, which functions as a connector between Ski and exosome complexes through its N-terminal domain (Halbach et al., 2013).

1.4. RNA Quality Control pathways

There are several RNA quality control mechanisms in the cell. The aim of these pathways is to assure that only the correctly synthesized mRNAs are translated into proteins. If aberrant transcripts are not targeted for degradation, they can turn into potential toxic proteins for the cell. Depending on the error that the mRNA is bearing, it will trigger different quality control mechanisms: nonsense-mediated decay (NMD), no-go decay (NGD) and non-stop decay (NSD; Figure 2; Siwaszek et al., 2014).

The NMD is activated when mRNAs have a premature termination codon, present introns in their 3'UTR, have very long 3'UTRs or when they bear an

upstream Open Reading Frame (ORF) in their 5'UTR (Baker and Parker, 2004; Kertesz et al., 2010). These events can be caused by an error in transcription or in splicing or due to genetic mutations. The ATP-dependent RNA helicase UPF1 is the main enzyme of the NMD (Bhattacharya et al., 2000). First, the complex formed by UPF2 and UPF3 recognizes the aberrant transcript (He and Jacobson, 1995). Subsequently, UPF1 binds to UPF2 and promotes the activation of the NMD. The N-terminal domain of UPF1 interacts with decapping factors, which will promote deadenylation-independent decapping followed by Xrn1 degradation of the transcript (Swisher and Parker, 2010). Alternatively, UPF1 can promote deadenylation and 3'-5' degradation of the mRNA (Mitchell and Tollervey, 2003). UPF1 also plays a role in ribosome release from the mRNA and recycling of the translation machinery (Franks et al., 2010; Ghosh et al., 2010).

NGD is triggered when the elongation complex gets stalled due to secondary structures in the mRNA or due to positively charged chains in the nascent polypeptide that strongly interact with the ribosome exit tunnel (Bengtson and Joazeiro, 2010; Dimitrova et al., 2009). Dom34/Hbs1 complex promotes the dissociation of the stalled ribosomal subunits (Shoemaker et al., 2010) and targets the mRNA for endonucleolytic cleavage (Doma and Parker, 2006; Schaeffer and van Hoof, 2011).

The NSD targets mRNAs that lack a stop codon due to mutations or a premature polyadenylation (Ozsolak et al., 2010). In this case, the exosome cofactor Ski7 recognizes a stalled ribosome with an empty aminoacyl-(RNA binding) site and triggers the recruitment of the exosome and further deadenylation-independent mRNA decay (Schaeffer and van Hoof, 2011; Shoemaker and Green, 2012). Again, the Dom34/Hbs1 complex releases the stalled ribosome.

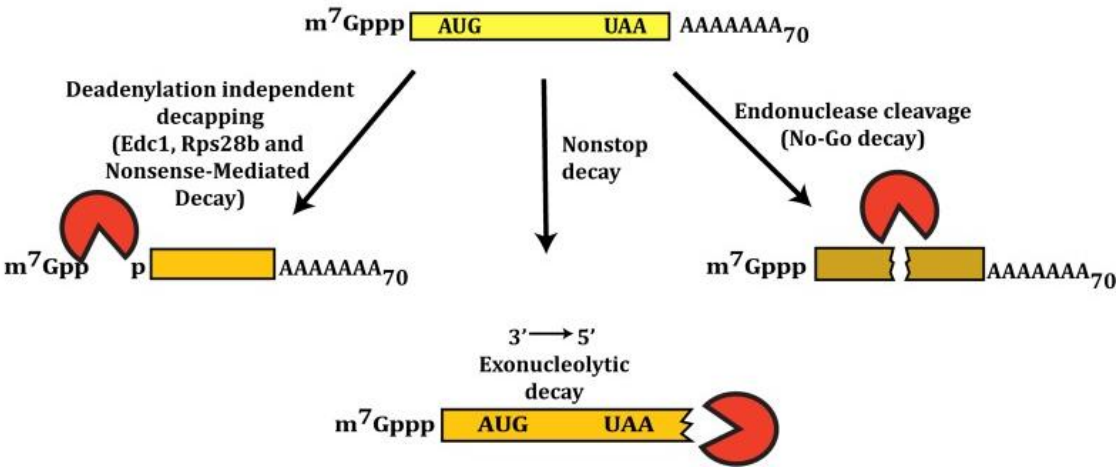

Figure 2: RNA Quality Control pathways. These pathways are responsible for the degradation of anomalous mRNAs that would translate into toxic proteins for the cell. The nonsense-mediated decay is triggered my mRNAs that bear premature stop codons (left); non-stop decay is activated by mRNAs that lack a stop codon (middle); no-go decay is triggered by strong ribosomal stalling during translation. Adapted from (Parker, 2012).

1.5. Nuclear RNA decay

Rat1 is the nuclear paralog of the cytoplasmic exonuclease Xrn1. Rat1 is in charge of the degradation of cellular mRNA precursors in the nuclear compartment. Besides, it is involved in transcription termination (El Hage et al., 2008), telomere maintenance (Luke et al., 2008) and maturation of snoRNAs and rRNAs (Wang and Pestov, 2011).

Rat1 and Xrn1 share a highly conserved N-terminal domain, especially at the catalytic domain. However, Rat1 lacks the unstructured C-terminal domain that is characteristic of Xrn1 (Jones et al., 2012). When forced to be expressed in the cytoplasm by deleting its nuclear localization signal (NLS), Rat1ΔNLS fully complements the role of Xrn1 in mRNA degradation and cellular growth (Johnson, 1997).

2. Xrn1, the major 5'-3' exonuclease

2.1. General features and structure of Xrn1

The 175 kDa 5'-3' exoribonuclease Xrn1 functions in all mRNA degradation pathways in the cell. Xrn1 is predominantly cytoplasmic and is highly conserved among all eukaryotes from yeast to humans. Especially the N-terminal domain, which contains the catalytic activity, is highly conserved (Jones et al., 2012). The structure of Xrn1 has been deciphered by crystallography assays in both *Kluyveromyces lactis* (J. H. Chang et al., 2011) and *Drosophila melanogaster* (Jinek et al., 2011). The N-terminal domain of Xrn1 is composed of two conserved regions (CR1 and CR2) that form together the catalytic domain (Figure 3). The CR1 contains the active site and is surrounded by CR2 which helps to form the correct structure of the active site (J. H. Chang et al., 2011). The access to the catalytic domain is tightly regulated. First, the active site is very narrow, which favors the interaction of Xrn1 with single-stranded RNA molecules and the disruption of secondary structures in the transcript while they enter through the access gap. Second, the catalytic domain is composed of a basic pocket that recognizes 5' monophosphorylated mRNAs and prevents the binding of bigger 5' capped mRNAs (J. H. Chang et al., 2011; Jinek et al., 2011).

The C-terminal domain instead is unstructured and less conserved among different species. It confers stability to the catalytic N-terminal domain. In fact, removal of the C-terminal of Xrn1 inhibits its catalytic activity and causes growth defects in cells. Interestingly, overexpression of the C-terminal is toxic for the cells as it decreases their viability (Page et al., 1998). Some studies postulate that the disordered C-terminal domain of Xrn1 functions as a putative protein interacting platform for other proteins involved in decay (Braun et al., 2012; Carpousis, 2007).

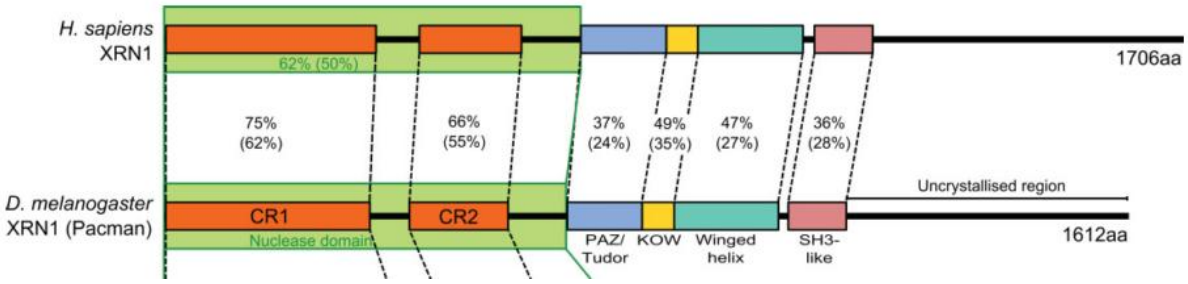

Figure 3: Xrn1 shows high conservation among eukaryotes. The N-terminal domain (in green) shows high conservation between *H. sapiens* and *D. melanogaster*. The central region of the protein has four domains that have been identified both in *H. sapiens* and *D. melanogaster*, although their conservation is lower. Adapted from (Jones et al., 2012).

2.2. Xrn1 interacting partners

Xrn1 is known to interact with several factors of the decapping machinery (Figure 4). In *Saccharomyces cerevisiae*, Xrn1 directly interacts with the C-terminal of Pat1 through a helical leucine-rich motif (HLM) that is located in an unstructured region close to the C-terminal of Xrn1. Pat1 also interacts with Dcp2 through the same binding surface, promoting in this way the successive recruitment of the decay machinery (Charenton et al., 2017). In *Drosophila melanogaster,* instead, Xrn1 directly interacts with Dcp1 through a proline-rich sequence present in its C-terminal domain (Braun et al., 2012). Additionally, Xrn1 interacts with Edc4 in an RNA-independent manner in humans.

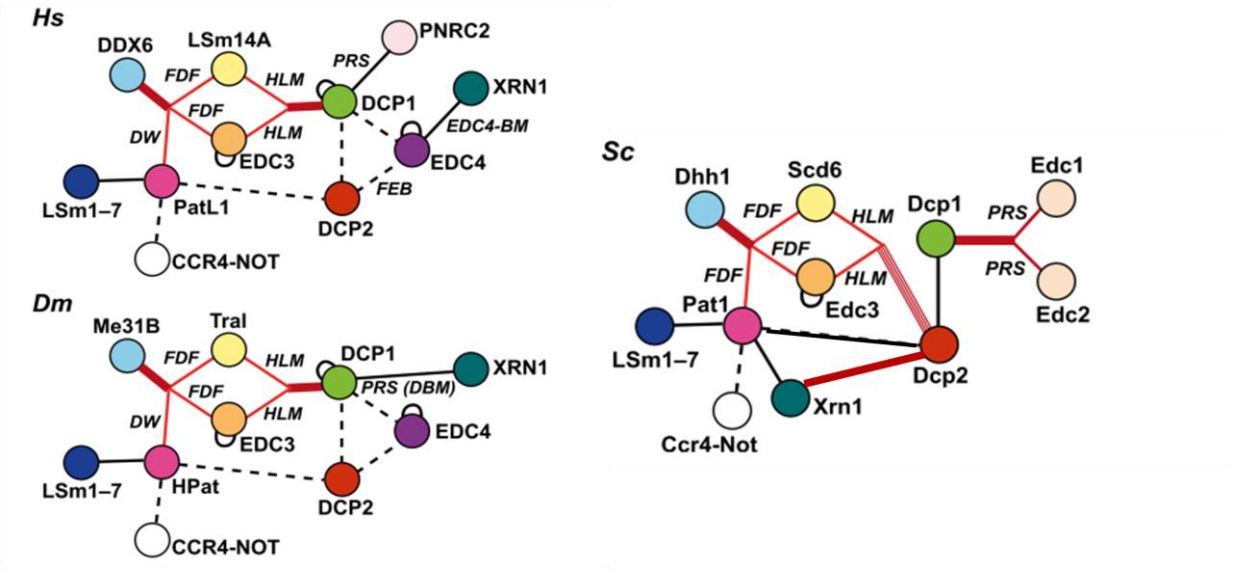

Figure 4. Interaction network of the decapping machinery. Interacting partners of the decay machinery in *Homo sapiens* (*Hs*), *Drosophila melanogaster* (*Dm*) and *Saccharomyces cerevisiae* (*Sc*). Proteins are represented with circles. Direct interactions are indicated with solid lines. Mutually exclusive interactions are represented with red lines. The motifs that mediate interactions are indicated in italics. Dotted lines indicate interactions that have not yet been demonstrated to be direct. Semicircles indicate oligomerization. Figure adapted from (Jonas and Izaurralde, 2013).

2.3. Described functions of Xrn1

Xrn1 plays an important role in the control of growth rates in unicellular eukaryotes such as *S. cerevisiae*. For example, Xrn1-depleted yeast cells show slower growth (Larimer and Stevens, 1990), less capacity to sporulate, and higher sensitivity to the microtubule-destabilizing drug benomyl (J. Kim and Kim, 2002). Also, cells that have Xrn1 mutated show defects in meiosis cell division (Tishkoff et al., 1991).

In multicellular organisms Xrn1 is involved in different developmental stages. For example, plants that lack Xrn4 (Xrn1 homolog in plants) present anomalous phenotypes such as late flowering (Geraldo et al., 2009). The loss of Xrn1 in *Caenorhabditis elegans* is lethal for the organism, suggesting a key function of Xrn1 during development that cannot be complemented by the 3'-5' decay pathway (Newbury and Woollard, 2004).

Xrn1 has also been associated to human diseases (Pashler et al., 2016). For example, Xrn1 is related to a type of cancer called osteosarcoma. Some case studies reported that osteosarcoma cell lines as well as osteosarcoma patient samples have mutations in the sequence of Xrn1 and show a decrease in Xrn1 RNA levels compared to healthy cells. These results suggest a role of Xrn1 in the regulation of tumor cell proliferation (Pashler et al., 2016; Zhang et al., 2002). Besides, Xrn1 appears to have an important function in the host response to viral infections. Recent studies have shown that Flaviviruses, among other viruses such as Hepatitis C, hijack and stall Xrn1 to prevent degradation of the viral RNA and to favor the progression of infection (Chapman et al., 2014; Guo et al., 2018; Moon et al., 2015; Thibault et al., 2015).

All these functions of Xrn1 can be related to its molecular function in mRNA decay and transcription.

2.3.1. The role of Xrn1 in decay

Xrn1 is the major 5'-3' exoribonuclease that degrades decapped cellular mRNAs that have been targeted for destruction (Sheth and Parker, 2003). Apart from its role in general RNA turnover, Xrn1 takes part in the nonsense-mediated mRNA decay and degrades RNAs that have been targeted via small interfering RNAs, microRNAs and long non-coding RNAs such as XUTs (Xrn1-sensitive unstable transcripts). Besides, Xrn1 directs maturation of ribosomal RNAs (rRNAs) and eliminates aberrant splicing intermediates that would turn into dysfunctional proteins in the cell (van Dijk et al., 2011; Geerlings et al., 2000; Geisler and Coller, 2012; Parker, 2012).

Xrn1 accumulates together with other factors from the decay pathway in processing bodies (P-bodies) and stress granules in yeast and human cells (Luo et al., 2018). This accumulation is increased under stress conditions. Both P-bodies and stress granules are membrane-less dynamic granules located in the cytoplasm. P-bodies are primarily composed of translationally repressed mRNAs and proteins involved in mRNA decay. They have been extensively

studied in the last years and the current proposed functions include post-transcriptional regulation, translational repression and mRNA decay (Luo et al., 2018). We will further describe stress granules in section 4.

2.3.2. The role of Xrn1 in transcription

In addition to its role in mRNA decay, Xrn1 plays an important role in transcriptional activation in yeast cells. Xrn1 shuttles together with the components of the decay complex to the nucleus by a mechanism that depends on its catalytic activity. Once in the nucleus, Xrn1 binds to the promoter of multiple genes and promotes their transcription initiation and elongation (Haimovich et al., 2013).

Several lines of evidence corroborate a role of Xrn1 in transcription. For example, Haimovich et al. performed Genomic Run-On experiments in *Saccharomyces cerevisiae* to obtain genome-wide data on the transcription rates of cellular mRNAs in cells lacking Xrn1, together with parallel experiments in which they depleted the other components of the decay complex. The results showed that, first, the transcription rates of cellular mRNAs were downregulated in *xrn1*Δ cells (Haimovich et al., 2013). Second, the decrease in mRNA transcription rates is compensated by an increase in their half-lives, leading to mRNA stability. The most Xrn1-dependent genes in transcription are highly transcribed genes mainly encoding ribosome biogenesis and translation factors (Medina et al., 2014). Third, Chromatin ImmunoPrecipitation assay (ChIP) showed that Xrn1, as well as Dcp2 and Lsm1, binds to chromatin 30 base pairs upstream to the transcription start site (TSS). Moreover, there is a direct correlation between the ability of Xrn1 to bind to a promoter and the transcription rate of that particular gene, indicating that the binding is functionally relevant to the role of Xrn1 in transcription. Fourth, *xrn1*Δ cells show an unusual Pol II accumulation in the 3' region of the ORF, whereas its elongation activity is not increased (Haimovich et al., 2013), suggesting a role of Xrn1 in transcription elongation step. Last, the role of Xrn1 in transcription is confirmed by several studies that show that Xrn1 physically and genetically

interacts with nuclear transcription factors (Collins et al., 2007; Costanzo et al., 2010; A. C. Gavin et al., 2006; Wilmes et al., 2008). A parallel study, however, described that Xrn1 depletion causes an increase in both decay rates and transcription rates. They propose that the role of Xrn1 in transcriptional repression is indirect and mediated by the transcriptional repressor Nrg1 (Sun et al., 2013).

Recent results in human liver carcinoma cells support the role of Xrn1 as a transcriptional activator (Singh et al., 2019). In this study no changes were observed in the mRNA abundance of human transcripts upon Xrn1 depletion. This is because Xrn1-knockdown leads to stabilization of the transcripts, which is compensated by a decrease in transcription rates of the same group of transcripts. The authors propose that Xrn1-mediated mRNA buffering is a conserved eukaryotic feature, since similar results have been observed in yeast and other mammals (Haimovich et al., 2013; Parker, 2012; Singh et al., 2019; Sun et al., 2013).

In conclusion, Xrn1 acts as a master regulator of cellular homeostasis by controlling the abundance of cellular mRNAs at both transcription and degradation steps. Whether Xrn1 also plays a role in other steps of gene expression, such as translation, remains to be elucidated.

2.4. The link between transcription and degradation processes

As described above, Xrn1 plays a key role in the degradation of cellular mRNAs in a 5’ to 3’ sense (Jones et al., 2012). Also, Xrn1 binds to the promoter of several genes and activates transcription initiation and elongation of cellular transcripts (Haimovich et al., 2013). Altogether, Xrn1 acts as a master regulator of cellular homeostasis, by controlling the abundance of cellular mRNAs at both transcription and degradation steps. Interestingly, this is not the only example that connects different steps of gene expression. For instance, Rpb4 and Rpb7 subunits of RNAPII form heterodimers (Kolodziej et al., 1990) that dissociate from the RNAPII (Orlicky et al., 2001) and shuttle to the cytoplasm (Selitrennik

et al., 2006). Once in the cytoplasm, they can interact with decapping factors such as Pat1 and promote decay of mRNAs that encode for ribosomal proteins and translation factors (Lotan et al., 2005, 2007).

Furthermore it has been described that the promoter of certain genes can have *cis*-regulatory elements that control the stability of cognate transcripts (Dori-Bachash et al., 2012; Trcek et al., 2011). For example, the transcription activator Rap1 binds to a specific binding site in the promoter of *RPL30* gene in *S. cerevisiae* and stimulates its degradation by recruitment of the decapping machinery (Bregman et al., 2011).

The interconnection between degradation and transcription steps seems crucial to keep a global transcriptome balance in the cell under physiological and stress conditions, as it allows rapid changes in the pool of available mRNAs by simultaneous shifts in transcription and degradation steps.

3. Introduction to translation

Protein translation is the most energy consuming process of gene expression in the cell. For example, a rapidly dividing yeast cell that is growing in a rich medium produces up to 13000 proteins per second (von der Haar, 2008). Thus, mRNA translation is subject to a very tight regulation in the cell. Protein synthesis englobes four steps: initiation, elongation, termination and ribosome recycling.

3.1. Translation initiation

Translation initiation is the most complex step of protein synthesis. First, the translation initiation factor eIF2 binds to both a GTP molecule and Met-tRNA to form the ternary complex (TC). Afterwards, the TC binds to the ribosomal 40S subunit to form the 43S pre-initiation complex (PIC). At this point, the eIF4F protein complex, that consists of the translation initiation factors eIF4E, eIF4A and eIF4G, recognizes the 7-methylguanylate cap structure that is present at the 5' end of the mRNA molecule and recruits the 43S PIC, forming the so-called 48S PIC. The ribosomal machinery is now ready to scan the mRNA sequence starting from its 5' end to find the translation initiation site, commonly an AUG triplet codon. The recognition of the translation initiation site leads to the release of several of the factors that were forming the complex. The translation factor eIF5B allows the binding of the ribosomal subunit 60S to the 48S PIC, forming the 80S ribosomal complex. Now the translation machinery is ready to enter the translation elongation step (Dever et al., 2016). Alternatively, translation can be initiated independent of the 5' cap structure. The mRNA molecules that follow this pathway do not require the presence of the 7-methylguanylate cap in their 5' end. Instead, these mRNAs contain a different type of secondary structures that are called internal ribosome entry sites (IRES) that directly recruit the ribosomes for further scanning of the mRNA molecule (Iizuka et al., 2015; Reineke et al., 2008).

Given the complexity of this step, translation initiation is regulated at multiple levels when the cell encounters any physiological change. For example, translation is globally downregulated in response to stress. We will further explain the consequences of cellular stress in translation in the section 4.

3.2. Translation elongation

Translation elongation starts with the binding of the translation elongation factor eEF1A to a molecule of GTP and an aminoacid-carrying transfer RNA (aa-tRNA). This complex then binds to the A site of the ribosome (Figure 5). The mRNA codon-tRNA anticodon match leads to the hydrolysis of the GTP molecule and the subsequent release of the eEF1A-GDP from the ribosome. Then, the peptide sequence bounded to the tRNA of the P site binds to the aa-tRNA of the A site. The elongation factor eEF2 promotes the movement of the nascent peptidyl-tRNA from the A site to the P site and of the deacylated tRNA from the P site to the E site of the ribosome (Dever et al., 2016). With the help of eEF3, an elongation factor that is only present in fungi, the E site deacylated tRNA is released and subsequent rounds of translation elongation follow (Triana-Alonso et al., 1995).

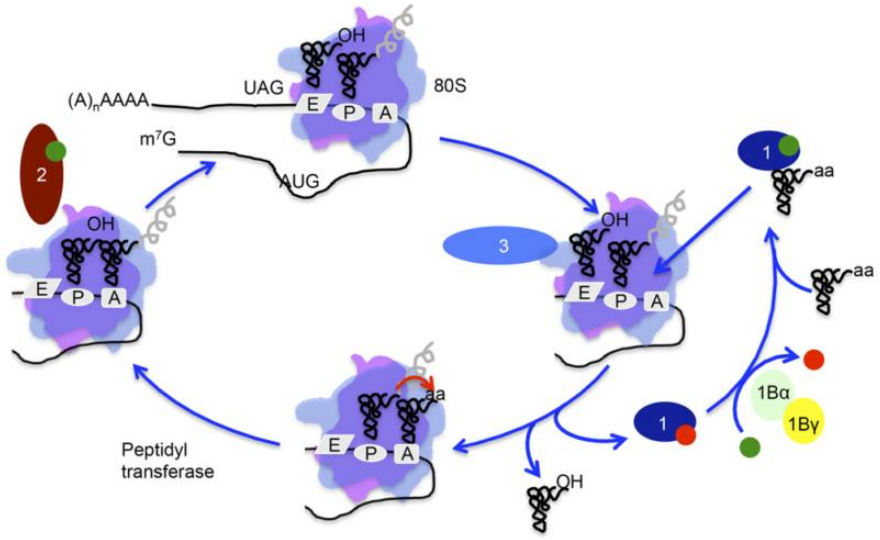

Figure 5. Model of translation elongation in yeast. A ribosome that is taking part in the translation elongation process has a peptidyl tRNA in the P site and a deacylated tRNA in the E

site. eEF1A (1) binds to GTP (green circle) and an aa-tRNA and the complex shuttles to the A site. When there is a codon-anticodon match, this leads to several conformational changes of eEF1A, which lead to hydrolysis of GTP and further release of eEF1A-GDP (red circle) from the A site tRNA. The peptide now binds to the A site aa-tRNA (red arrow). The ribosomal translocase eEF2 (2) promotes the movement of the peptidyl-tRNA from the A site to the P site and of the deacylated tRNA from the P site to the E site. The elongation factor eEF3 (3) interacts with eEF1A and helps in the release of the E site deacylated tRNA. Adapted from (Dever et al., 2016).

3.3. Translation termination and recycling

Translation termination and ribosome recycling involve the release of the newly synthesized polypeptide and the 40S and 60S ribosomal subunits for further cycles of translation. Translation termination begins when a stop codon enters the A site of the ribosome. The release factor eRF1 recognizes the termination codon with its tRNA-like structure in the N-terminal domain. The C-terminal domain of eRF1 instead serves as a protein-interacting platform. When the release factor eRF3 binds to eRF1, this complex facilitates the recognition of the termination codon. After eRF3 is released, the factor Rli1 binds to eRF1 and promotes the hydrolysis of the aminoacyl bond that links the polypeptide to the peptidyl-tRNA (Dever et al., 2016; Preis et al., 2014) and the release of the 60S ribosomal subunit. Finally, proteins Tma64 and Tma20 are in charge of accomplishing the last step of ribosome recycling, which consists of releasing the mRNA and deacylated tRNA from the 60S ribosomal subunit (Dever et al., 2016).

3.4. Localized translation at the endoplasmatic reticulum

Proteins are synthesized mostly by ribosomes that are free in the cytosol or bound to the surface of the endoplasmic reticulum (ER). Translation in each of these two major compartments has unique features that are still under investigation. Depending on their subcellular distribution, mRNAs are exposed to distinct regulatory factors and translational components, which consequently

affect protein synthesis (Reid and Nicchitta, 2015). In addition, protein folding also differs between the cytosol and the ER. For instance, aggregates of cytosolic proteins are generated by ER-bound ribosomes and are sequestered in the ER membrane (Zhou et al., 2014).

Although it was first described that only mRNAs that encode for secretory and integral membrane proteins were translated in the ER (Palade, 1975; Siekevitz and Palade, 1960), several studies indicate that the role of the ER in translation is much broader, taking part in the synthesis of the whole transcriptome (reviewed in Reid and Nicchitta, 2015). In fact, around 75% of all yeast proteins are synthesized associated to the ER (Zhou et al., 2014).

The routes of targeting mRNAs to the ER for translation include signal recognition particle (SRP)-dependent and -independent pathways. The signal recognition particle (SRP) is a ribonucleoprotein complex that recognizes a signal sequence in the nascent poypeptide chain that is being translated and thus targets the ribosomal complex to the ER. The SRP receptor, located in the ER membrane, is in charge of attaching the ribosomal complex to the ER for further localized translation (Ast et al., 2013; Nyathi et al., 2013). The SRP-independent pathways to recruit mRNAs to the ER are less understood. These include the interaction between cis elements within the message and trans-acting RNA-binding proteins, ribosome-mediated mRNA targeting, post-translational targeting and RNA-based localization (Ast and Schuldiner, 2013; Kraut-Cohen et al., 2013). This tight regulation of the recruitment of specific mRNAs to the ER, a differentially regulated translation compartment, opens a new layer of translational regulation. Thus, the compartmentalization of mRNAs between cytosol and ER represents another mechanism of post-transcriptional regulation.

4. BMV/yeast model system

4.1. Brome mosaic virus genome

The Brome Mosaic Virus (BMV) is a positive-strand RNA virus that is pathogenic in plants. It belongs to the *Bromoviridae* family and the *Alphavirus*-like superfamily and it was first isolated from bromegrass in 1942 (Lane, 1974).

The genome of BMV consists of three RNAs which are capped at their 5'end and have a tRNA-like structure in their 3'UTR (Figure 6; Rietveld et al., 1983). RNA1 and RNA2 are monocistronic and essential for replication, whereas RNA3 is dicistronic and non-essential (Ahlquist, 1992). RNA1 encodes protein 1a (109 kDa), whose N-terminal domain has GTP binding activity and is required for viral RNA capping whereas the C-terminal domain has an RNA helicase domain (Ahola et al., 2000; Ahola and Ahlquist, 1999; Kong et al., 1999). RNA2 encodes protein 2a (94 kDa), the RNA-dependent RNA polymerases. Protein 1a recruits itself, protein 2a and the viral RNAs to the ER, where it induces the formation of membrane-associated replication complexes (Schwartz et al., 2002). RNA3 encodes the cell-to-cell movement protein 3 (32 kDa) and the coat protein (20 kDa), which are needed for a systematic infection. The coat protein arises from the subgenomic RNA4, which is generated from the negative-strand RNA3.

BMV RNAs possess several *cis*-acting elements that coordinate their translation and replication. For example, RNA2 has specific sequences in the non-coding region of its 5' end that control the abundance of 2a polymerase (Noueiry et al., 2000). Besides, a conserved element located in the 5'UTR of RNA1 and RNA2 and in the intergenic region of RNA3 is in charge of the recruitment of the viral RNAs to the replication complex in the ER (Baumstark and Ahlquist, 2001; Chen et al., 2001; Sullivan and Ahlquist, 1999). The highly conserved tRNA-like structure at the 3'end of BMV and other plant virus RNAs is recognized by the host tRNA-nucleotidyl transferase, which confers stability to the viral RNA by adding a 3'-CCAOH group to the structure (Ahlquist et al., 1981; Bastin and

Hall, 1976; Rietveld et al., 1983). The 3' ends of BMV RNAs function as promoters of the negative-strand RNAs (Chapman and Kao, 1999; Dreher et al., 1984), which in turn have promoter activity in their 3' ends to synthesize the genomic positive-strand RNAs. RNA3 negative-strand has a promoter in its intergenic region to produce subgenomic RNA4.

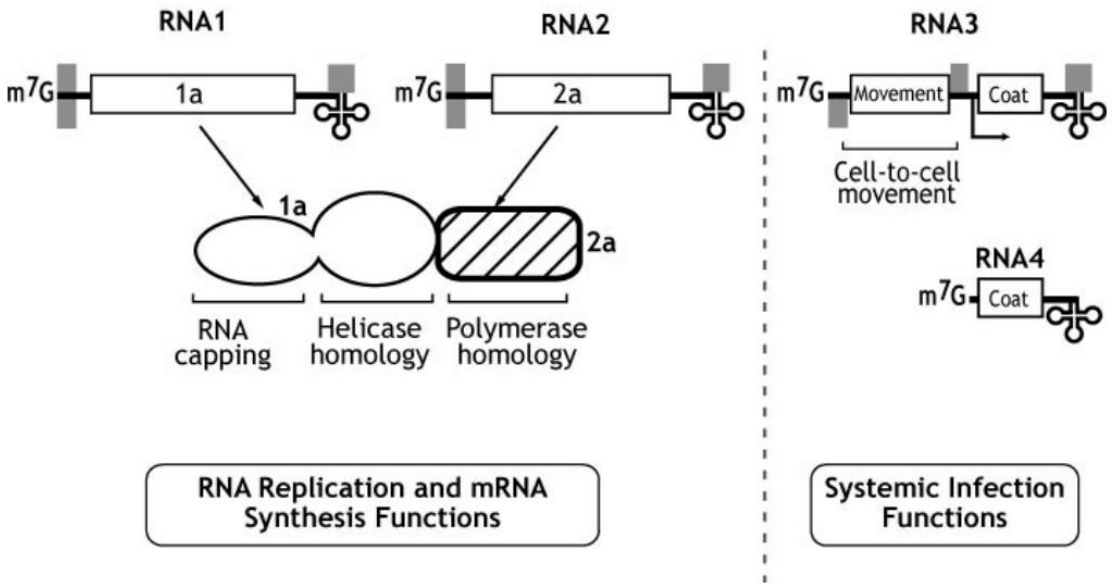

Figure 6. BMV genome. ORFs are represented with open boxes and non-coding regions with solid black lines. Grey boxes represent replication elements. The arrow in RNA3 represents the start site of the RNA4 (Noueiry and Ahlquist, 2003).

4.2. BMV life cycle

BMV follows a typical (+)RNA virus life cycle. Upon entering the host cell, the BMV (+)RNAs are translated into proteins (Figure 7). These viral proteins recruit together with some host proteins the viral RNAs to the ER, where the negative-strand RNA intermediates are synthesized to serve as templates for further replication of (+)RNAs, which in the end are encapsidated for a new cycle of infection (Ahlquist, 2006).

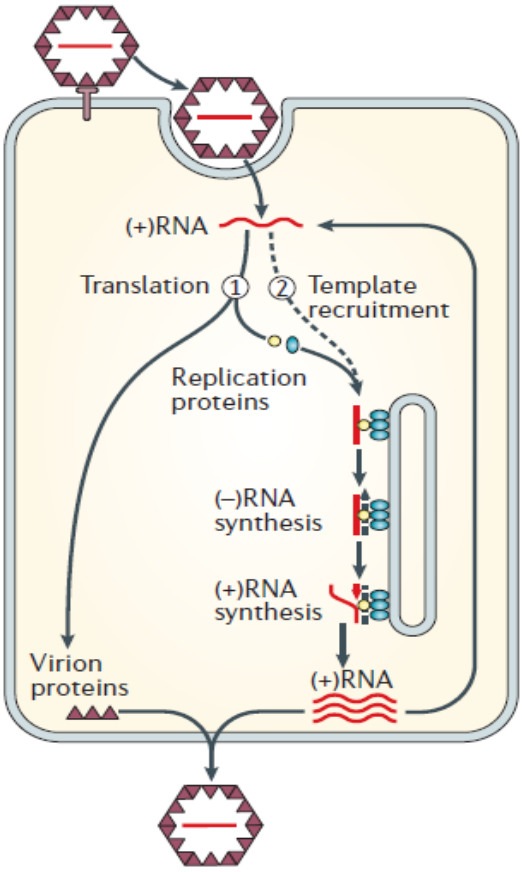

Figure 7. BMV life cycle. When the virus enters in the cell, (+)RNAs are translated into proteins. These proteins then recruit the viral RNAs to replication complexes at the ER where (-)RNA is generated which subsequently serves as template to greatly amplify the viral RNA genome. In the last step, the viral RNA progeny is encapsidated and released from the cell to start a new round of infection. Adapted from (Ahlquist, 2006).

4.3. *Saccharomyces cerevisiae* as a model host for (+)RNA viruses

Saccharomyces cerevisiae is a powerful model organism for the elucidation of a broad range of cellular processes and for the study of viral replication. *S. cerevisiae* is eukaryotic and its genome was first sequenced in 1996 (Goffeau et al., 1996). Its genome is composed of 16 chromosomes with around 6600 protein coding genes with little redundancy. In fact, early analyses of the sequencing of yeast genomes showed that 31% of their protein coding genes have homologs in the human genome (Botstein et al., 1997). In addition, *S. cerevisiae* is easy to grow and manipulate in laboratory conditions, since along the years researchers have developed several tools to efficiently handle yeast, such as (i) commercially available tagged strains, (ii) mutant and non-essential deletion strains, and (iii) DNA microarrays chips (Hanson, 2018). Altogether, *S. cerevisiae* has developed to be an excellent model system to study conserved processes of more complex eukaryotic organisms, such as humans.

There are numerous viruses that can replicate in *Saccharomyces cerevisiae*, including DNA and RNA viruses that infect plants (BMV), animals (Flock House virus (FHV)) and humans (Human Papillomavirus (HPV)) (Zhao, 2017). These virus/yeast systems share some characteristics, such as (i) the viral RNA-dependent RNA polymerase can be expressed from a yeast plasmid in trans, (ii) the genomic viral RNA conserves the original UTRs and can be expressed from a plasmid or electroporated, (iii) viral replication can be measured by the use of a reporter whose expression depends on the replication of the virus. Altogether, viral studies in yeast have greatly contributed to the study of virus-host interactions.

4.4. BMV replicates efficiently in *Saccharomyces cerevisiae*

Replication of BMV in yeast fully recapitulates all steps of the viral life cycle. Using plasmids expressing different genes of BMV these steps can be dissected and studied separately. For instance, to study translation, yeast cells are transformed with a plasmid expressing the viral protein 1a or 2a. The viral genetic information is transcribed into a viral RNA with its natural UTRs. Translatability is determined by the quantification of protein expression levels divided by the abundance of RNA levels. In order to study recruitment, yeast cells are transformed with a plasmid containing the genetic information to express viral RNA3 alone or together with protein 1a. In the presence of protein 1a, RNA3 gets recruited to replication complexes in the ER (Schwartz et al., 2002). Recruitment to the ER renders RNA3 more stable as it is spatially less accessible to the host degradation machinery (Janda and Ahlquist, 1998). This can be detected by an increase in the amount of RNA3 by Northern Blot analysis. Finally, to study replication, the co-expression of RNA1, RNA2 and RNA3 is required. In order to uncouple the effect of replication from translation and recruitment, RNA1 and RNA2 are expressed from cellular promoters, whereas wild-type RNA3 is introduced in the cell by electroporation or *in vivo* transcription from a plasmid (Ishikawa et al., 1997). In this way, the production of replication-dependent sgRNA4 can be measured.

5. Introduction to yeast stress responses

A biological stress is defined as any intrinsic or extrinsic stimulus that provokes a cellular response (Yaribeygi et al., 2017). In fact, cells are constantly facing changes in their environment. For instance, they may undergo starvation, be exposed to non-optimal temperature conditions, grow in presence of toxins, or suffer from changes in water availability. The survival of an organism under stress conditions is determined by its capacity to adapt to the environmental change (Hohmann and Mager, 2003). For this, cells have developed a wide variety of responses to ensure their survival under perturbed circumstances. The first step that a cell undergoes after sensing an environmental stress is to arrest cell growth (Brauer et al., 2008), followed by changes in its gene expression landscape (de Nadal and Posas, 2015) that will lead to the specific expression of proteins with stress-protective functions (Toone and Jones, 1998).

5.1. Stress-activated signaling pathways

When cells are growing under physiological conditions, the cellular machinery favors synthesis of growth-related genes, whereas stress-protective genes are expressed at very low concentrations (Broach, 2012; Smets et al., 2010). Upon facing suboptimal environmental conditions, specific sensors trigger the activation of a stress-activated signaling pathway that will lead to a transient arrest of growth and cell cycle, followed by distinct changes in transcription, translation, protein function and localization that changes the metabolism of stressed cells (Chasman et al., 2014).

In yeast, several stress-activated signaling pathways are well described and characterized. These pathways are evolutionarily conserved among eukaryotes, as multicellular organisms can experience stress conditions as well. For example, cells that form the roots of a plant must deal with osmotic imbalances due to water availability (Moshelion et al., 2004). Likewise, mammalian renal cells are exposed to high urea concentrations that activate stress signaling pathways (Burg and Ferraris, 2008). Due to the conservation of these pathways

among eukaryotic organisms, *Saccharomyces cerevisiae* has been used as a genetic model system to study eukaryotic stress-activated signaling pathways (Toone and Jones, 1998).

5.1.1. Environmental stress response

Depending on which stress cells are facing, they can trigger (i) a generic response that is shared by many different stresses, or (ii) a specific response that focuses on a particular stress (de Nadal and Posas, 2010; Weake and Workman, 2010). Genes harboring similar expression patterns upon several distinct stresses are classified as environmental stress response (ESR) genes. These genes include around 300 stress-defensive genes that are induced (iESR) to promote cell protection and repair, as well as around 600 genes related to ribosome biogenesis and protein synthesis functions that are repressed (rESR) in order to delay proliferation and promote recycling of transcription and translation machineries in favor of iESR synthesis (Causton et al., 2001; Gasch et al., 2000; Ho and Gasch, 2015). Although the ESR was first described to act in several environmental stresses such as osmotic, oxidative or heat stress, further studies corroborated the broad effect of ESR due to both external and internal changes (Gasch et al., 2001).

The major activators of the ESR are Msn2 and Msn4, two redundant transcription factors (TFs) (Boy-Marcotte et al., 1998; Görner et al., 2002; Hasan et al., 2002; Kandror et al., 2004). Under various stress circumstances, Msn2/Msn4 shuttle to the nucleus and bind to the stress-response elements (STREs) located in the promoter regions of stress-activated genes (Görner et al., 1998; Martínez-Pastor et al., 1996; Schmitt and McEntee, 1996; Wieser et al., 1991). Under optimal conditions, the protein kinase PKA represses the ESR by inhibiting the activation of Msn2/Msn4 (Morano et al., 2012). However, when cells are subjected to perturbations of their environmental conditions, the PKA and mTOR (target of rapamycin) pathways are shut down, which leads to the activation of Msn2/Msn4 TFs. In fact, Msn2/Msn4-dependent iESR genes represent approximately 80% of the global transcriptional response to a specific

stress (Gasch et al., 2000). The remaining 20% of the transcriptional response are modulated by one or more stress-activated signaling pathways, each activating a subset of specific transcription factors depending on the exact type of stress (Capaldi et al., 2008; Jacquet et al., 2003).

As an example, yeast cells grown under higher than optimal temperatures activate the heat-shock response (HSR) pathway. Here, the rapid heat shock response is mediated by the Msn2/Msn4 and Hsf1 transcription factors. Hsf1 binds to the heat-shock elements (HSE) located in the promoter regions of genes encoding for heat-shock proteins (HSP) and participates in protein folding, cell membrane permeability, protein stability and gene expression (Morano et al., 2012). Importantly, the Msn2/Msn4 and Hsf1 transcription factors have different functions during heat shock adaptation: Hsf1 is essential for *Saccharomyces cerevisiae* to withstand short-term exposure to high temperatures, whereas Msn2/Msn4 are necessary under prolonged heat conditions (Yamamoto et al., 2008). As another example, the oxidative stress response (OSR) pathway is triggered when cells accumulate reactive-oxygen species due to their internal metabolism or due to external factors such as radiation, exposure to herbicides and/or other chemical products that produce DNA damage. The OSR favors the induction of detoxifying enzymes and activation of this pathway is controlled by several transcription factors, such as Yap1, Hsf1, Msn2/Msn4, among others. Interestingly, the cellular responses to heat and oxidative stress conditions are very similar, as heat shock causes production of reactive-oxygen species (Morano et al., 2012). The interconnections between different stresses highlight the ability of eukaryotic cells to sense, distinguish and appropriately respond to distinct conditions that threaten their survival.

5.1.2. Mitogen-activated protein kinase signaling pathways

One of the main sets of yeast signaling pathways are the so-called mitogen-activated protein kinase (MAPK) cascades (Herskowitz, 1995). These pathways are activated as a fast response to changing environmental conditions to ensure

survival of the organism. Due to their key role in stress adaption, MAPK cascades are highly conserved throughout the evolutionary tree (Avruch, 2007). A MAPK cascade consists of the sequential activation of at least three kinases (Figure 8). The MAPK kinase kinase (MAPKKK or MAP3K) is activated when cells recognize a trigger stimulus either by another upstream kinase or by an activator protein. The MAPKKK will then promote phosphorylation of MAPKK (MAP2K) in one or more Serine/Threonine residues of its activating loop. Lastly, the MAPK will similarly be activated by its upstream acting MAPKK and will be the effector protein prompting changes in metabolism and gene expression according to the initial stimulus (Widmann et al., 1999).

There are five different MAPK signaling pathways described in *Saccharomyces cerevisiae*, each triggered differently depending on the exact environmental stress (Gustin et al., 1998): (i) the filamentous growth or pseudohyphal development pathway (Kss1 MAPK is triggered) is activated in diploid cells under specific culture conditions and triggers a dimorphic switch to form pseudohyphae able to invade solid media; (ii) the mating-pheromone response (Fus3 MAPK is triggered) helps haploid cells with different mating types to mate by arresting cell cycle, among other functions; (iii) the spore wall assembly pathway (Smk1 MAPK is triggered) stimulates diploid cells to form spores that are resistant to a variety of stresses; (iv) the cell integrity pathway (Mpk1/Slt2 MAPK is triggered) stimulates the cell wall synthesis in cells that are undergoing cell division; and (v) the HOG pathway (Hog1 MAPK is triggered), which is stimulated under osmolarity-changing environments (Chen and Thorner, 2007; Gustin et al., 1998).

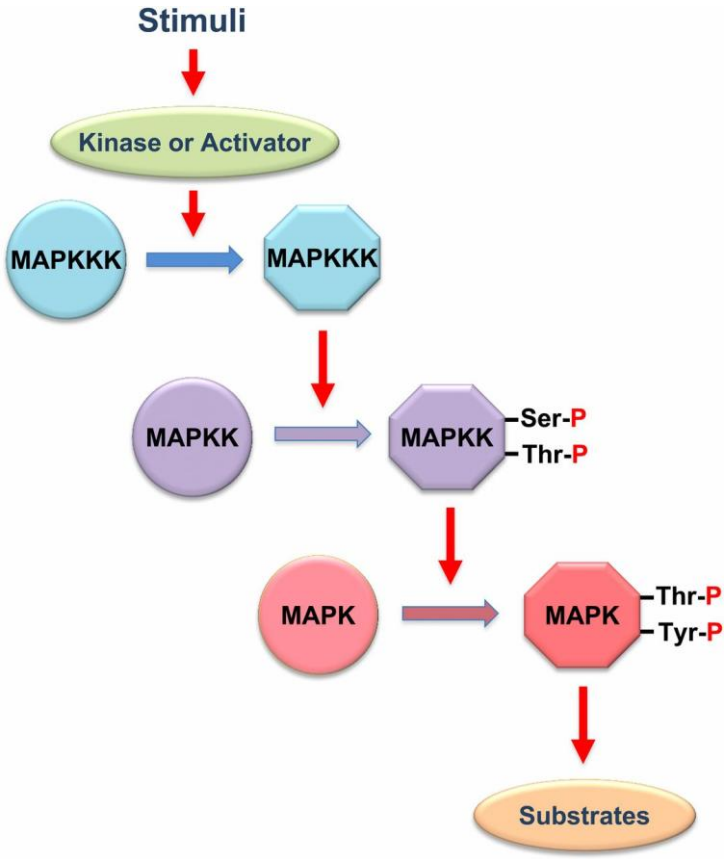

Figure 8. MAPK pathways cascade. Circles represent the inactive forms of the kinases, whereas octagons represent their active forms. Red arrows indicate the site of activity of the upstream kinase. Adapted from (Saito and Posas, 2012).

5.2. Hyper-osmotic stress and Hog1 MAPK

Saccharomyces species live of the sugar uptake from decomposing fruits. Once the fruits dry, the encountered sugar concentration increases dramatically, creating an adverse osmotic environment for the yeast cells. When the external osmolarity increases, it provokes a water outflow from the cells to the external medium and, within seconds, the cells shrink in volume and the intracellular ion concentration increases as a consequence (Wood, 2011). This process is known as hyperosmotic stress or osmostress and, just as it starts, yeast cells need to rapidly adapt to the new environmental conditions by producing glycerol to counter the osmotic pressure, transiently arresting the cell cycle and changing their gene expression patterns (Figure 9; Hohmann, 2002; Mager and Varela, 1993).

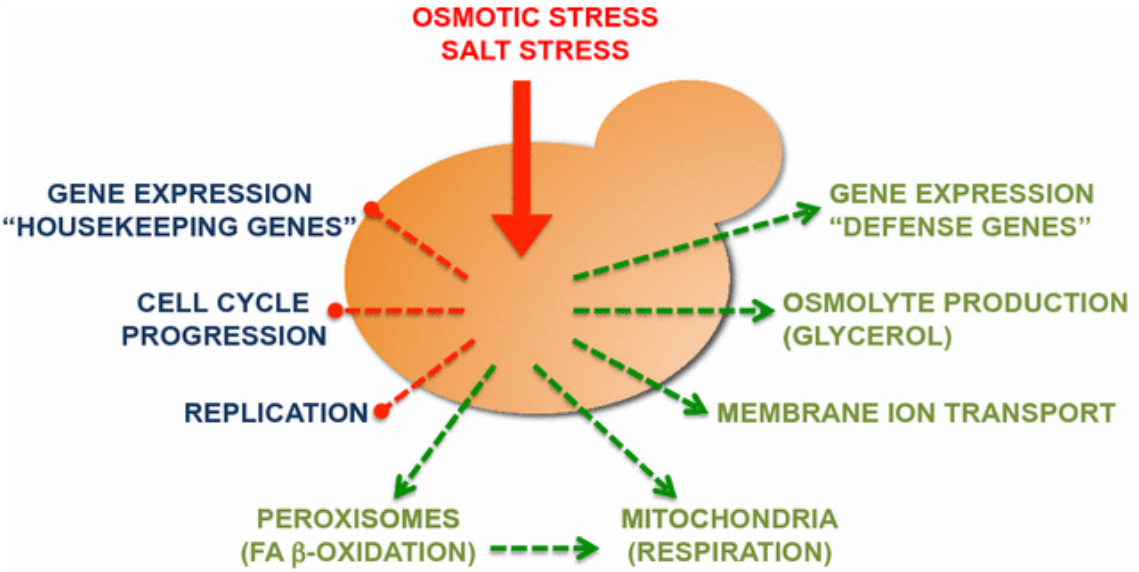

Figure 9. Adaptive mechanisms of budding yeast to hyperosmotic stress. Upon stress, glycerol synthesis is increased to balance the osmotic pressure, while membrane anion transport is stimulated for ion homeostasis regulation. In addition, mitochondrial metabolism is reinforced and global gene expression shifts from cell-growth and 'housekeeping' genes to stress-defensive genes, provoking an arrest in cell cycle and replication. Adapted from (Pascual-Ahuir et al., 2018).

A rapid response to osmotic stress is particularly important in unicellular organisms that are in direct contact with the external environment. Nevertheless, multicellular organisms such as mammals may suffer from osmostress under certain conditions (Ho, 2006). For example, the human retinal pigmented epithelial cells (RPE) constitute the most external layer of the retina and serve as a barrier for water and osmolyte movements. When RPEs are under hyperosmotic conditions, they activate the p38 mitogen-activated protein kinase cascade (homologous to Hog1 pathway in yeast) and regulate cell proliferation (Arsenijevic et al., 2013).

Under osmotic stress conditions, the High Osmolarity Glycerol (HOG) signaling pathway is triggered, leading to the activation of the Hog1 MAPK and its downstream targets. The Hog1 MAPK cascade is highly conserved among fungal species and Hog1 can get phosphorylated by two redundant, yet mechanistically different modules, the Sln1 branch and the Sho1 branch (Figure

10; Dan et al., 2001; Posas et al., 1996; Posas and Saito, 1997; Raitt et al., 2000).

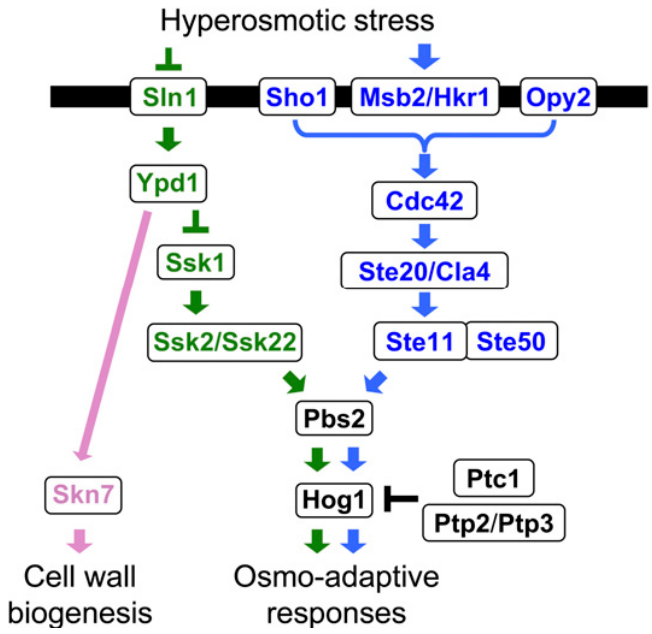

Figure 10. Diagram of the HOG signaling pathway in *Saccharomyces cerevisiae*. Proteins colored in black are shared between the two branches. Arrows indicate activation, whereas T-shaped bars represent inhibition. Adapted from (Haruo Saito and Posas, 2012).

5.2.1. The Sln1 branch of the HOG pathway

The Sln1 branch of the HOG pathway starts with the recognition of the external signal by the sensor histidine kinase (SHK) Sln1 (Posas et al., 1996; Saito, 2001). This protein contains a sensor domain in its N-terminal end (Maeda et al., 1994; Ota and Varshavsky, 1993) and a HK catalytic domain and a receiver (REC) domain in the C-terminal end. When Sln1 is activated upon an external stimulus, the Sln1 HK catalytic domain auto-phosphorylates the REC domain of Sln1 (Posas et al., 1996). From there, the phosphoryl group is transferred to Ypd1 protein. Next, Ydp1 transfers the phosphoryl group to the REC domain of Ssk1, which in turn activates two homologous and functionally redundant MAPKKKs called Ssk2/Ssk22 (Posas and Saito, 1998). Once Ssk2/Ssk22 are activated, they initiate a cascade in which the MAPKK Pbs2 and MAPK Hog1 are sequentially phosphorylated (Boguslawski, 1992; Brewster et al., 1993).

Besides controlling Sln1-Ydp1-Ssk1 activation under hyper-osmotic conditions, Sln1 also regulates the Sln1-Ydp1-Skn7 cascade, which is activated during hypo-osmotic stress (Posas et al., 1996). In contrast to Ssk1, Skn7 is localized in the nucleus and features a DNA-binding N-terminal domain as well as a REC domain in the C-terminal end. Due to this spatial separation, Ydp1 is localized in the cytoplasm for the phosphorylation of Ssk1, whereas it shuttles to the nucleus to transfer the phosphoryl group to Skn7 (Ault et al., 2002; Li et al., 1998; Lu et al., 2003). In this way, Sln1 signaling integrates and controls the cellular response to both possible osmotic stresses.

5.2.2. The Sho1 branch of the HOG pathway

The Sho1 branch of the HOG pathway is less studied. When cells recognize an external signal, the putative osmo-sensors Msb2 and Hkr1 initiate the Sho1 cascade (Tatebayashi et al., 2007). The interaction between Msb2 and Hkr1 with Sho1 leads to the association of the Ste20 and Cla4 kinases to the membrane-bound small G-protein Cdc42 (Lamson et al., 2002). When Ste20/Cla4 are active, they phosphorylate the MAPKKK Ste11, which in turn phosphorylates Pbs2, which in turns phosphorylates Hog1 MAPK (Drogen et al., 2000).

Hog1 is mostly found in the cytoplasm under physiological conditions but rapidly shuttles to the nucleus upon osmotic stress where it phosphorylates its target substrates, such as transcription factors and cell-cycle regulators. Once cells have adapted to the osmotic stress, Hog1 shuttles to the cytoplasm and controls long-term survival by its metabolic functions (Bouwman et al., 2011; Ferrigno et al., 1998).

5.3. Mechanism of adaptation to hyper-osmotic stress

Yeast cells employ different mechanisms to survive in high osmolarity conditions. The HOG pathway coordinates the action of several cell machineries and leads to changes in cellular metabolism and protein expression dynamics (Figure 11). Here I will review the cellular mechanisms of adaptation to osmostress found in yeast.

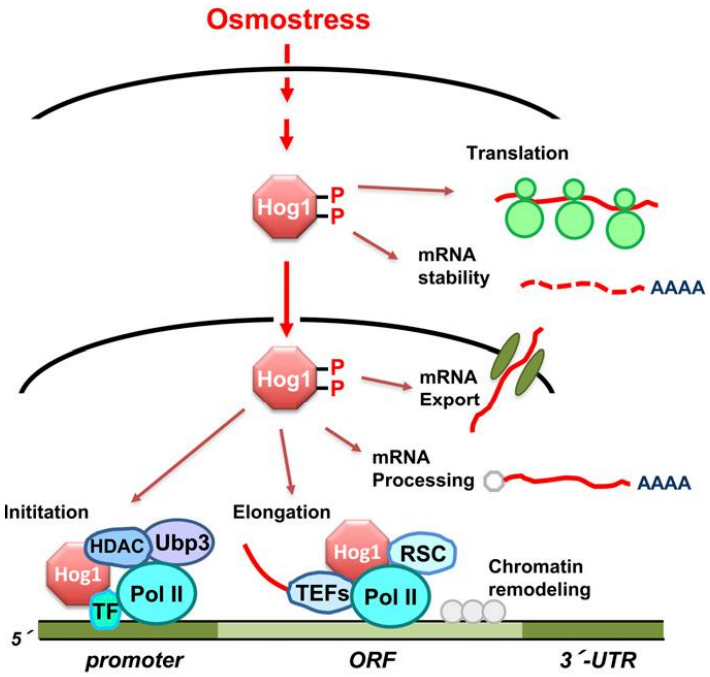

Figure 11. Regulation of gene expression by the Hog1 MAPK. Under osmotic stress conditions, Hog1 travels to the nucleus where it activates TFs by phosphorylation. Additionally, it binds to the promoter and coding regions of stress-responsive genes and stimulates their transcription initiation and elongation. Besides, Hog1 seems to be involved in the nuclear export, mRNA stability and translation of stress genes. Adapted from (Saito and Posas, 2012).

5.3.1. Transcription

Genome-wide transcriptional studies revealed that 5-7% of the genome undergoes changes of expression after hyper-osmotic stress. Specifically, induced genes were associated to carbohydrate and amino acid metabolism, ribosome biogenesis, signal transduction and stress protection groups (Causton et al., 2001; Gasch et al., 2000; Pokholok et al., 2006; Posas et al., 2000; Rep

et al., 2000; Taymaz-Nikerel et al., 2016). Moreover, the transcriptional induction of the majority of genes was dependent on Hog1 (Posas et al., 2000; Rep et al., 2000).

Hog1 plays a very important role in transcription initiation by the direct phosphorylation and successive activation of several unrelated transcription factors. These TFs in turn regulate the expression dynamics of osmo-responsive genes (Miller et al., 2011; Molin et al., 2009; Romero-Santacreu et al., 2009). For example, Hog1 regulates the activity of Hot1, Smp1, Msn1, Msn2, Msn4, as well as Sko1 (de Nadal and Posas, 2010). The latter acts as a transcriptional repressor under physiological conditions due to its interaction with the Tup1/Ssn6 repressor complex. When Hog1 phosphorylates Sko1, it prompts a conformational change in Sko1 that releases it from the Tup1/Ssn6 repressing complex and thereby activates it (Garcia-Gimeno and Struhl, 2000; Pascual-Ahuir et al., 2001; Proft and Struhl, 2002). Overall, the collaborative interactions of the various Hog1-dependent transcription factors enable the dynamic transcriptional response necessary for survival and adaption to osmotic stress (Ni et al., 2009).

Apart from its effect in transcription due to direct phosphorylation of transcription factors, Hog1 is also able to associate with chromatin at osmo-responsive gene loci (Reiser et al., 1999). This association is dependent on its catalytic activity and is mediated via physical interactions to transcription factors. For instance, Hog1 binds to the promoter of *STT1* by Msn2/Msn4 TFs, whereas it binds to *STL1* gene promoter through the interaction with Hot1 (Alepuz et al., 2001).

Transcription elongation of osmo-responsive genes is also tightly regulated by Hog1. On one hand, Hog1 binds to the coding regions of elongating genes and travels together with the elongating RNA Pol II along their CDS, favoring their transcription (Pascual-Ahuir et al., 2006; Pokholok et al., 2006; Proft et al., 2006). On the other hand, Hog1 interacts with the RSC chromatin-remodeling complex and modifies the nucleosome structure of osmo-defensive genes to increase the association of RNA Pol II and thereby increase the expression of stress genes (Mas et al., 2009).

5.3.2. Translation

Translational efficiency suffers a global reduction within minutes after osmotic shock, most likely due to a dramatic drop in amino acid uptake as well as a transcriptional downregulation of ribosomal genes (Norbeck and Blomberg, 1998; Uesono and Toh-e, 2002; Warringer et al., 2010). Moreover, cells experience a decrease of growth-related proteins and an increase in stress-related protein levels (de Nadal et al., 2011). For most osmo-responsive genes, increases in RNA levels due to transcriptional activation are accompanied by increases in protein levels due to translational activation (Halbeisen and Gerber, 2009). Nonetheless, some groups of genes show a translation regulation independent of their transcriptional regulation. For instance, while some transcripts are translationally upregulated even though their RNA levels remain stable, others show translational repression when their mRNA levels are transcriptionally elevated. These translationally upregulated osmogenes depend on Hog1 to be recruited to polysomes, although the mechanism remains unclear (Warringer et al., 2010).

The role of Hog1 in protein synthesis is mainly related to long-term adaptation since polysome formation is delayed in *hog1*Δ cells exposed to high osmolarity (Norbeck and Blomberg, 1998; Uesono and Toh-e, 2002). Specifically, Hog1 regulates the activation of Rck2 kinase, which in turn phosphorylates the eEF2 translation elongation factor to favor translation of osmo-responsive proteins (Bilsland-Marchesan et al., 2000; Teige et al., 2001).

An additional layer of translation regulation are stress granules (SG) formed during osmostress. Stress granules are mRNA-protein assemblies that are composed of non-translating mRNAs, translation initiation factors and other proteins related to the mRNA life cycle (Giménez-Barcons and Díez, 2011). They are formed when translation initiation is repressed and lack any association with ribosomes (Mazroui et al., 2006). Their relevance for the cellular stress response is still unclear, although they are thought to play a role by i) concentrating proteins that are activated by enzymatic cascades, ii) limiting the interactions of SG components with the cytosol, and iii) favoring the

formation of translation initiation complexes by promoting the interaction of mRNAs with translation factors (Buchan et al., 2008; Giménez-Barcons and Díez, 2011; Protter and Parker, 2016).

Overall, these results illustrate that translation is another tightly regulated process under osmotic stress conditions.

5.3.3. Glucose metabolism and cell cycle

Within the first minutes of stress, cells accumulate compatible osmolytes such as glycerol and trehalose as a mechanism to reestablish osmotic balance (Albertyn et al., 1994; Klipp et al., 2005). Hog1 activates the TFs necessary to induce expression of enzymes responsible for the synthesis of glycerol (Albertyn et al., 1994; Martínez-Pastor et al., 1996; Ruis and Schüller, 1995). For example, the activity of the 6-phosphofructo-2-kinase (Pfk2), a key activator of glycolysis, is regulated by Hog1 (Dihazi et al., 2004). In addition, the export of glycerol to the external media is abrogated under stress to favor internal accumulation of the osmolyte. Since the yeast cellular membrane has low permeability for glycerol, the osmolyte is transported through specific channels such as Fps1, which closes in a Hog1-independent manner upon stress (Tamás et al., 1999).

Cell-cycle progression is transiently inhibited upon osmotic stress in order to allow cells to adapt to the new environmental conditions before undergoing mitosis (Clotet and Posas, 2007). The haploid yeast cell cycle is divided into four phases (S for synthesis, M for mitosis, G1 for Gap1 between M and S-phase, and G2 for Gap2 between S and next M-phase) and the progression along phases is regulated by a single cyclin dependent kinase (CDK) called Cdc28. During hyper-osmotic shock, Hog1 modulates every phase of the cell cycle by regulating the expression of cell-cycle regulators and cyclins (Duch et al., 2012). For instance, Hog1 delays G1/S transition by directly phosphorylating Sic1, which in turn inhibits Cdc28 (Escoté et al., 2004; Zapater et al., 2005). In addition, Hog1 helps to coordinate transcription and replication in cells that face

osmostress during S-phase to prevent collisions of engaged DNA and RNA polymerases. Under physiological conditions, cells have developed several mechanisms to facilitate the simultaneous activity of both the transcription and replication machineries during all phases of the cell cycle. However, under osmotic stress, the rapid changes in transcription can result in genomic instability when both machineries are located in close proximity (Aguilera, 2002, 2005). To avoid such collisions between the transcription and replication machineries, Hog1 phosphorylates a component of the replication machinery called Mrc1, leading to a slower progression of the replication machinery and thus allowing the timely transcription of osmo-responsive genes (Duch et al., 2018, 2013).

Overall, yeast cells orchestrate a rapid response to osmotic stress through the coordinated action of several cell machineries.

5.4. The role of Xrn1 in environmental stress responses

Glucose deprivation and certain other stresses cause activation of another protein kinase called Snf1, which regulates transcription and mRNA stability (Hedbacker and Carlson, 2008). Interestingly, mass spectrometry analyses in yeast revealed that Ccr4, Dhh1 and Xrn1 are required for the correct decay of Snf1-dependent genes. Furthermore, Xrn1 requires a Snf1-dependent phosphorylation in its C-terminal domain to promote glucose-induced *ADH2* mRNA decay, and it is also involved in regulating transcription of Snf1-dependent genes. Overall, Xrn1 is required for proper mRNA homeostasis under glucose deprivation stress (Braun et al., 2014).

Whether Xrn1 is also required for proper responses to other environmental stresses such as hyper-osmotic shock remains unclear.

OBJECTIVES

The exonuclease Xrn1 regulates gene expression by coupling mRNA decay with mRNA transcription. Originally identified as the major 5'-3' exonuclease in the cytoplasm, Xrn1 has been shown to also shuttle to the nucleus to stimulate transcription of some mRNAs and hence to mediate a crosstalk between decay and transcription. In our group we have recently discovered that Xrn1 furthermore plays a role in translation regulation and thereby closes the circle of gene expression. Specifically, we demonstrated that Xrn1 promotes translation of BMV RNA2. However, the role of Xrn1 in cellular mRNA translation and the molecular mechanisms underlying this newly discovered function of Xrn1 is unknown. Given this central position of Xrn1 connecting the three major steps of gene expression, it is likely that Xrn1 plays a key role in the response to osmotic stress. How Xrn1 functions to adapt the cell to altered conditions has not been studied.

The specific aims of this PhD thesis are:

1) To elucidate the molecular mechanisms underlying the function of the exonuclease Xrn1 in viral and cellular mRNA translation

2) To uncover the function of Xrn1 upon altered environmental conditions

RESULTS

Publication I:

The exonuclease Xrn1 activates transcription and translation of mRNAs encoding membrane proteins

Nature Communications 10:1298–1313 (2019)

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Blasco-Moreno B, de Campos-Mata L, Böttcher R, García-Martínez J, Jungfleisch J, Nedialkova DD, et al. The exonuclease Xrn1 activates transcription and translation of mRNAs encoding membrane proteins. Nature communications. 2019;10(1):1298–1298. DOI: 10.1038/s41467-019-09199-6

Publication II:

Xrn1 links the Snf1 and Hog1 pathways to control gene expression upon hyper-osmotic shock (in preparation)

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Key words: Xrn1, osmostress, transcription, translation, degradation, stress granul

Abstract

Cells have developed a wide variety of response mechanisms to ensure their survival in constantly changing environments. When facing an increase in external osmolarity, *Saccharomyces cerevisiae* cells activate several interconnected signaling pathways that lead to an adjustment of the gene expression landscape. Based on genome-wide RNA sequencing analyses coupled with biochemical and functional assays, we show in this study that the exonuclease Xrn1 modulates cellular transcriptional and translational responses upon hyper-osmotic shock. Xrn1 localizes to stress-induced aggregates rapidly after NaCl uptake in a manner dependent on its unstructured C-terminal domain and mediated by the major signal integrator Snf1 adenosine monophosphate-activated protein kinase. Under these conditions Xrn1 maintains a diminished exonuclease activity and assists in the transcriptional and translational activation of a subset of osmo-induced genes that are enriched for proteins interacting with Hog1, the main mitogen-activated protein kinase involved in osmoregulation. Our results reveal a role of the exonuclease Xrn1 as linker of the Snf1 and Hog1 pathways to control gene expression upon osmotic stress.

Introduction

The ability of cells to sense and appropriately respond to environmental cues depends on complex and intertwined multi-layered signaling pathways that are conserved throughout the evolutionary tree (Avruch, 2007; Plotnikov et al., 2011). Among these pathways, different mitogen-activated protein kinase (MAPK) cascades have been widely studied, as they represent response mechanisms to specific environmental signals that range from alterations in ambient temperature, pH, oxygen concentration or radiation to nutrient deprivation and extracellular osmolarity (Avruch, 2007; Plotnikov et al., 2011).

For instance, to counter the osmotic imbalance caused by hyperosmotic shock, *Saccharomyces cerevisiae* cells induce glycerol production via the high

osmolarity glycerol (HOG) pathway and its MAPK Hog1 (Finan et al., 2009; Nadal-Ribelles et al., 2015; Saito and Posas, 2012). Moreover, they initiate a stress-dependent gene expression program by tightly regulating transcription, translation as well as RNA stability (Nadal-Ribelles et al., 2012; De Nadal and Posas, 2015; Romero-Santacreu et al., 2009; Saito and Posas, 2012; Uesono and Toh-E, 2002). Besides MAPK signaling, a major signal integrator is the Snf1 adenosine monophosphate-activated protein kinase (AMPK), which is activated upon nutrient deprivation as well as other environmental stresses and interacts with MAPK pathways (Coccetti et al., 2018; Pastor et al., 2009). Snf1 maintains energy homeostasis by controlling diverse cellular processes, including metabolism and gene expression (Coccetti et al., 2018; Hedbacker and Carlson, 2008; Lee et al., 2012; Shashkova et al., 2015).

Interestingly, many of the described mechanisms of gene expression under osmostress overlap known functions of Xrn1, a master regulator of RNA fate in the cell. Xrn1 is the major cytoplasmic 5'-to-3' exoribonuclease and tightly controls RNA steady-state levels by independently regulating transcription, translation as well as degradation (Blasco-Moreno et al., 2019; Haimovich et al., 2013; Nagarajan et al., 2013; Pérez-Ortín et al., 2013). Moreover, the Lsm1-7/Pat1 complex, another key component of RNA decay that interacts with Xrn1 and regulates the decapping process, has previously been reported to bind osmostress-induced mRNAs and modulate their translation (Garre et al., 2018). Given this evidence, we set out to elucidate the role Xrn1 plays in the cellular response to hyperosmotic shock and its potential connection to stress-activated kinase signaling.

Here we show that a fraction of Xrn1 colocalizes with Dhh1 to form aggregates upon osmostress in a manner dependent on Snf1 activity and the C-terminal region of Xrn1. Under these conditions, Xrn1 exonuclease activity is diminished. The remaining functionally active Xrn1 helps to induce expression of Hog1 interactors, which could benefit long-term survival in stress conditions. These results indicate that Xrn1 functions in homeostasis extend to osmostress and are modulated by Snf1-mediated relocalization.

Results

Xrn1 is involved in the osmostress response and localizes to stress granules

Xrn1 plays a multifunctional role in yeast gene expression under physiological conditions. Since gene expression is tightly regulated upon osmotic stress, we examined whether Xrn1 is involved in the osmostress response by testing whether Xrn1 knockdown affects cellular viability in growth assays during hyperosmotic shock. For this, we fused Xrn1 to an auxin-inducible degron (AID) that stimulates rapid degradation of Xrn1 (Blasco-Moreno et al., 2019; Nishimura and Kanemaki, 2014). Auxin addition led to Xrn1 degradation (**Fig. S1A**) and conferred sensitivity to NaCl uptake in a dose-dependent manner (**Fig. 1A-D**), indicating that Xrn1 plays a role in the cellular osmotic stress response.

To test whether Xrn1 protein levels remained stable after osmotic stress, we performed western blot (WB) analyses of Xrn1-AID cells at 0.4 M NaCl and found that Xrn1 levels significantly decreased shortly after NaCl addition (**Fig. 2A**). In contrast, the DEAD-box helicase Dhh1, a decapping activator acting in complex with Xrn1, showed no such decrease (**Fig. 2A**). Since AID-tagging targets proteins for ubiquitination, we repeated the experiment in WT cells, which corroborated our results (**Fig. 2B**). Given these unexpected findings, we questioned whether Xrn1 was a target for specific degradation by the ubiquitin(Ub)-proteasome pathway upon NaCl uptake. For this, we blocked degradation of ubiquitin-conjugated proteins by adding MG132 proteasome inhibitor to permeable yeast cells bearing a deletion of *PDR5* gene (Collins et al., 2010). Addition of MG132 did not affect Xrn1 protein levels (**Fig. 2C**), indicating that Xrn1 is not targeted by the Ub-proteasome pathway. To independently validate this result, we performed an immunoprecipitation (IP) assay with a yeast strain carrying a GFP-tagged Xrn1 and examined its ubiquitination status using an anti-ubiquitin antibody. Reassuringly, our IP showed no evidence of ubiquitination on Xrn1 protein after NaCl uptake (**Fig. 2D**).

Curiously, we noted that Xrn1 levels remained stable after NaCl addition in cell extracts prepared for the IP assay (**Fig. 2D**). In contrast to our standard lysis protocol for WB assays in which cell breakage was performed using glass beads, the sample preparation protocol for IP assays instead included cell disruption via cryogenic grinder. We therefore speculated whether upon NaCl addition Xrn1 might be aggregating in an insoluble fraction of the cytosol that is inaccessible to the solvents used in our WB assays. To test this hypothesis, we carried out microscopy experiments with fluorescently-labelled Xrn1 and Dhh1, which revealed that both proteins colocalize in foci upon hyper-osmotic shock (**Fig. 3A**). Both proteins showed differing dynamics, as Dhh1 localizes to foci earlier than Xrn1, while Xrn1 is retained longer (**Fig. S2A**). As additional validation for our microscopy results, we also explored the localization of other known components of processing bodies (P-bodies) and stress granules (SG) (Dcp1/2, Upf1) (**Fig. S2B**), all of which localized to aggregates as expected.

Based on these results we hypothesized that a significant fraction of Xrn1 is being sequestered from the cytosolic pool in order to regulate its functions in gene expression. We therefore measured the catalytic activity of Xrn1 by using a reporter based on RNA2 of brome mosaic virus (BMV) and compared RNA degradation in WT and *xrn1*Δ cells during osmostress and basal conditions. In agreement with a reduction of catalytic activity under osmostress conditions, WT cells exposed to osmostress showed a reduction in RNA2 degradation compared to non-stressed cells. However, degradation in WT cells was clearly higher than in *xrn1*Δ cells in both conditions (**Fig. 4A**). We then quantified Xrn1 protein abundance after 20 min of osmotic shock in WT cells and found that only around 45% of Xrn1 remained in the cytosol (**Fig. 4B**). Furthermore, we observed stress-induced RNA2 degradation in both WT and *xrn1*Δ cells, implicating the involvement of other degradation factors such as the exosome complex in regulating RNA levels upon osmostress. In summary, during hyperosmotic shock Xrn1 colocalizes with Dhh1 and forms aggregates, reducing the available amount of catalytically active enzyme in the cytosol. In summary, after hyperosmotic shock Xrn1 colocalizes with Dhh1 in stress

granules and this Xrn1 redistribution correlates with a reduction of Xrn1 catalytic activity.

Osmostress-induced Xrn1 aggregation is mediated by its disordered C-terminal domain

Protein aggregation is often mediated by intrinsically disordered regions that mediate protein-protein interactions (Uemura et al., 2018). Xrn1 harbors a disordered region in its C-terminus (Braun et al., 2012) that is crucial for its regulatory functions in transcription and translation (Blasco-Moreno et al., 2019). The nuclear paralog of Xrn1, Rat1, is structurally similar to Xrn1 but lacks this C-terminal region. When forced to localize in the cytoplasm by deleting its nuclear localization signal (NLS), Rat1ΔNLS functionally replaces the catalytic function. However, replacement of the Xrn1 translation function requires the fusion of the Xrn1 C-terminus to Rat1ΔNLS (Rat1ΔNLS-XC). To explore whether the Xrn1 C-terminus is also required for Xrn1 aggregation under stress conditions we expressed in *xrn1*Δ cells Rat1ΔNLS or Rat1ΔNLS-XC (**Fig. 5A-B**) and measured aggregation by following protein levels in WB analyses. While Rat1ΔNLS-XC levels follow similar patterns as those for Xrn1, no effect was observed on Rat1ΔNLS levels indicating that the C-terminus of Xrn1 mediates its aggregation. We further confirmed Rat1ΔNLS lack of aggregation by microscopy analyses (**Fig. S3**).

Xrn1 is strongly phosphorylated upon hyper-osmotic shock (Janschitz et al., 2019; Romanov et al., 2017). As the AMPK (5´AMP-activated protein kinase) Snf1 phosphorylates the same Xrn1 phosphosites under glucose deprivation (Braun et al., 2014), we next investigated whether knockouts of Snf1 or of the MAPK (mitogen-activated protein kinase) Hog1, a well-described kinase in osmostress, abrogated Xrn1 aggregation. While Hog1 did not show any involvement (**Fig. 5C**), *snf1*Δ reduced Xrn1 aggregation in WB experiments (**Fig. 5D**). Thus, Xrn1 aggregation is indeed mediated by phosphorylation of its C-terminal domain by Snf1.

A subset of osmo-responsive genes is Xrn1-dependent

To elucidate the functions of Xrn1 under high osmolarity conditions we combined our auxin-inducible degron system with RNA-sequencing and examined how Xrn1-KD affects RNA steady-state levels after 10 and 30 minutes of osmotic shock. By comparing the identified osmo-genes with a consensus of four publicly available datasets, we ensured that our degron cells exhibited a normal osmostress response (see Methods for details) (**Fig. 6A-B, S4A-B, Table S1**). We next analyzed the influence of Xrn1 knockdown on subsets of osmo-responsive genes. In the case of osmo-induced genes, 75 and 73 (6.96 % and 4.57 %) genes showed significantly reduced induction in Xrn1-KD cells at 10 and 30 minutes, respectively (**Fig. 6C-D**). For osmo-repressed genes, 61 and 177 (6.01 % and 10.66 %) genes were less repressed at 10 and 30 min, respectively. Independent qPCR-based experiments for selected genes confirmed these results, which indicated that Xrn1 plays a role both in transcription and degradation of a subset of osmo-responsive genes (**Fig. S4C-D**).

To clarify whether Xrn1 also acts in the translation of these genes, we calculated their translatability before and after stress both in WT and xrn1Δ cells based on WB and qPCR experiments. Indeed, Xrn1 depletion reduced the translatability of the selected genes in both non-stressed and stressed cells, yet the translational defects were bigger under osmotic stress conditions (**Fig. 7A-B, Fig S5**).

Xrn1 aids in transcription and translation of a subset of osmogenes

To further characterize the different gene subsets we examined significantly enriched functional terms (GO, KEGG, REACTOME). After 10 min of NaCl exposure, we found that Xrn1-dependent osmo-induced genes were mainly enriched in terms related to glycolysis and protein folding, while Xrn1-independent genes showed enrichment for various metabolic processes including glycolysis and TCA cycle as well as stress response terms, cell wall organization and MAPK signaling (**Table S2**). These functional enrichments were not preserved after 30 min of NaCl exposure, as Xrn1-dependent osmo-

induced genes then showed enrichment for sodium ion transport and salt stress response terms, while metabolism terms were not prominently featured. Similarly, Xrn1-independent genes at 30 min were related to catabolic processes such as the proteasome, carbohydrate metabolism and stress-response terms.

For osmo-repressed genes, at 10 min Xrn1 dependence was associated with terms related to cell cycle control, while Xrn1-independent genes were enriched for ribosome biogenesis, transcription of rRNAs and tRNAs by Pol I and Pol III and showed few cell cycle-related terms. In contrast, after 30 min both Xrn1-dependent and -independent genes showed enrichment for ribosome-related terms, with independent genes additionally being enriched for nonsense-mediated decay, cell cycle and various metabolism terms (**Table S3**).

Osmogenes targeted by Xrn1 are enriched for Hog1 interactors

These differences in functional enrichment hinted at specific subsets of genes being controlled by Xrn1, leading us to hypothesize that some physical properties could differ among the different groups of transcripts. Therefore, we compared Xrn1-dependent and -independent RNAs regarding their lengths, the lengths of their CDSs and UTRs and RNA structure as measured by PARS (Kertesz et al., 2010). Xrn1-dependent osmo-induced transcripts at 10 min featured significantly longer CDSs (1567.5 nt vs. 1156.5 nt) and 3' UTRs (158 nt vs. 124 nt), while the CDSs of osmo-repressed transcripts tended to be shorter than their Xrn1-independent counterparts (615 nt vs. 1194 nt; **Fig. S6, Table S4A**). Similarly, after 30 min the CDSs of repressed RNAs were shorter (612 nt vs. 1146 nt), as were their 5' UTRs (33 nt vs. 48 nt). Meanwhile, for osmo-induced Xrn1-dependent transcripts, only the CDSs were significantly longer (1528.5 nt vs. 1107 nt; **Fig. S7, Table S4B**).

Looking at PARS values, Xrn1-dependent transcripts that were induced after 10 min of NaCl were significantly more structured in their CDS and 3' UTR compared to Xrn1-independent transcripts (CDS: 0.589 vs 0.319; 3' UTR: 0.071 vs. 0.019; **Fig. S8, Table S4A**). Moreover, this higher structure could also be

observed before the translation initiation site (**Fig 8A**), while no tendency for higher structured RNAs could be seen at 30 min (**Fig. S9 and 8B, Table S4B**).

The features of Xrn1-dependent RNAs after 10 min of osmostress mimicked the ones we identified for translationally regulated RNAs (Blasco-Moreno et al., 2019). We therefore also tested for enrichment between our gene groups and genes whose regulation in transcription and degradation depend on Xrn1 (Medina et al., 2014), being the “synthegradon” group the most Xrn1-responsive transcripts that mainly include highly transcribed genes encoding ribosome biogenesis and translation factors, whereas the “anty-synthegradon” group contains the least responsive genes. At 10 min osmostress, the odds ratio to find members of the synthegradon among Xrn1-dependent osmo-induced genes was 3.731 ($p = 0.01192$), while no such enrichment could be seen after 30 mins (**Table S5**). In contrast, anti-synthegradon members were depleted from Xrn1-dependent osmo-induced genes at 10 min, but enriched in Xrn1-dependent osmo-repressed genes ($OR = 3.092$, $p = 0.05877$).

This dynamic behavior implicated an early regulatory involvement of Xrn1 during the osmostress response. Since transcription is largely dispensable at such short time frames (Mettetal et al., 2008; Westfall et al., 2008), it is possible that Xrn1 helps to organize cellular adaptation to persistent stress. Following this line of thought, we wondered whether known Hog1 interacting proteins were significantly over-represented among Xrn1-dependent osmo-induced genes. After 10 min Xrn1-dependent osmo-induced genes are significantly enriched for Hog1 interacting proteins ($OR = 1.969$, $p = 0.03897$), while at 30 mins Xrn1-dependent osmo-repressed genes showed a significant under-representation ($OR = 0.4942$, $p = 0.02017$; **Table S5**). In contrast, the two groups of Xrn1-dependent osmogenes did not show significant enrichment / depletion of known Snf1 interacting proteins, albeit similar trends could be observed (10 min induced: $OR = 1.749$, $p = 0.06937$; 30 min repressed: $OR = 0.6949$, $p = 0.1491$).

In summary, during the early hyper-osmotic shock response, Xrn1 induces the expression of a subset of osmogenes enriched for Hog1 interacting proteins.

These genes are involved in diverse cellular functions but display properties common to RNAs that are translationally up-regulated by Xrn1.

Discussion

Suboptimal growth conditions like high osmolarity require the adaptation of gene expression dynamics to ensure cell survival. In this study we investigate the role of Xrn1, a master regulator of RNA fate, during the yeast cell osmostress response. Unexpectedly, we identified Xrn1 as a modulator of transcriptional and translational responses upon hyper-osmotic shock. In addition, a fraction of Xrn1 is sequestered in stress-induced aggregates and colocalizes with Dhh1, leading to a diminished Xrn1 exonuclease activity. While stress granule formation of Xrn1 has been reported after heat shock (Grousl et al., 2015), this is the first time such aggregation has been found after hyper-osmotic shock.

Xrn1 colocalizes with Dhh1 after hyper-osmotic shock yet both proteins show differing dynamics, as Dhh1 localizes to foci earlier than Xrn1 while Xrn1 is retained longer. Besides, Xrn1 but not Dhh1 showed reduced protein levels in our western blot analysis, suggesting that Dhh1 remained more accessible to the used lysis solvents than Xrn1. In conjunction with the reported dependence of aggregate formation on Dhh1 ATPase activity (Mugler et al., 2016), these findings could indicate Xrn1 as a possible target of Dhh1-directed sequestration by aggregate formation after osmostress. Moreover, as we observed a faster Dhh1 aggregation, Xrn1 might be recruited to these aggregates once they have formed due to Dhh1 activity.

Interestingly, Dhh1 mediates P-body formation during glucose starvation (Mugler et al., 2016), while such conditions reportedly lead to Xrn1 accumulation at plasma membrane-associated eisosomes (Grousl et al., 2015). Since Xrn1 does not exhibit this plasma membrane association in osmostress, differential regulatory mechanisms that share key coordinating factors might be at play. Such a condition-specific response would not be surprising, as glucose depletion is a gradual process, whereas hyper-osmotic shock is an immediate environmental stress that requires rapid adaptation for cell survival. Thus an active sequestration of Xrn1 could be necessary to avoid detrimental effects

caused by concentrating intracellular agents due to the osmostress-induced water efflux (Petelenz-Kurdziel et al., 2011)

Remarkably, the chimeric fusion Rat1ΔNLS-XC, that bears the C-terminus of Xrn1, but not Rat1ΔNLS showed stress-induced sequestering upon osmotic shock, further suggesting that the disordered C-terminal region of Xrn1 is key for Xrn1 aggregation. We therefore focused on putative regulatory sites that could be targeted by MAP or other stress-activated kinases and found that Xrn1 relocalization was dependent on Snf1 presence, suggesting an active regulation of Xrn1 sequestering in stress conditions. Moreover, two residues located in the C-terminal domain that were identified to be phosphorylated upon hyper-osmotic shock (Janschitz et al., 2019; Romanov et al., 2018) were also found to be phosphorylated by Snf1 during glucose starvation (Braun et al., 2014). Based on this evidence, the suppression of Xrn1 aggregation in *snf1*Δ cells further strengthens a connection between the C-terminus of Xrn1, its sequestration upon stress and a regulatory role of stress-activated kinases. Our findings thus not only point to a more general mechanism of stress adaptation involving Xrn1, but also towards a broader role of Snf1 in coordinating gene expression and mRNA turnover during diverse cellular stress responses beyond glucose starvation.

Interestingly, shortly after NaCl exposure Xrn1 controls a subset of osmostress genes both transcriptionally and translationally. These genes feature properties akin to Xrn1-dependent target genes for translation as previously reported by us (Blasco-Moreno et al., 2019), such as a higher structural region situated upstream of the translation initiation site. It is thus likely that mRNA secondary structures confer Xrn1 dependence for this subset of genes as previously described (Blasco-Moreno et al., 2019). As these genes were enriched for Hog1 interacting proteins, they could resemble a long-term stress adaptation strategy allowing a rapid regulation of stress genes upon sequential encounters of hyper-osmotic conditions. In line with this thought, the enrichment seen at 10 minutes after stress is mostly lost after prolonged exposure (30 min), whereas the amount of accessible Xrn1 remained at comparable levels. Furthermore, the

translocation of Hog1 to the nucleus has been shown to be dispensable for an early osmostress response, yet it led to decreased viability upon repeated osmotic shocks (Westfall et al., 2008). Conjointly, these findings reinforce the hypothesis that metabolic adaptation is one of the major protectors of yeast cell survival when encountering a hyper-osmotic shock, while the transcriptional and translational response serve as the basis for adaptation to repeated or long-term stress conditions. Moreover, our results indicate a previously unknown crosstalk between the Snf1 and Hog1 pathways upon hyper-osmotic shock.

Methods

Cell culture and degron system

Yeast cells were grown in YAPD medium at 30ºC on a rotary shaker. Cultures of yeast cells transformed with plasmids were grown in synthetic selective medium. Glucose (2%) was used as a carbon source unless indicated otherwise. Cells were grown from solid media to liquid media during the day and were diluted to grow overnight. The following day they were diluted and grown until they reached similar doubling times and an OD_{600} of 0.5-0.6. At this point, 0.4 M NaCl dissolved in media was added to growing cells. Cells were harvested at the indicated time points by centrifugation. For big volume experiments, cells were harvested with a very fast vacuum filtration system.

In order to transiently deplete endogenous Xrn1 from yeast cells, we used a yeast strain in which *TIR1* gene was integrated and Xrn1 was fused to an auxin-inducible degron (AID) (Nishimura and Kanemaki, 2014). This strain enables the quick degradation of Xrn1 upon auxin addition to the media through a protein degradation pathway typical of plants (Nishimura and Kanemaki, 2014). When AID-tagged yeast cells reached OD_{600} of 0.5-0.6, 500 µM auxin was added to the media for 30 min. Xrn1-KD cells were then treated with 0.4 M NaCl as mentioned before. Yeast strains and plasmids are listed in Table S6.

Cloning of Xrn1 derivative plasmids

The plasmids used in this study were generated as described in (Blasco-Moreno et al., 2019). The experiments performed with Rat1ΔNLS-FLAG

plasmid refer to pLCM6 from (Blasco-Moreno et al., 2019). Rat1ΔNLS-XC-FLAG chimera (pLCM9) was generated by fusing the N-terminal domain (1–884 aminoacids) of Xrn1 to the C-terminal domain (733–1528 aminoacids) of Rat1ΔNLS. Also, we depleted a loop in the CDS of Rat1ΔNLS (Δ24–42 amino acids) because it was hampering the interaction between the N-terminal and the C-terminal domains of the chimera (Blasco-Moreno et al., 2019). All the plasmids used in this study are listed in Table S6.

Knockout strains generation

The knockout strains for translation experiments were generated in BY4741 cells. The selected gene for depletion was replaced by URA by homologous recombination. First, the URA sequence was amplified from a plasmid by PCR (with KOD Hot Start DNA polymerase from Millipore) with primers that overlapped with upstream and downstream sequences of the selected gene. Next, the amplified sequence was transformed into WT BY4741 cells and the cells were grown in -URA selective media to select for positive homologous recombination. The correct replacement of URA was verified by PCR.

Protein expression levels measurement by Western blot

To check for protein expression levels, cells were grown and harvested as specified in previous section. Two total OD (Optical Density) units were harvested by centrifugation. The pellet was resuspended in lysis buffer supplemented with protein inhibitors. Then the cells were lysed with equivalent amount of glass beads by two rounds of two minutes vortexing. After, 2% SDS buffer was added to each sample and the sample was heated to 95ºC for 10 min. Lastly, samples were loaded on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and were separated according to their molecular weight. Next, samples were immunoblotted into a nitrocellulose membrane for 90 min at 100 V on ice, as previously described (Ishikawa et al., 1997). Antibodies against GFP (Clontech), FLAG (Sigma-Aldrich), PGK (Invitrogen), Xrn1 (gift from Arlen Johnson), Dhh1 (Santa Cruz Biotechnology) and BMV 2a (Noueiry and Ahlquist, 2003) were used. Detection of proteins was performed with an Amersham Imager 600.

Proteasome inhibition by MG132 drug

BY4741 yeast cells lacking *PDR5* gene (a gift from Francesc Posas lab) were grown until they reached an OD_{600} of 0.5. Half of the culture was treated with 50 µM MG132 for 1 h while the other half was left growing untreated. Both cultures were harvested after 1 h (t=0) and then were shocked with 0,4 M Nacl for 10 min. Protein expression levels were measured as described in previous section.

Immunoprecipitation for ubiquitin detection

BY4741 WT yeast cells and cells carrying a genomic GFP-tag fusion of Xrn1 were grown (1200 ml culture) in exponential phase until an OD_{600} of 0.5 was reached. Then, 400 ml of unstressed culture was harvested by vacuum filtration and the remaining culture (800 ml) was treated with 0.4 M NaCl and harvested after 5 and 10 min time-points (400 ml culture harvested for each time-point). Cells were lysed with the Freezer/Mill (SPEX SamplePrep) with two cycles of 2 min at 5 cps with a 2 min cooling-down step in between. Half volume of the lysed sample was dissolved in 500 µl lysis buffer (50 mM Tris-HCl pH = 7.5, 150 mM NaCl, 0.1% NP-40, 1 mM EDTA, 5 mM *N*-Ethylmaleimide (NEM) and protease inhibitors), then it was incubated for 5 min at 4ºC in a rotating mixer and lastly centrifuged at 16100 x g for 5 min at 4ºC. After recovering the soluble fraction, total protein amount was measured by Pierce TM BCA Protein Assay Kit (ThermoFisher). As a control for the input sample, 150 µg of total protein were kept. For the immunoprecipitation, 3.5 mg of total protein were used. Protein extracts were then incubated with 15 µl of GFP-trap_A beads (Chromotek) for 1 h at 4 °C shaking in a rotating mixer. Three washes with 500 µl of wash buffer (10 mM Tris-HCl pH = 7.5, 200mM NaCl, 0.5 mM EDTA, 1 mM PMSF, 5mM NEM, Protease inhibitor Cocktail) were performed and beads were pelleted in-between by centrifugation (2500 × g, 2 min, 4 °C). Beads were resuspended in 20 µl of wash buffer and 10 µl of 3x loading dye were added. Samples were eluted from the beads by boiling at 95 °C for 5 min. Lastly, samples were loaded on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and were separated according to their molecular weight. Next, samples were immunoblotted into a nitrocellulose membrane for 90 min at 100 V on ice, as previously described (Ishikawa et al., 1997). After

immunoblotting the membrane was washed with 0.5% glutaraldehyde for 15 min to link the proteins to the membrane. Antibodies against Ubiquitin (gift from Francesc Posas), Xrn1 (gift from Arlen Johnson) and PGK (Invitrogen) were used. Detection of proteins was performed with an Amersham Imager 600.

Microscopy experiments

Prior to use, chambers (Lab-TEK chambered Coverglass) were washed with 100% ethanol and coated for an hour with 1 mg/ml sterile filtered Concanavalin A. Coated slides were then washed with distilled water and dried. Yeast cells were seeded in YAPD to the chambers at an OD_{660} of 0.2 (400 µl/well) and were allowed to settle for 30 min. Non-attached cells were washed away with low fluorescence media and cells were allowed to adapt for 30 more minutes before imaging. Stressed samples were treated with NaCl dissolved in low fluorescence media in a final concentration of 0.4 M NaCl. Cells were imaged with a Nikon Eclipse T*i* microscope and Hamamatsu orcaR2 digital camera at 100x magnification using mCherry-LAMP and GFP-LAMP channels for the indicated time points. The software used for imaging was NIS Element AR - Nikon. Last, images were analyzed using ImageJ (FIJI) program.

BMV RNA2 degradation assay

To evaluate BMV RNA2 degradation rates under osmotic stress conditions, WT and *xrn1*Δ cells were transformed with 2a plasmid and were grown in selective media supplemented with 2% galactose. When cells reached an OD_{600} of 0.5, 3 OD units were harvested by centrifugation (16100 x g, room temperature, 30 sec) and frozen directly in liquid nitrogen (time point=0). The rest of the culture was centrifuged twice and the media was changed to pre-warmed selective media supplemented with 2% glucose, in order to stop transcription of BMV RNA2. Cells were left growing in glucose for 5 min on a rotary shaker. Next, 0.4 M NaCl dissolved in media was added to growing cells. Cells corresponding to three OD units were harvested after 20 min and 60 min of glucose addition and immediately frozen. Total RNA was extracted by a hot-phenol method, sample concentration was measured by a Nanodrop device and 100 ng/µl dilutions were prepared for every sample. Then 100 ng RNA was reverse transcribed

into first-strand cDNA with SuperScript III Reverse Transcriptase (Invitrogen). Lastly, BMV RNA2 levels were quantified by quantitative PCR using the Power Sybr Green PCR Master Mix (Applied Biosystems).

RNA-sequencing

Xrn1-AID cells were grown until they reached OD_{600} of 0.5. At this point, half of the culture was treated with 500 µM auxin for 30 min (Xrn1-KD cells), while the other half continued growing (WT cells). Then, time-point 0 was harvested by rapid vacuum filtration system. Next, 0.4 M NaCl dissolved in media was added to the culture and the cells were harvested at 10 and 30 min time-points. The samples were treated with Proteinase K (New England Biolabs) to get rid of the proteins, and then total RNA was extracted by a phenol-chloropropane method. After, remaining DNA was digested by Turbo DNase (Ambion) and RNA was extracted again with acid phenol-chloroform method. RNA concentration was measured by Nanodrop The libraries were generated with 2.8 µg of the RNA sample by the TrueSeq Stranded mRNA Sample kit (Illumina). The libraries were sequenced by HiSeq 2500 System (Illumina) in reads of 50 base-pair length (1x50).

Data analysis

RNA-sequencing reads were mapped to the sacCer3 genome build using TopHat2 (v2.1.0, settings: “--max-multihits 1 --library-type fr-firststrand --b2-very-sensitive --no-coverage-search”) and quantified using featureCounts (v1.5.1, “-Q 1 -s 2”) (Kim et al., 2013; Liao et al., 2014). Differential expression testing was performed using an interaction term in DESeq2 (v1.22.2) to discern the contribution of Xrn1 in the transcriptional response to osmotic stress (Love et al., 2014). We used the gProfileR package (v0.6.7) for functional enrichment and ggplot2 (v3.1.1) for visualizations (Raudvere et al., 2019; Reimand et al., 2007; Wickham, 2016). RNA structures were estimated using published PARS (parallel analysis of RNA structure) values (Kertesz et al., 2010). To define consensus osmo-stress genes, we used three available RNA-seq datasets (GSE80512 (Studer et al., 2016), GSE98352 (Silva et al., 2017) and GSE130549-unreleased, personal communication) allowing comparisons of

stressed and unstressed cells (0.4 M NaCl, either 10 or 15 min exposure). Consensus genes were defined as genes exceeding absolute $\log_2$ fold changes greater than 1 in at least three independent comparisons (same fold change direction) when testing for differential expression using DESeq2 (lfcThreshold = 1, padj < 0.05). Lists of Hog1 and Snf1 interacting proteins were retrieved from www.yeastgenome.org (date of access: 07.09.2019) and interacting genes marked as “Physical” were used for enrichment testing using Fisher’s exact test. Unless otherwise stated a Wilcoxon-Mann-Whitney test was employed to identify statistically significant differences in features.

Validation of RNA-seq by quantitative PCR

In order to confirm the transcriptome results by qPCR, the genes were first selected for validation according to their (i) RNA overexpression levels after osmotic stress induction and (ii) Xrn1-dependence for RNA overexpression. Then, specific primers were designed by Primer Express Software v3.0.1 (Thermo Fisher Scientific) to detect selected genes. Yeast cells were grown and three OD units were harvested as described in Cell Culture and Degron System section. Total RNA was extracted by a hot-phenol method, sample concentration was measured by Nanodrop and 100 ng RNA was reverse transcribed into first-strand cDNA with SuperScript III Reverse Transcriptase (Invitrogen). Lastly, the RNA levels of the selected genes were quantified by quantitative PCR using the Power Sybr Green PCR Master Mix (Applied Biosystems).

Translation assay by WB analysis

To evaluate the translation of genes that had been validated by quantitative PCR, we selected the genes according to their GFP-tagged strain availability in the Yeast GFP Clone Collection (the strains used in this study were a gift from Francesc Posas and Oriol Gallego). Once selected, knockout strains of each gene were generated as described in the Knockout Strains Generation section. GFP-tagged and delta strains were grown and two OD units were harvested for protein extraction and three OD units for total RNA extraction. Total protein protein was extracted from equivalent number of cells as described in the

Protein Expression Levels Measurement by Western Blot section. Antibodies against GFP (Clontech) and PGK (Invitrogen) were used. Proteins were detected with an Amersham Imager 600. Total RNA from yeast cells was isolated as described in Validation of RNA-seq by quantitative PCR section. Translation was calculated by the comparison between protein expression levels measured by western blot and RNA expression levels measured by quantitative PCR.

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Figure 1:

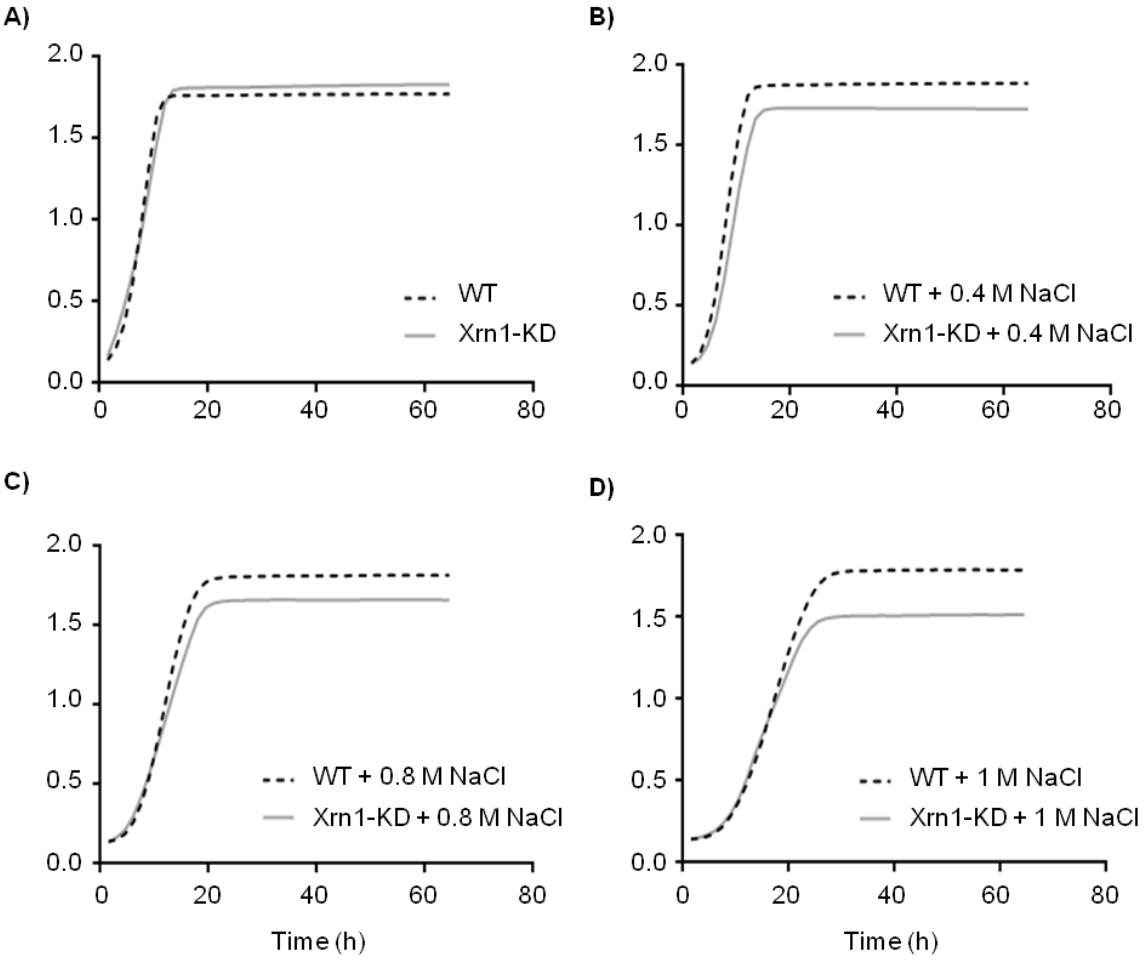

Figure 1. Xrn1 knockdown confers sensitivity to NaCl uptake in a dose-dependent manner. Representative examples of growth curves at 30ºC for WT and auxin-induced Xrn1-KD without stress and at 0.4, 0.8 and 1 M NaCl.

Figure 2:

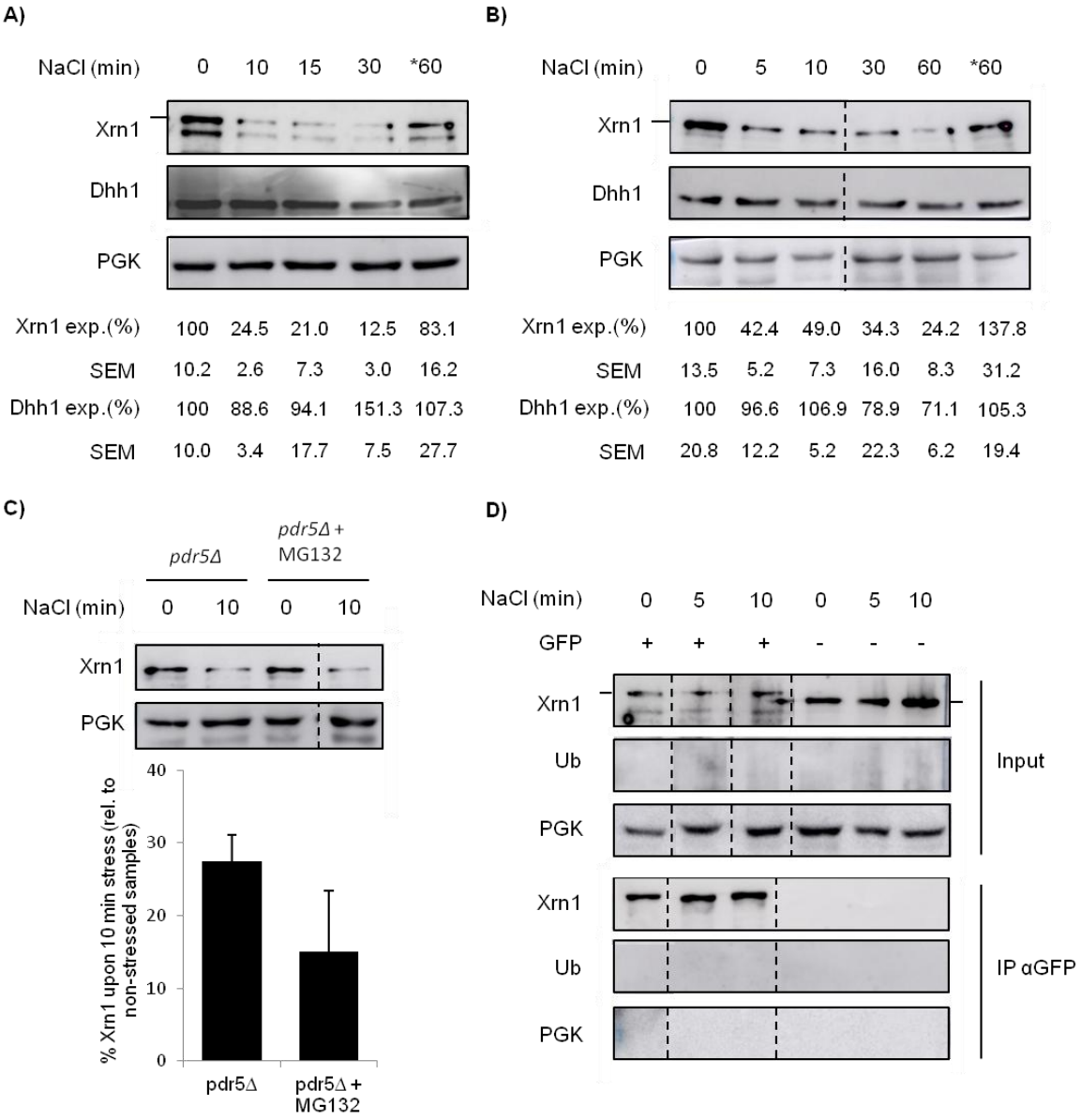

Figure 2. Xrn1 physical state changes upon NaCl addition. **(a, b)** Xrn1 but not Dhh1 protein levels appear to decrease upon NaCl uptake. **(a)** Western blot assay of Xrn1-AID cells not treated with auxin upon 0.4 M NaCl addition. **(b)** Western blot assay of BY4741 WT cells upon 0.4 M NaCl addition. *60 refers to 60 min recovery with media without Nacl **(c, d)** Xrn1 is not degraded by proteasome pathway upon NaCl uptake. **(c)** Western blot assay of *pdr5*Δ cells +/- MG132 drug upon 0.4 M NaCl uptake. (d) Western blot analysis of immunoprecipitation assay. A yeast strain expressing Xrn1-GFP fusion was grown. As a negative control, BY4741 WT cells lacking any GFP tag were used. Immunoprecipitations were carried out with GFP-trap beads. Results represent averages of n=3 biological replicates.

Figure 3:

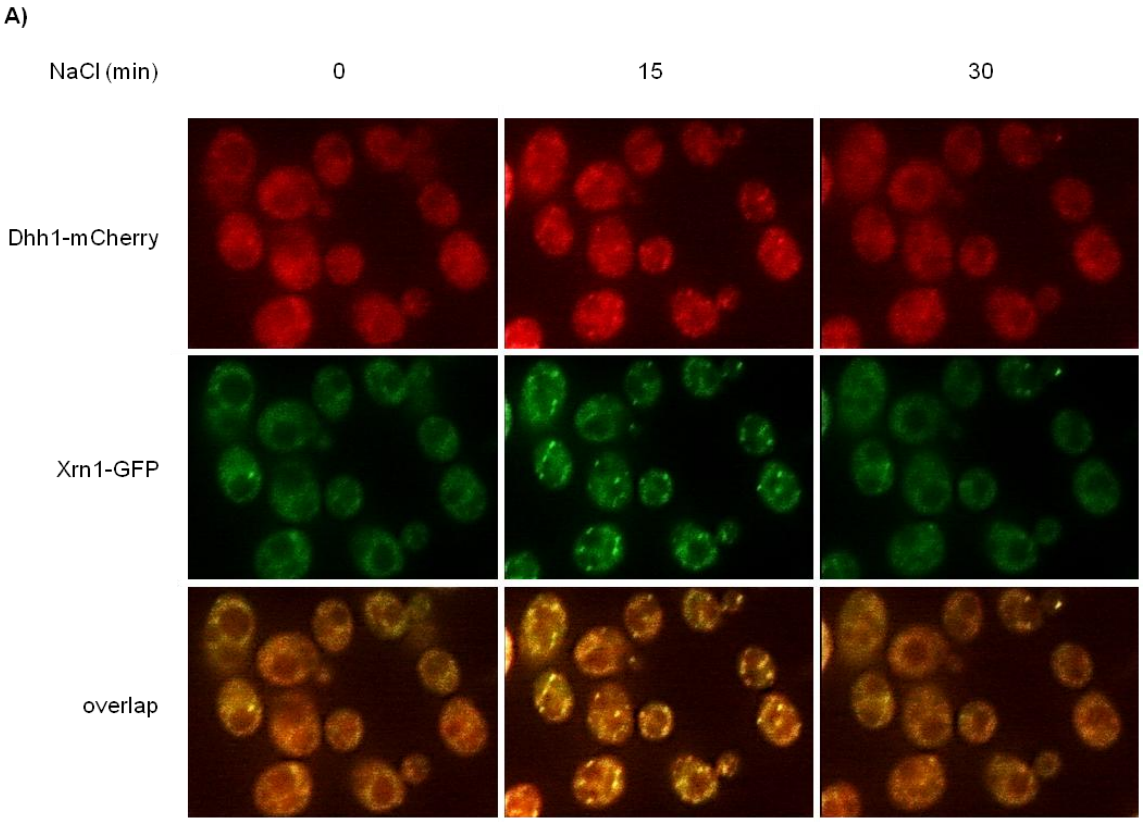

Figure 3. Xrn1 and Dhh1 colocalize in distinct foci upon hyper-osmotic shock. Yeast cells expressing Xrn1-GFP and Dhh1-mCherry fusion proteins were grown and their localization was assessed by microscopy upon 0.4 M NaCl addition. Cells were imaged at a 100 x magnification using mCherry-LAMP and GFP-LAMP channel.

Figure 4:

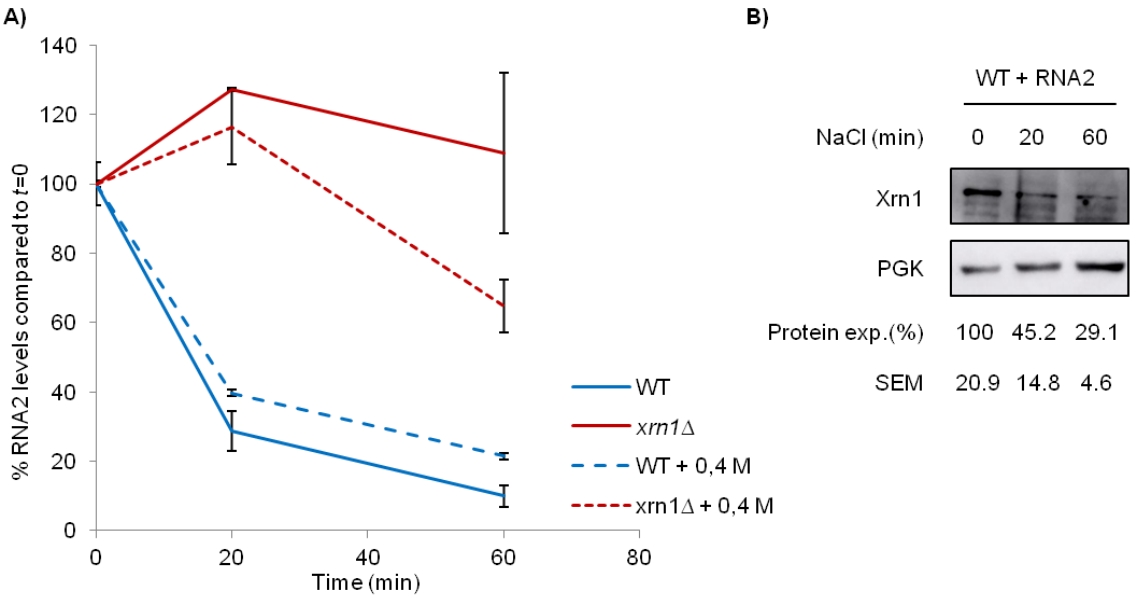

Figure 4. Xrn1 relocalization during hyper-osmotic shock reduces the catalytic activity of Xrn1. BY4741 WT and *xrn1*Δ cells expressing BMV RNA2 under *GAL1* promoter were grown in galactose. Next, transcription of RNA2 was shut-off upon glucose addition and BMV RNA2 stability was determined by monitoring RNA2 levels by quantitative PCR at 20 min and 60 min time-points post-glucose addition **(a)**. **(b)** Xrn1 protein expression levels from the same experiment were measured by Western blot assay. Results represent averages of n=3 biological replicates.

Figure 5:

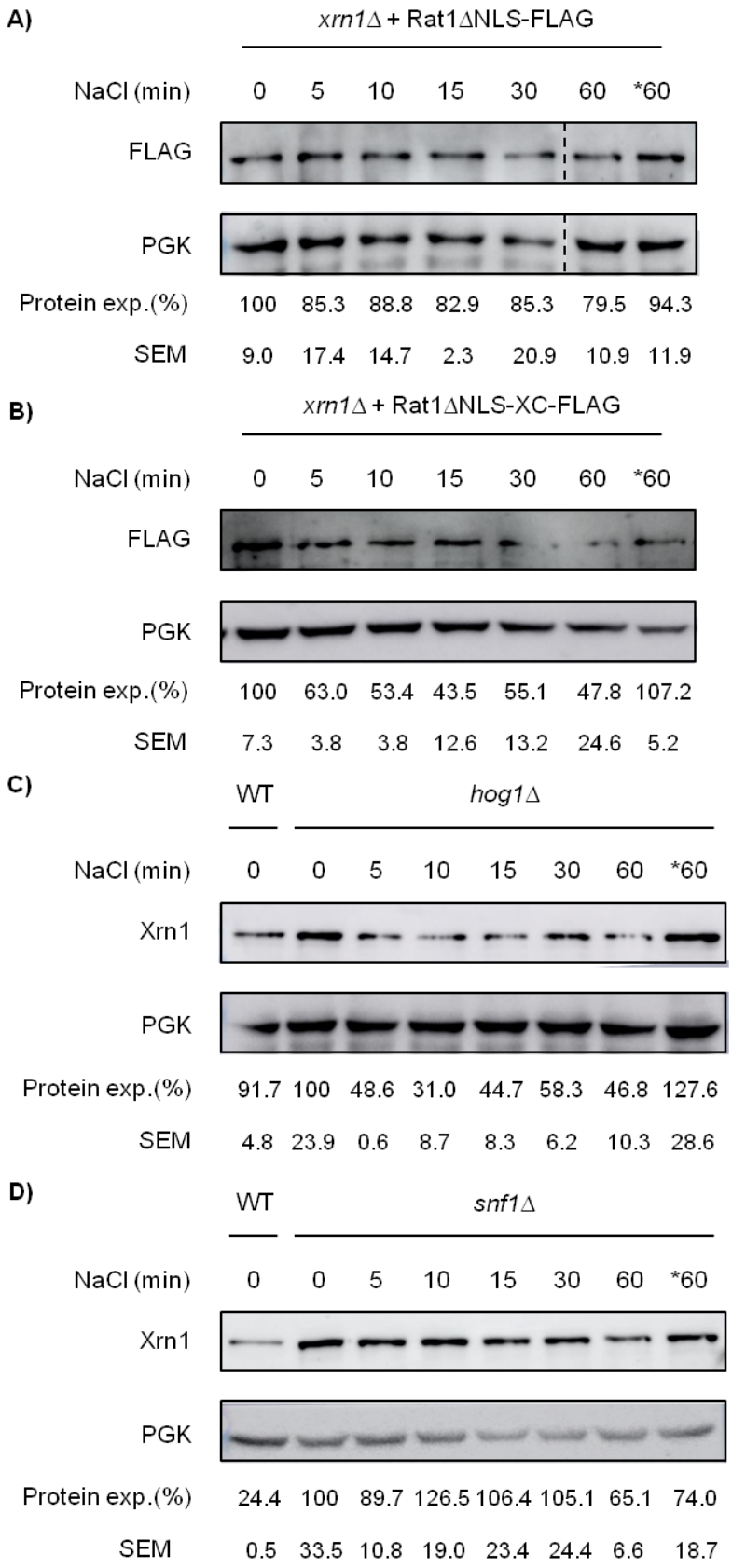
A)
xrn1Δ + Rat1ΔNLS-FLAG
NaCl (min) 0 5 10 15 30 60 *60
FLAG
PGK
Protein exp.(%) 100 85.3 88.8 82.9 85.3 79.5 94.3
SEM 9.0 17.4 14.7 2.3 20.9 10.9 11.9
B)
xrn1Δ + Rat1ΔNLS-XC-FLAG
NaCl (min) 0 5 10 15 30 60 *60
FLAG
PGK
Protein exp.(%) 100 63.0 53.4 43.5 55.1 47.8 107.2
SEM 7.3 3.8 3.8 12.6 13.2 24.6 5.2
C)
WT
hog1Δ
NaCl (min) 0 0 5 10 15 30 60 *60
Xrn1
PGK
Protein exp.(%) 91.7 100 48.6 31.0 44.7 58.3 46.8 127.6
SEM 4.8 23.9 0.6 8.7 8.3 6.2 10.3 28.6
D)
WT
snf1Δ
NaCl (min) 0 0 5 10 15 30 60 *60
Xrn1
PGK
Protein exp.(%) 24.4 100 89.7 126.5 106.4 105.1 65.1 74.0
SEM 0.5 33.5 10.8 19.0 23.4 24.4 6.6 18.7

Figure 5. Xrn1 aggregation depends on the presence of its C-terminal and is Snf1-mediated. Protein expression levels were measured by Western blot assay in BY4741 *xrn1*Δ cells expressing Rat1ΔNLS **(a)** and Rat1ΔNLS-XC **(b)** plasmids. Xrn1 protein expression levels were measured by Western blot assay in BY4741 *hog1*Δ **(c)** and *Snf1*Δ **(d)** cells. *60 refers to 60 min recovery with media without Nacl. Results represent averages of n=3 biological replicates.

Figure 6:

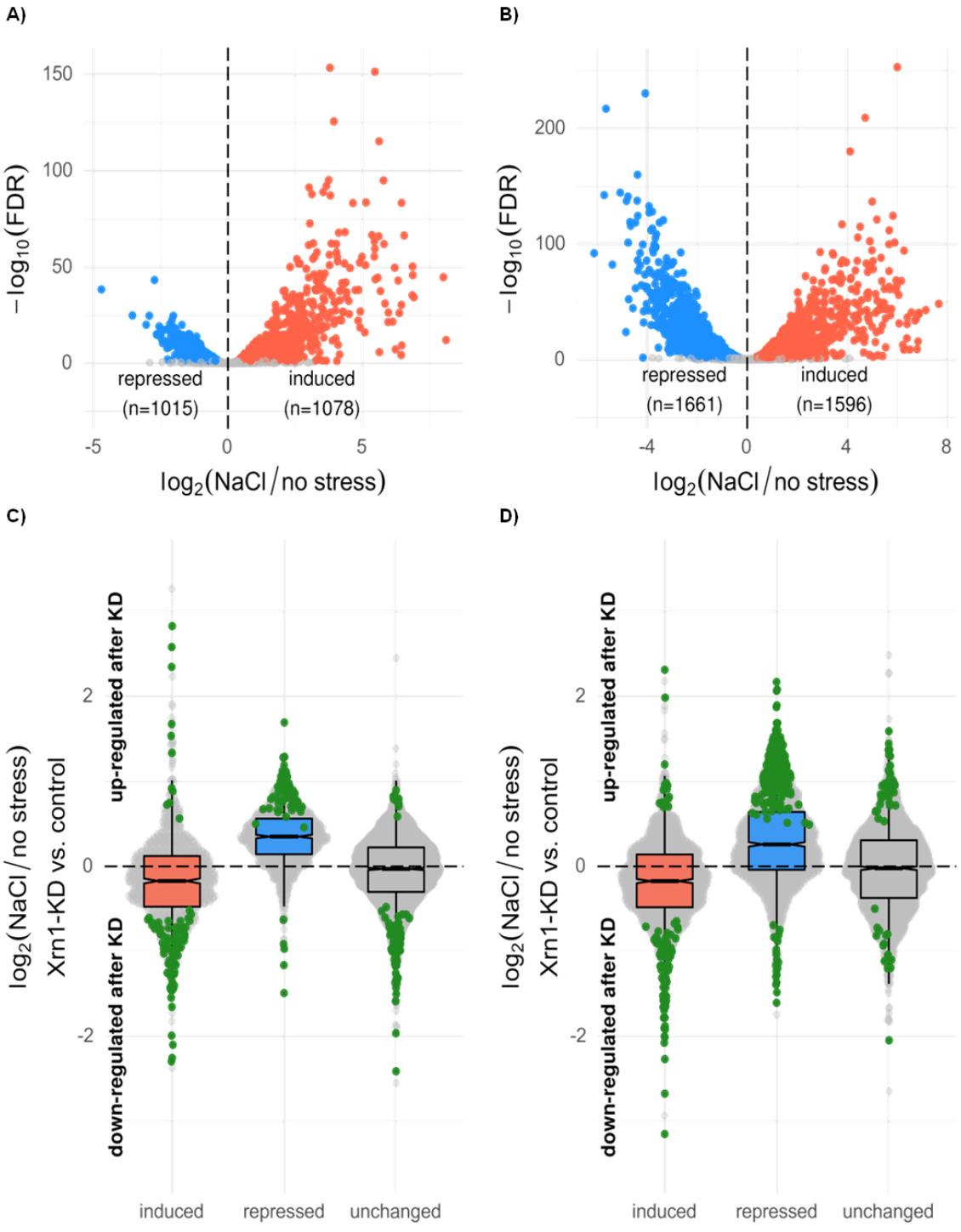

Figure 6: Visualization of RNA-seq results. Volcano plots for induced (red) and repressed (blue) genes after **(a)** 10 min and **(b)** 30 min of osmostress. Violin plots for **(c)** 10 min and **(d)** 30 min represent the effect of Xrn1-KD on gene behavior depending on their osmostress response in

WT cells. Genes with significant differences between Xrn1-KD and WT cells are marked with green dots, all other genes are marked with grey dots.

Figure 7:

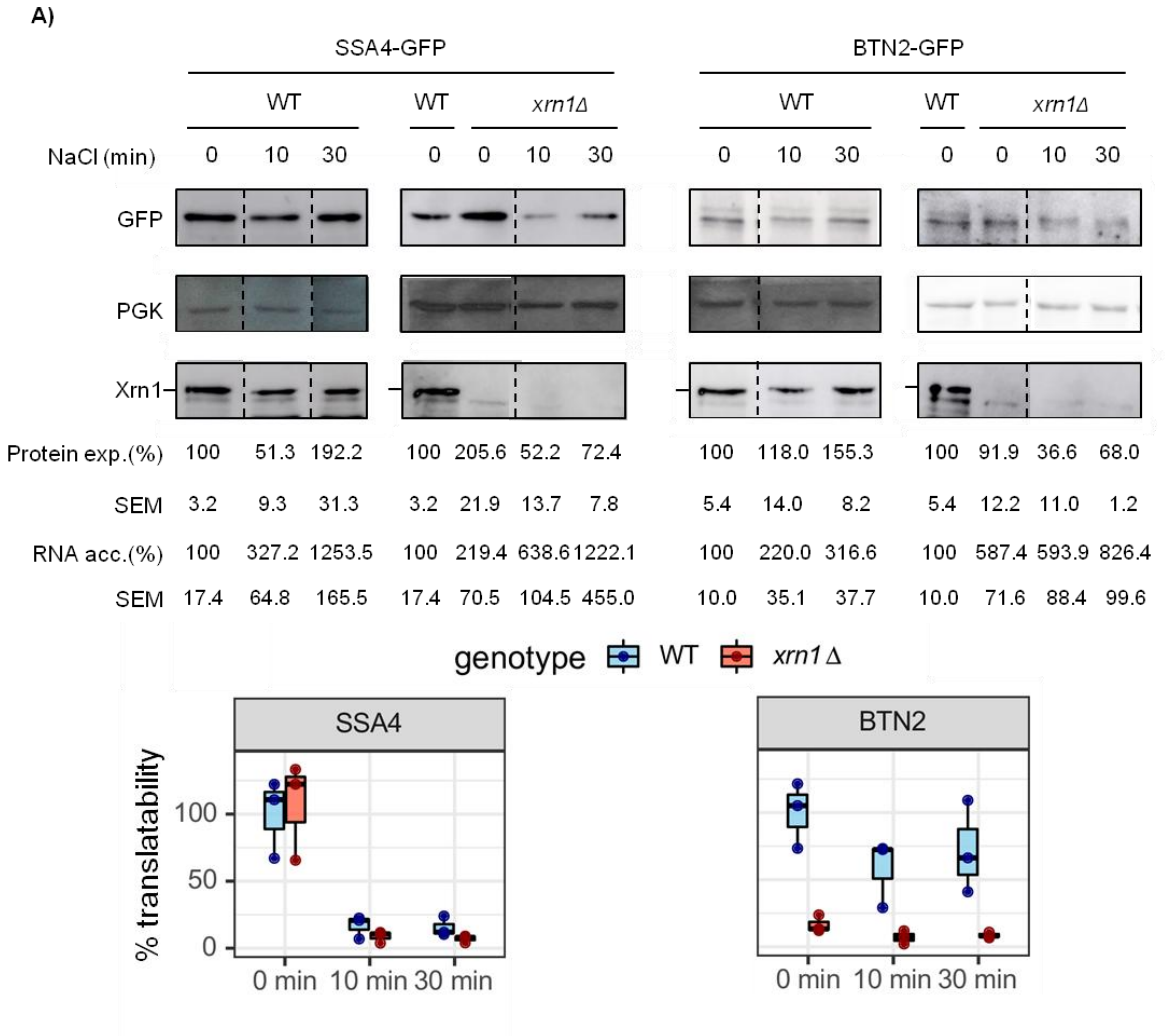

Figure 7. Role of Xrn1 on mRNA translatability under osmotress. BY4741 yeast cells expressing SSA4-GFP or BTN2-GFP fusion proteins were grown. Protein expression levels were measured by Western blot and detected by anti-GFP antibody (upper pannel) and RNA levels were measured by quantitative PCR. Translatability (bottom pannel) was calculated by changes in protein levels divided by changes in RNA levels. Boxplots of mRNA translatability in WT (blue) or xrn1Δ (red) cells across all queried time points, with individual replicates indiciated as dots. Results represent averages of n=3 biological replicates.

Figure 8:

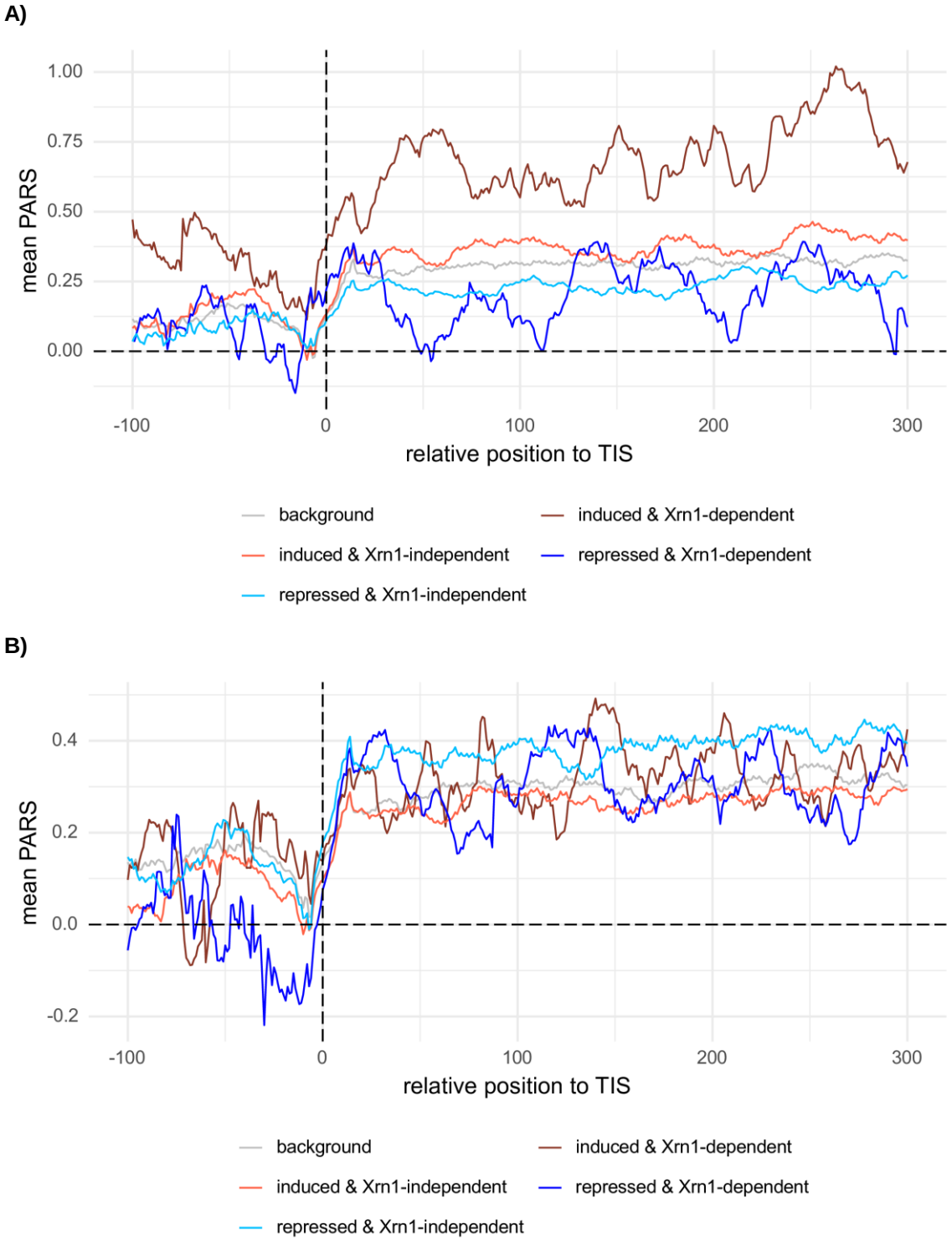

Figure 8: Average RNA structure around the translation initiation site (TIS) across different groups of transcripts as measured by PARS [PARS citation] at **(a)** 10 min and **(b)** 30 min of osmostress. PARS profiles were smoothed by calculating the mean PARS across a sliding

window of ±5 nt for each nucleotide position prior to averaging PARS among different groups. For visualization, nucleotide positions were converted to relative distances to the TIS.

Figure S1:

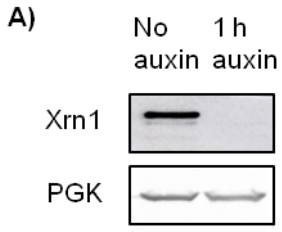

Figure S1. Xrn1 is efficiently depleted upon auxin addition. Xrn1-AID cells were grown and treated with 500 µM auxin for 1 h. Xrn1 protein expression levels were measured by Western blot assay

Figure S2:

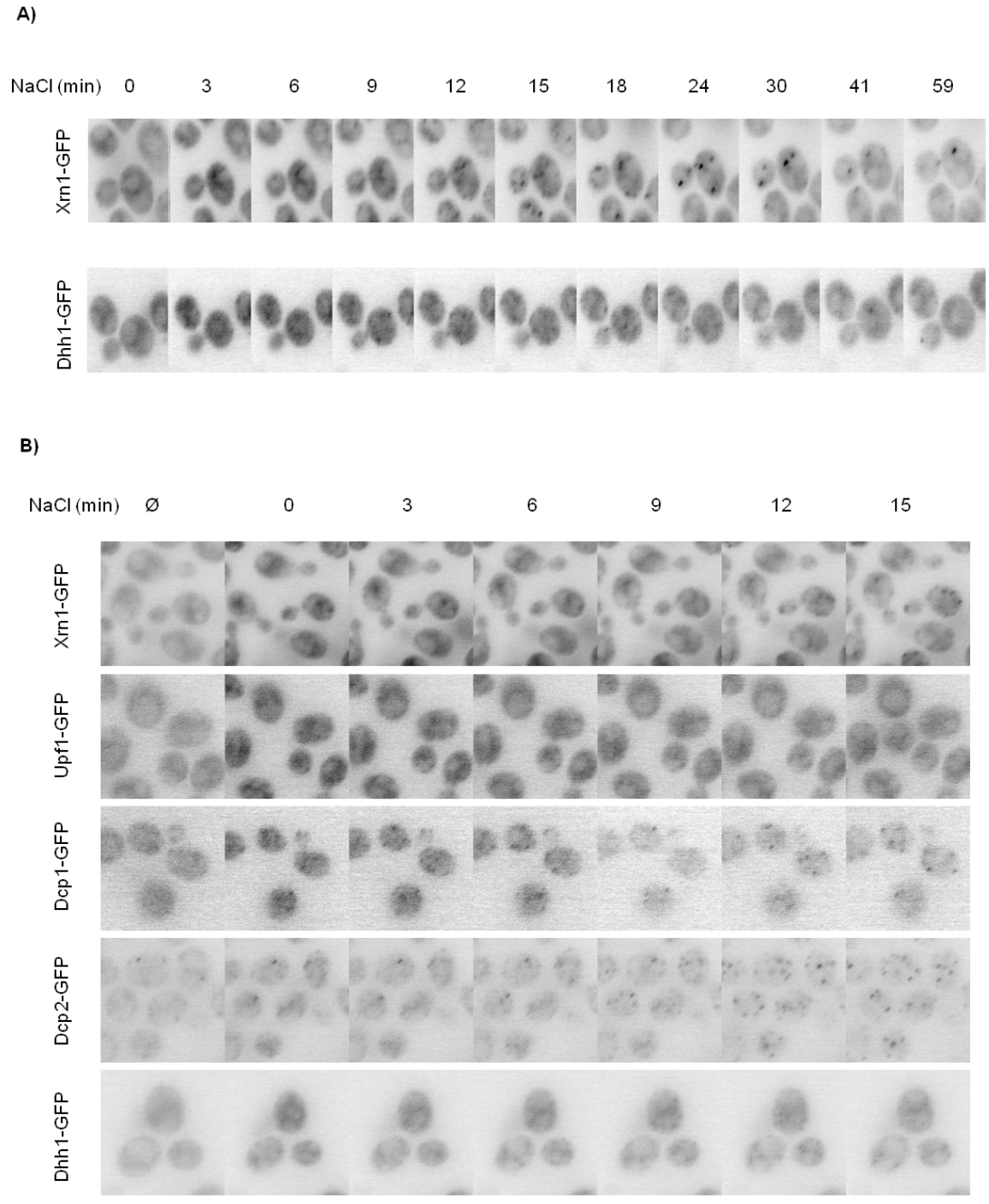

Figure S2. Localization of Xrn1 and known components of P-bodies and stress granules upon hyper-osmotic shock. **(a)** Xrn1 and Dhh1 localization and dynamics. Yeast cells expressing Xrn1-GFP or Dhh1-GP fusion proteins were grown and their localization was assessed by microscopy upon 0.4 M NaCl uptake. **(b)** Localization of GFP-tagged proteins were grown and their localization was assessed by microscopy.

Figure S3:

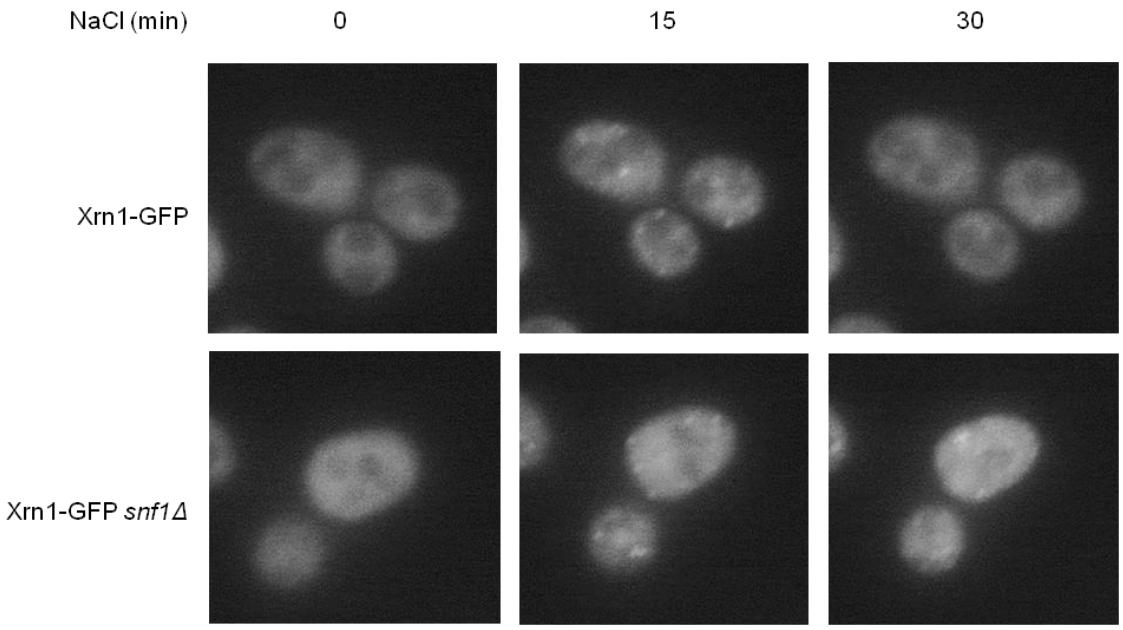

Figure S3: Rat1ΔNLS doesn't localize to stress-induced aggregates. XRN1-GFP WT and snf1Δ cells were grown and their localization was assessed by microscopy upon 0.4 M NaCl addition. Cells were imaged at a 100 x magnification using mCherry-LAMP and GFP-LAMP channel.

Figure S4:

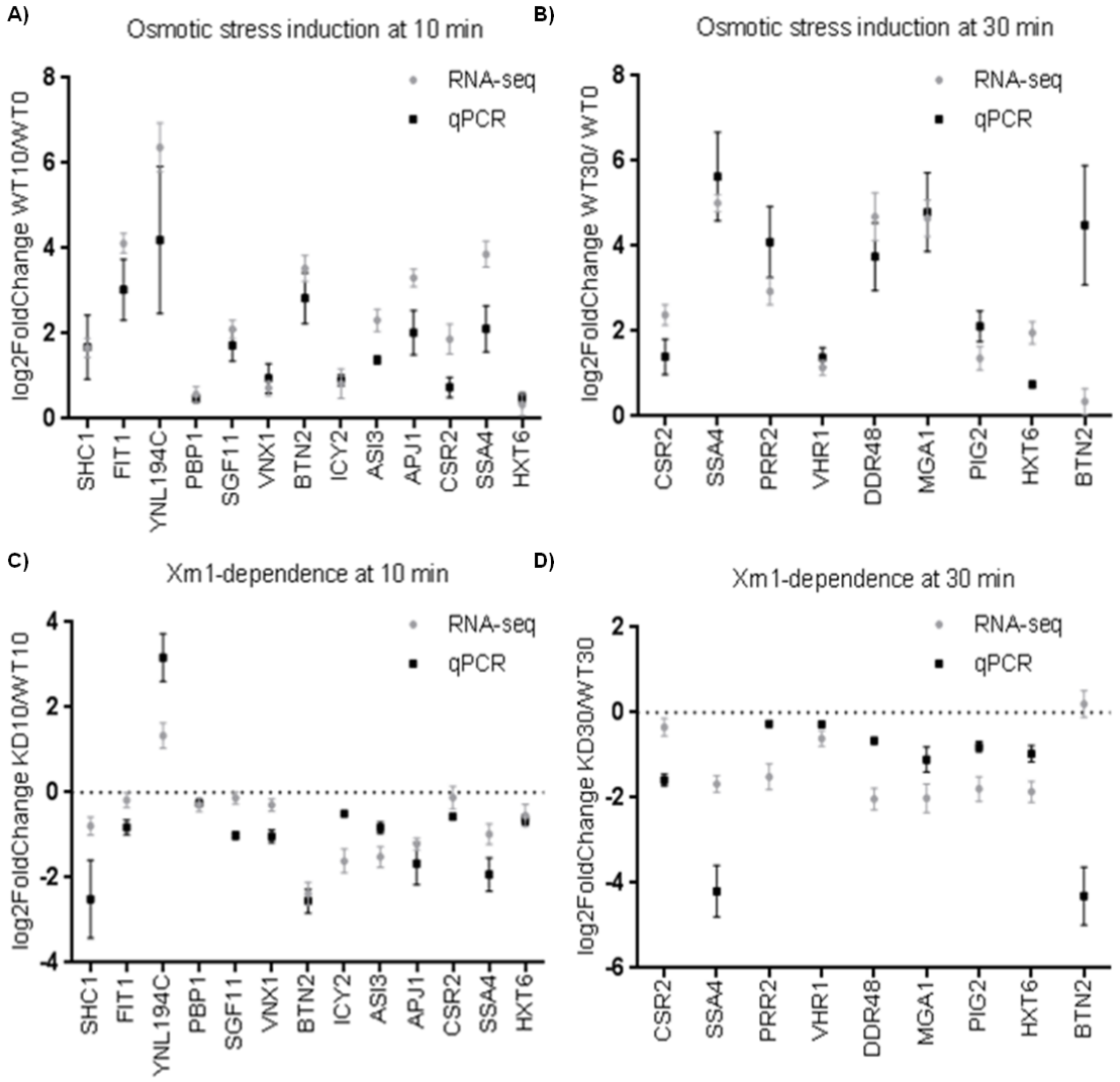

Figure S4. Validation of Xrn1-dependent osmo-induced genes. Xrn1-AID cells were grown in +/- auxin conditions and treated with 0.4 M NaCl for 10 and 30 min. RNA values of several genes were measured by quantitative PCR and represented together with the corresponding RNA values obtained by RNA-seq. **(a, b)** Osmotic stress induction was measured by comparing the changes in RNA levels after NaCl induction (WT10 or WT30) to unstressed samples (WT0) of WT cells. **(c, d)** Xrn1-dependence was measured by comparing the changes in RNA levels of stressed *xrn1*Δ cells to stressed WT cells. Results represent averages of n=3 biological replicates

Figure S5:

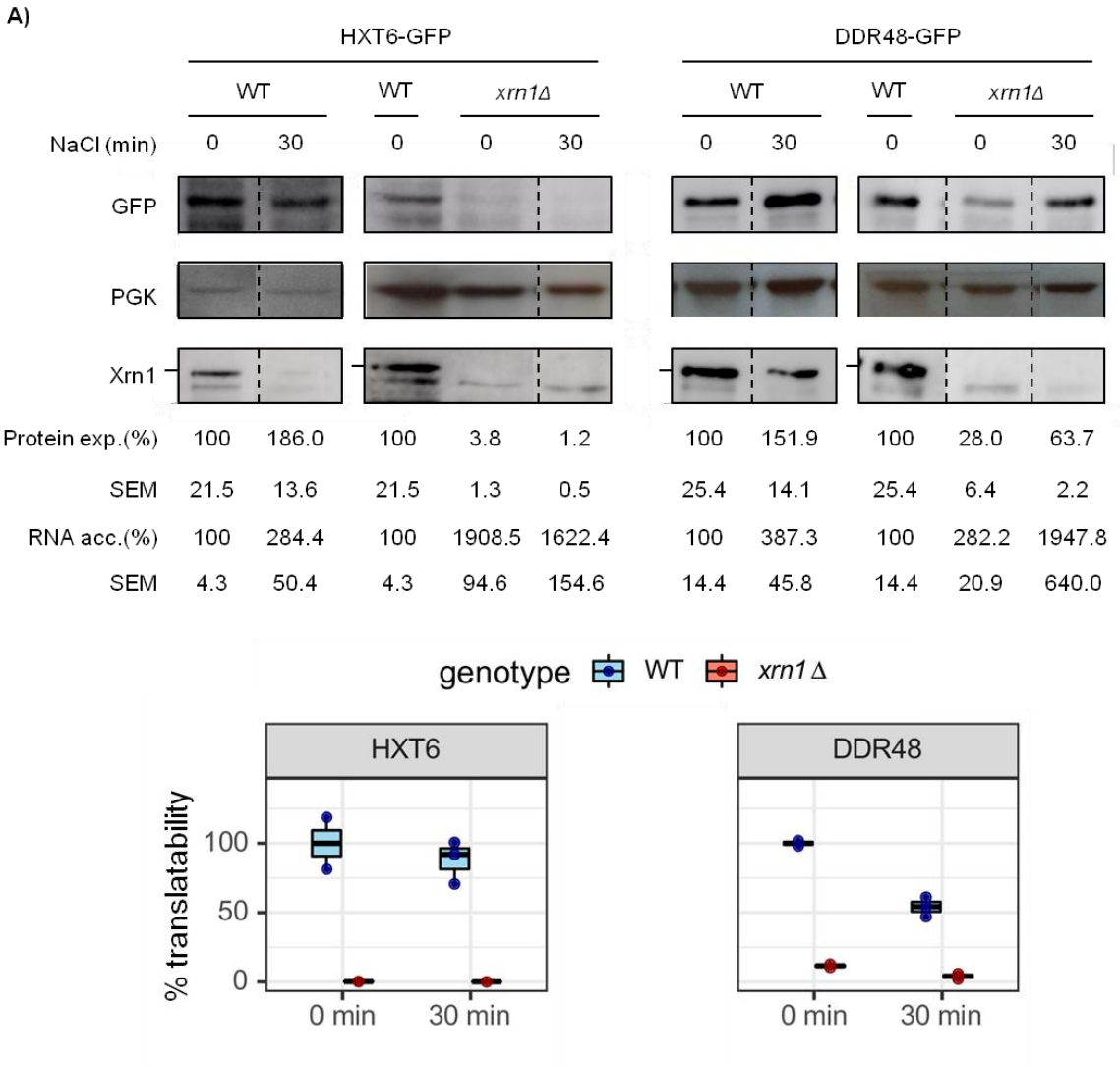

Figure S5. Xrn1 promotes translation of Xrn1-dependent osmostress genes on transcription. BY4741 yeast cells expressing GFP-tagged fusion proteins were grown. Protein expression levels were measured by Western blot and detected by anti-GFP antibody (upper pannel) and RNA levels were measured by quantitative PCR. Translatability (bottom pannel) was calculated by changes in protein levels divided by changes in RNA levels. Boxplots of mRNA translatability in WT (blue) or *xrn1***Δ** (red) cells across all queried time points, with individual replicates indicated as dots. Results represent averages of n=3 biological replicates.

Figure S6:

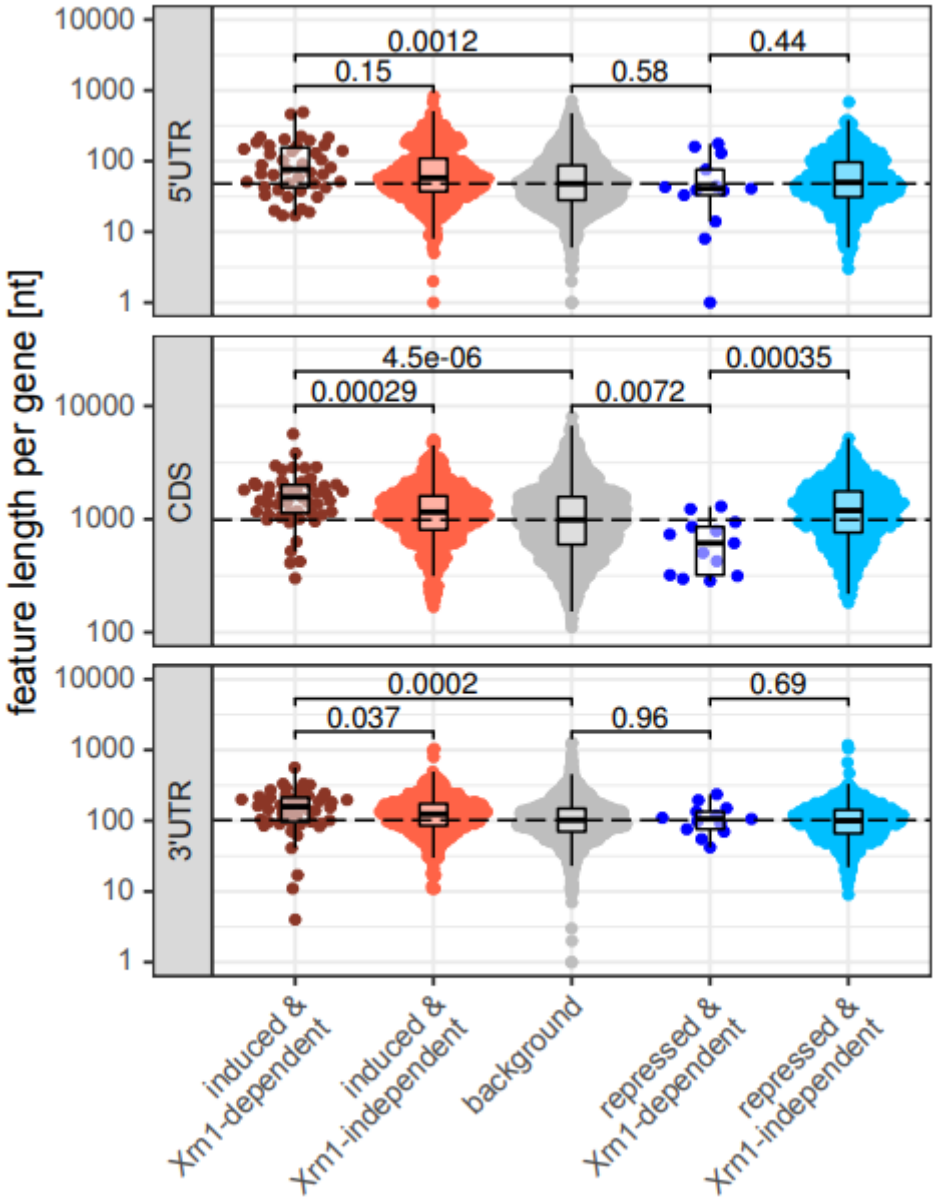

Figure S6: Length of mRNA features across different groups of transcripts at 10 min of osmostress. For easier comparability, dashed horizontal lines indicate the median value of the background group for each feature, respectively. All p-values were calculated using a Wilcoxon-Mann-Whitney test.

Figure S7:

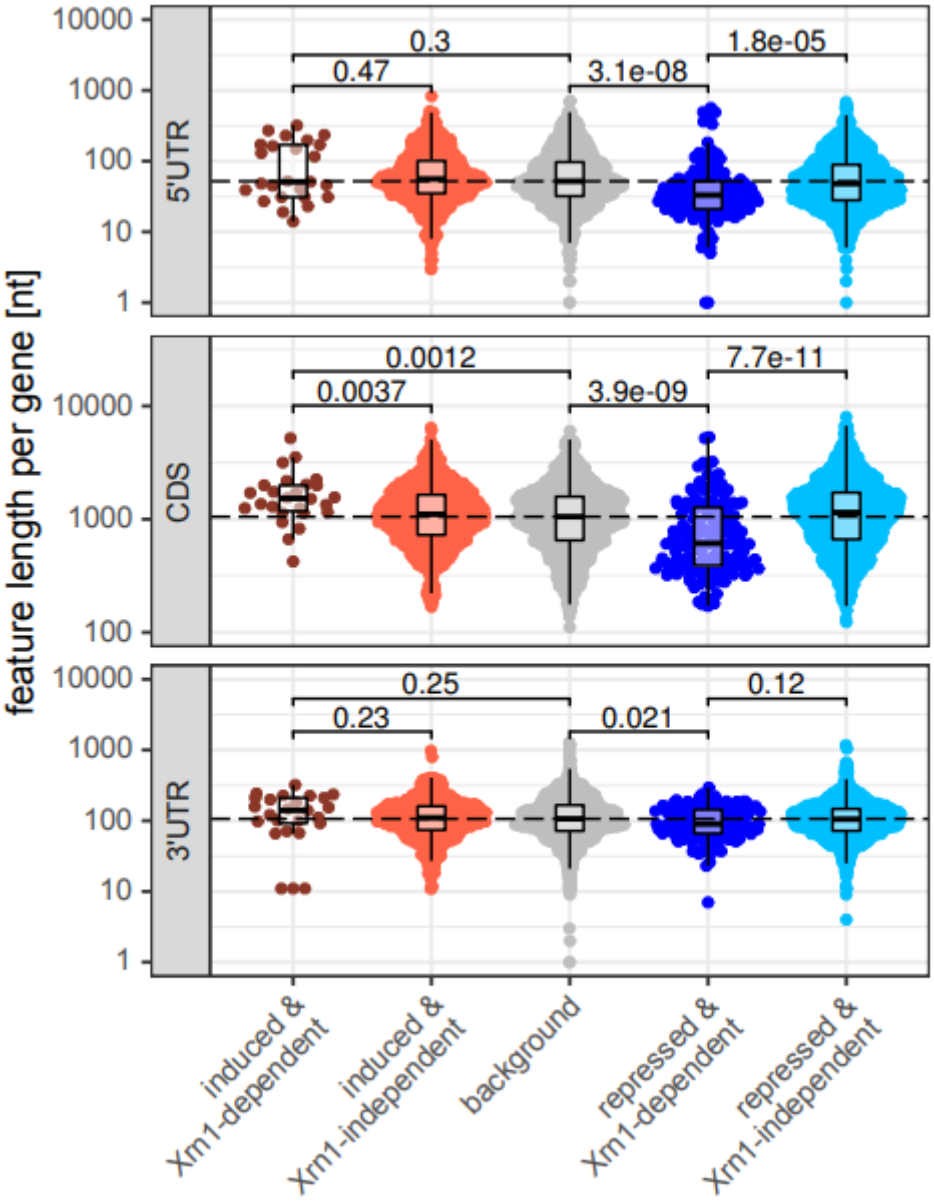

Figure S7: Length of mRNA features across different groups of transcripts at 30 min of osmostress. For easier comparability, dashed horizontal lines indicate the median value of the background group for each feature, respectively. All p-values were calculated using a Wilcoxon-Mann-Whitney test.

Figure S8:

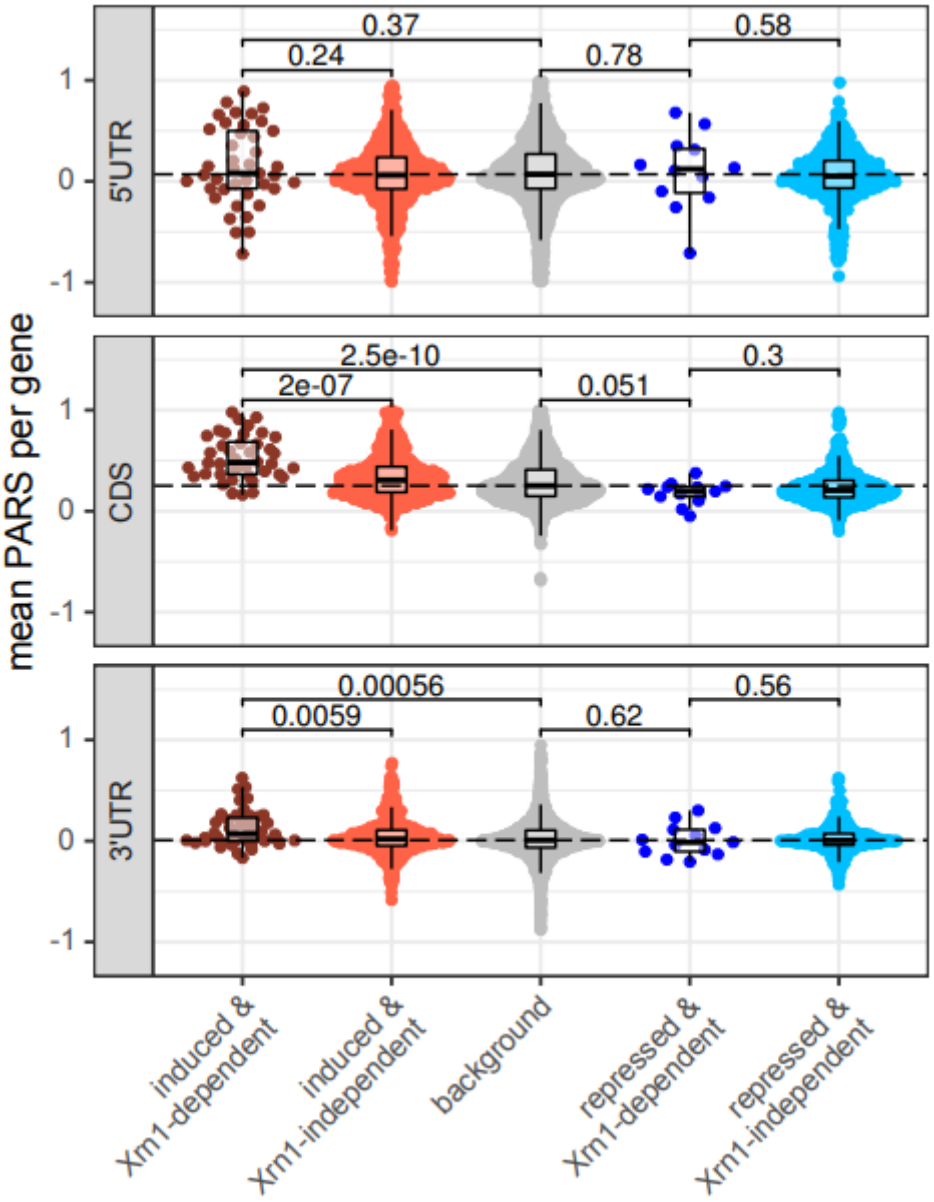

Figure S8: Average RNA structure for mRNA features across different groups of transcripts at 10 min of osmostress as measured by PARS [PARS citation]. For easier comparability, dashed horizontal lines indicate the median value of the background group for each feature, respectively. All p-values were calculated using a Wilcoxon-Mann-Whitney test.

Figure S9:

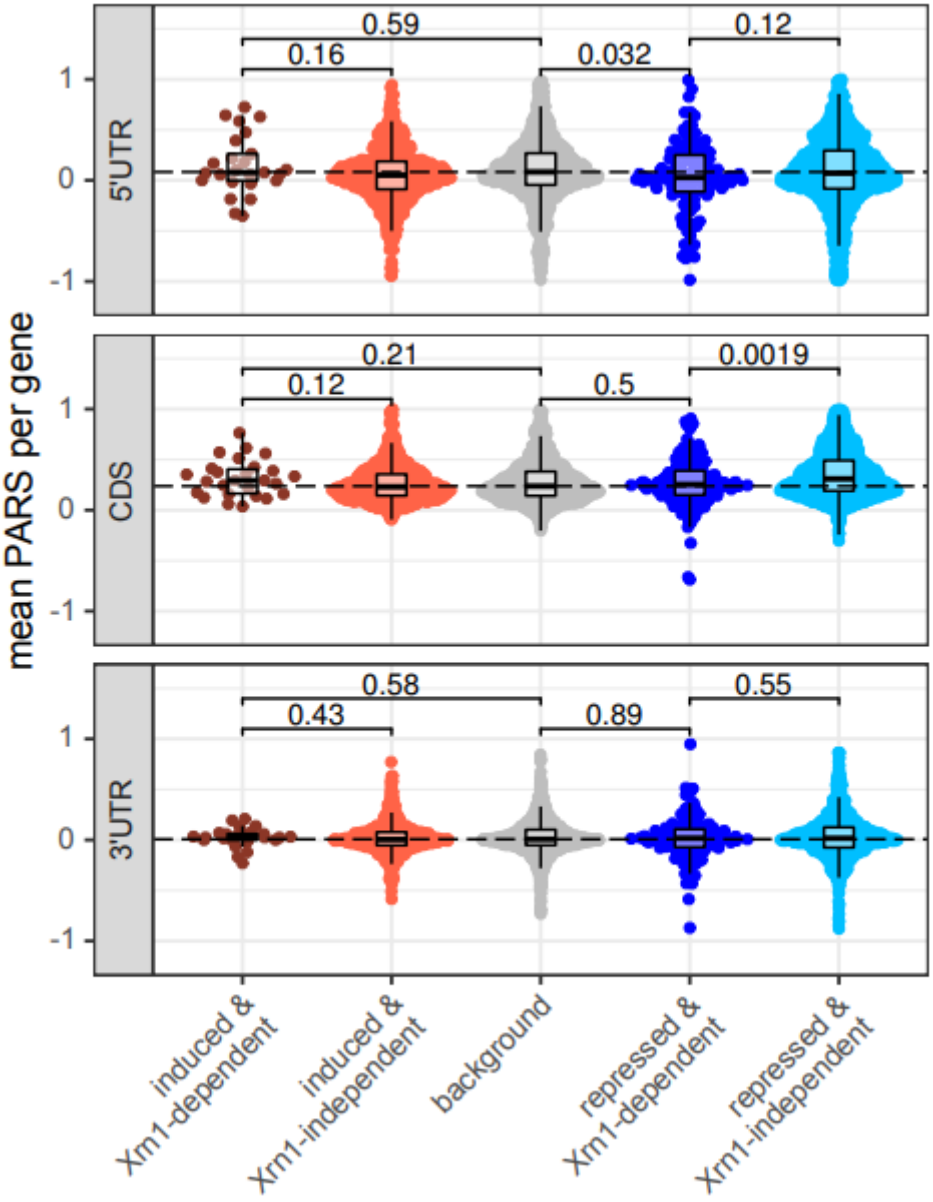

Figure S9: Average RNA structure for mRNA features across different groups of transcripts at 30 min of osmostress as measured by PARS [PARS citation]. For easier comparability, dashed horizontal lines indicate the median value of the background group for each feature, respectively. All p-values were calculated using a Wilcoxon-Mann-Whitney test.

SUPPLEMENTARY TABLES

Supplementary tables are enclosed in CD format.

Table S1: Overview of RNA-seq results of both examined timepoints, stored in separate sheets. Columns contain: gene_ID / gene - systematic gene ID and official gene name; LFC_interaction / FDR_interaction - DESeq2 $\log_2$ fold change and false discovery rate for each gene when using an interaction term to discern significant Xrn1 contributions to the osmostress response; LFC_NaCl_VS_basal_WT / FDR_NaCl_VS_basal_WT - DESeq2 $\log_2$ fold change and false discovery rate for each gene when comparing stressed and non-stressed WT cells; significant_Xrn1_contribution - classifier, whether the interaction-based results for an Xrn1 contribution to the gene's expression were considered significant; osmostatus - classifier, whether a gene was considered as osmogene or not based on comparing stressed and non-stressed WT cells.

Table S2: Results of functional enrichment analysis after 10 min of osmostress using gProfileR, each RNA group has been stored in a separate sheet. Columns contain: p.value - adjusted p-value for enriched term; term.size - number of genes associated to term; query.size - number of valid gene symbols in group; overlap.size - overlap between query and genes associated with term; term.id - systematic term ID; domain - source domain of enriched term, i.e. either KEGG, REACTOME or GO, with GO_BP: biological process, GO_MF: molecular function, GO_CC: cellular component; term.name - description of enriched term ID.

Table S3: Results of functional enrichment analysis after 30 min of osmostress using gProfileR, each RNA group has been stored in a separate sheet. Columns contain: p.value - adjusted p-value for enriched term; term.size - number of genes associated to term; query.size - number of valid gene symbols in group; overlap.size - overlap between query and genes associated with term; term.id - systematic term ID; domain - source domain of enriched term, i.e. either KEGG, REACTOME or GO, with GO_BP: biological process, GO_MF: molecular function, GO_CC: cellular component; term.name - description of enriched term ID.

Table S4: Overview of statistical test results and average values for each group of genes organized in separate sheets, corresponds to Figures S5-8. A) Columns contain: timepoint - time of NaCl addition; feature - mRNA feature considered for test; group1 / group2 - the two groups of genes compared against one another; pV_length - Wilcoxon-test p-value comparing the feature's lengths between both groups; FC_length - fold change of length values (group1 / group2); pV_PARS - Wilcoxon-test p-value comparing the feature's mean PARS between both groups; meanDiff_PARS - mean difference of PARS values (group1 - group2). B) Columns contain: timepoint / feature - as in A; group - group of genes; mean_PARS /

median_PARS - mean & median PARS of feature among gene group; mean_length / median_length - mean & median length of feature among gene group;

Table S5: Results of enrichment analysis for Xrn1-dependent genes. Using Fisher's exact test, each group of genes was compared against the corresponding Xrn1-independent control group, e.g. osmo-induced_xrn1-induced vs. osmo-induced_xrn1-unchanged and osmo-repressed_xrn1-repressed vs. osmo-repressed_xrn1-unchanged. Columns contain: p-value - p-value from Fisher's exact test; OR - odds ratio, an OR >1 indicates enrichment, whereas an OR <1 indicates depletion relative to the control group; 95% CI - 95% confidence interval of OR.

Table S6. Supplementary Methods. Sheet 1 shows the yeast strains used in this study, sheet 2 shows the plasmids used in this study and sheet 3 shows the antibodies used in this study.

GENERAL DISCUSSION

In this PhD thesis we show in a yeast model system that the exonuclease Xrn1 interconnects under physiological conditions all stages of gene expression by promoting transcription, translation and degradation of a subset of cellular mRNAs with shared structural characteristics. Besides, under hyper-osmotic shock conditions Xrn1 regulates transcription and translation of genes involved in stress response. Overall, our results unveil an unprecedented role for Xrn1 as a key regulator of gene expression under both physiological and suboptimal environmental conditions.

Multiple evidences support the role of Xrn1 in translation of viral and cellular RNAs. First, polysome profiling shows that the depletion of Xrn1 leads to a shift of BMV RNA2 from polysomes towards monosomes and free RNPs, indicating that Xrn1 functions in translation initiation. Second, Xrn1 protein co-fractionates in this analysis with the 40S and 60S ribosomal subunits. Moreover, Xrn1 interacts in a RNA-independent manner with both ribosomal subunits. This observation is in agreement with a recent study that characterizes the interaction of Xrn1 with the mRNA exit site of the ribosome (Tesina et al., 2019). The authors argue that the 80S-Xrn1 complex ensures rapid degradation of the transcripts after the last round of translation. Moreover, our results raise the possibility of a role in translation mediated by the direct interaction of Xrn1 with ribosomal subunits. Third, using the auxin-induced degron system we show that BMV RNA2 translation is immediately inhibited upon Xrn1 depletion. These results argue against the possibility of explaining the observed translation defects by indirect adaptive effects of the cells in Xrn1 absence. Fourth, a mutant of the nuclear paralog of Xrn1, called Rat1ΔNLS, which localizes in the cytoplasm, fully rescues the function of Xrn1 in degradation but not in translation. These results confirm that the role of Xrn1 in translation is specific and not indirectly mediated by changes in mRNA abundance caused by Xrn1 depletion. Fifth, Xrn1 but not Rat1ΔNLS directly interacts with the translation initiation factor eIF4G. The genetic and physical interaction of eIF4G with Xrn1 has already been described via high-throughput screenings in other studies (Gavin et al., 2006; Wilmes et al., 2008). Interestingly, this interaction is mediated by the C-terminal domain of Xrn1 and is necessary to promote Xrn1-

mediated translation. Indeed, the C-terminus of Xrn1, which is not present in Rat1, is intrinsically disordered and includes short linear motifs (SLiMs), a characteristic that has been postulated to mediate interaction with other proteins (Tompa, 2012). Sixth, the role of Xrn1 in translation is extended to a subset of genes that are induced upon osmotic shock, corroborating that Xrn1 function in translation is vital for cell survival. All together these data demonstrate that the function of Xrn1 in gene expression can be extended to a role in translation.

Suboptimal growth conditions like high osmolarity require the adaptation of gene expression dynamics to ensure cell survival. In this thesis we demonstrate that the exonuclease Xrn1 modulates not only translational but also transcriptional activation of osmo-induced genes upon high osmolarity. Therefore, Xrn1 shuttles to the nucleus and activates transcription of genes mainly related to glycolysis, protein folding, sodium ion transport and salt stress response. Interestingly, these genes are enriched for Hog1 interactors, being Hog1 the main mitogen-activated protein kinase involved in osmoregulation. Similarly, Xrn1 activates both transcription and degradation of mRNAs that are induced upon glucose deprivation and that depend on the major signal integrator Snf1 adenosine monophosphate-activated protein kinase (Braun et al., 2014). In addition, we observe that upon osmotic shock a fraction of cytosolic Xrn1 is rapidly sequestered to stress-induced aggregates. Spatial segregation of Xrn1 upon environmental stresses had been described previously. For instance, Xrn1 associates with stress granules (SG) after robust heat shock (Grousl et al., 2015). The same authors show that the exonuclease accumulates in cortical eisosomes in post-diauxic cells (Grousl et al., 2015). However, a characteristic that is unique to osmotic stress is that cells lose nearly 30% of its initial volume due to water loss, thus changing the cytosolic physical distribution of cells.(Petelenz-Kurdziel et al., 2011).The function of the formation of cytosolic aggregates upon environmental stresses is a matter of major controversy. Stress granules are composed of untranslating mRNAs that are stalled in translation initiation and therefore are targeted for degradation (Protter and Parker, 2016). Therefore, some authors propose that active degradation of target mRNAs takes place in stress granules. All together, we

hypothesize that Xrn1 sequestration upon hyper-osmotic stress in cytosolic aggregates promotes specific degradation of mRNAs that are recruited in stress-induced aggregates. Besides, it prevents the exonuclease from over-accumulating in the cytosol, which would cause unspecific degradation of stress-induced mRNAs that are necessary to fulfil a correct response to stress, as well as non-targeted translation of mRNAs that should be shut down upon stress addition. Strikingly, Snf1 is involved in the phosphorylation of the SG component eIF4G, which in turn regulates the degradation of specific mRNAs under glucose starvation conditions likely through the sequestration of Dhh1 to SGs (Chang and Huh, 2018). Since Xrn1 and eIF4G directly interact in physiological conditions (Blasco-Moreno et al., 2019), we speculate that upon hyper-osmotic shock Snf1 mediates eIF4G/Dhh1/Xrn1 recruitment to stress granules. In accordance with these results, microscopy imaging revealed that Xrn1 colocalizes with Dhh1 in our set-up. However, both proteins showed differing dynamics, as Dhh1 localizes to foci earlier than Xrn1 while Xrn1 is retained longer. Besides, Xrn1 but not Dhh1 showed reduced protein levels upon osmotic shock in our western blot analysis, suggesting that Dhh1 remained more accessible to the used lysis solvents than Xrn1. Of note, Dhh1 ATPase activity is known to regulate processing bodies in yeast cells (Mugler et al., 2016). Based on these observations, our findings may indicate that Dhh1 first aids in the formation of cytosolic aggregates and then mediates Xrn1 accumulation in the granules with the help of eIF4G. Notably, although western blot and microscopy imaging performed with Rat1ΔNLS showed no aggregation upon NaCl uptake, the chimeric fusion Rat1ΔNLS-XC, that bears the C-terminal of Xrn1, did show sequestering to stress-induced aggregates upon osmotic shock indicating that Xrn1 relocalization is mediated by its unstructured C-terminal domain. Furthermore, Xrn1 spatial segregation was dependent on the presence of the phosphokinase Snf1, suggesting that phosphorylation of Xrn1 by Snf1 might regulate its sequestration and hence its activity under stress conditions. In line with this hypothesis two residues in the C-terminal domain of Xrn1 are known to be phosphorylated upon hyper-osmotic shock (Janschitz et al., 2019; Romanov et al., 2018). Furthermore these two phosphosites are

phosphorylated by Snf1 during glucose starvation (Braun et al., 2014). Interestingly, Snf1 phosphorylates mRNPs that are components of stress granules under glucose deprivation (Chang and Huh, 2018). Based on this, we conclude that Xrn1 aggregation is regulated via its C-terminal domain likely by Snf1. Our results point towards a previously unknown crosstalk between the Hog1 and Snf1 stress-activated signalling pathways upon hyper-osmotic shock and mediated by the exonuclease Xrn1. In agreement with this there are few evidences of Hog1 and Snf1 interconnections in other environmental stress conditions. For instance, Snf1 inhibits ER stress responses mediated by Hog1 through negatively regulating transcription of Ssk1 upstream activator of HOG pathway (Mizuno et al., 2015). In addition, Snf1 is essential for Hog1 activation upon glucose deprivation in a manner dependent on the kinase Ssk1 (Piao et al., 2012). All together, our findings not only reveal a more general mechanism of stress adaptation involving Xrn1, but also towards a broader role of Snf1 in coordinating gene expression and mRNA turnover during diverse cellular stress responses beyond glucose starvation.

Xrn1 stimulates expression of cellular mRNAs that possess highly structured sequences as a shared feature. Specifically, Xrn1-dependent genes on translation contain long and highly structured 5'UTRs. On average, the structural context of cellular transcripts around the translation initiation site (TIS) diminishes to favor recruitment of the translation machinery to the mRNA and start translation initiation (Gu et al., 2010). Xrn1-dependent mRNAs, however, exhibit higher structure in the nucleotides preceding the TIS, hindering the proper binding of ribosomal subunits to the transcript. Interestingly, the genes that are induced upon osmotic stress and are dependent on Xrn1 for their transcriptional activation are also characterized by a higher structural context around the TIS. These results suggest that Xrn1 stimulates the expression of mRNAs with complex structural traits by facilitating the recruitment of the translation machinery to the translation initiation sites. Interestingly, Xrn1 shares the same mechanism among genes involved in different yet specific biological processes that are activated upon physiological and altered conditions.

Xrn1 functions as a master regulator of gene expression by linking transcription, degradation and translation processes in physiological and suboptimal environmental conditions. The role of Xrn1 in degradation and transcription is extended to the vast majority of mRNAs, although it preferentially affects both the synthesis and decay of highly transcribed genes that encode for ribosome biogenesis and translation factors (Medina et al., 2014). The term “Xrn1 synthegradon” has been established to refer to this group of genes. However, the role of Xrn1 in translation is limited to a subgroup of mRNAs with highly structural traits that encode for membrane proteins (Blasco-Moreno et al., 2019). Interestingly, there are several observations that confirm that the role of Xrn1 in different steps of gene expression is interconnected. First, global co-translational degradation by Xrn1 is more frequent in genes related to vacuole transport in yeast (Pelechano et al., 2015) and the endomembrane system in *A. thaliana* (Merret et al., 2015). This observation provides a link between the role of Xrn1 in co-translational mRNA decay and translational activation of mRNAs targeted to the ER for translation. We infer that this linkage has evolved to regulate proper gene expression of membrane proteins. These proteins contain hydrophobic domains that strongly tend to aggregate. Consequently, their expression levels and localization must be finely tuned to avoid aggregations that might be toxic. Second, the genes that are translationally activated by Xrn1 show lower transcription rates and increased half-lives in Xrn1 knock-down cells, confirming that Xrn1 regulates transcription, degradation and translation of the same group of genes. Third, BMV RNA2 directly electroporated in the cytosol of yeast cells is not dependening on Xrn1 for translation, meaning that Xrn1 needs to promote transcription of an RNA to further regulate translation of the same transcript. Fourth, the catalytic domain of Xrn1 needs to be active so that Xrn1 can shuttle to the nucleus and promote transcription (Haimovich et al., 2013). Accordingly, the Xrn1^{D208A} mutant, that binds to uncapped mRNA as efficiently as Xrn1 but cannot degrade it (Solinger et al., 1999), is deficient in transcription and nuclear shuttling as well as in cytoplasmic translation. These findings confirm that the different functions of Xrn1 in gene expression are linked. Other proteins described to interconnect

transcription, translation and degradation are Rpb4 and Rpb7, two subunits of RNAP II. Interestingly, Xrn1 and Rpb7 genetically interact (Lotan et al., 2007), suggesting that Rpb7 and Xrn1 may work together. The advantages of interconnecting all steps in gene expression may include cell economy. In addition, this mechanism would ensure that a gene is transcribed only when translation is working properly and is translated only when the mRNA can be duly degraded when the time comes.

Xrn1 sequence and structure are highly conserved across species. The N-terminal domain of the protein exhibits catalytic activity and consequently shows the biggest preservation, since Xrn1 function in degradation is shared among all eukaryotic homologs (Jones et al., 2012). As found for yeast Xrn1, the human exonuclease (XRN1) has been recently reported to regulate mRNA homeostasis by acting both in transcription and degradation of mRNAs in human liver carcinoma HepG2 cells (Singh et al., 2019). The authors claim that the functions of Xrn1 in transcription and decay are a conserved eukaryotic feature. An interesting possibility is that the role of the exonuclease in translation activation is also extended among eukaryotes, although this has not been proven yet. Of note, the HepG2 human cell line is a cancer cell line and does not display the same gene expression dynamics as healthy human cells (Tyakht et al., 2014). In fact, the cellular response to cancer is somehow comparable to that of other cellular stresses, given that persistent cell stress is associated to an increased sensitivity to cancer diseases (Poljšak and Milisav, 2012). Considering this it is not surprising that Xrn1 buffers mRNA levels of human cancer cells, since we also observe that Xrn1 assists in gene expression responses to hyper-osmotic shock in a yeast model system.

Xrn1 functions in complex with other decay factors in all steps of gene expression. Haimovich and co-workers showed that not only Xrn1 but also Pat1, Dhh1, Dcp2 and Lsm1 can shuttle to the nucleus, bind to promoters and activate transcription (Haimovich et al., 2013). However, the role of Xrn1 is of major importance since shuttling of the other decay factors depends on the enzymatic activity of Xrn1. These results suggest that Xrn1 may be responsible

for the correct formation of the complex necessary to shuttle to the nucleus and, consequently, act in transcription. Similarly, Xrn1 acts in a timely manner together with multiple decay factors in mRNA degradation to ensure proper degradation. For instance, the recruitment of Xrn1 to the mRNA is mediated by the interaction with Pat1 and is preceded by the recruitment of Dcp2 through the same binding platform in Pat1 (Charenton et al., 2017), thus ensuring that Dcp2 first trims the 5' cap before Xrn1 degrades the decapped mRNA. In addition, multiple evidences indicate that Xrn1 also functions together with other decay factors in translation regulation. First, Dhh1, Lsm1, Pat1 and Dcp2 activate, as Xrn1, translation of BMV RNA2 (Jungfleisch et al., 2015 and unpublished data aroused from this thesis). Second, further studies on Dhh1 demonstrated that Dhh1 activates translation of yeast mRNAs with similar features to those activated by Xrn1, including long and highly structured CDSs and 5'UTRs and enrichment in membrane proteins (Blasco-Moreno et al., 2019; Jungfleisch et al., 2017). Third, as found for Xrn1, polysome profiling analyses reveal a role for Dhh1 in translation initiation, and immunoprecipitation assay indicated a RNA-independent interaction of Dhh1 with translation initiation factors. Nevertheless, additional evidences indicate that although all these factors function in similar processes the mechanism is specific for each one. First, in contrast of Xrn1, dependence on Dhh1 for BMV RNA2 translation include not only the 5´ UTR and CDS but also the 3´UTR (Jungfleisch et al., 2017). Second, even though Xrn1 and Dhh1 activate translation of transcripts with similar features, the overlap between Xrn1- and Dhh1-activated genes is small (35 shared genes out of 172 genes activated by Xrn1 and 210 genes activated by Dhh1). Interestingly, several decay factors have been identified to play key roles under suboptimal environmental conditions as well. For instance, several members of the Lsm1-7/Pat1 complex preferentially bind mRNAs that are induced after osmotic shock and regulate stress-induced global translation repression (Garre et al., 2018). Similarly, Xrn1 degrades ribosomal protein genes to ensure translation inhibition (Garre et al., 2013). Furthermore, the decapping activators Edc3 and Lsm4 block deadenylation of stress-induced genes to increase their stability in osmotic stress conditions (Huch and Nissan, 2017). Altogether, these

results suggest that during the complete cycle of gene expression decay factors act together with Xrn1 as a core component forming a highly dynamic complex whose composition is timely modulated to ensure proper gene expression under changing conditions.

Based on the knowledge achieved in this thesis and the current bibliography, we propose a model in which Xrn1 shuttles to the nucleus in complex with several decay factors and stimulates transcription initiation and elongation of genes by binding to their promoter (Blasco-Moreno et al., 2019; Haimovich et al., 2013). Subsequently, Xrn1 is exported to the cytoplasm already bound to the newly synthesized mRNA or in its free conformation where it interacts with translation initiation factor eIF4G and presumably with some other decay factors to perform its role in translation by facilitating the interaction of the ribosomal subunit 40S with the translation initiation site of mRNAs with highly structural traits. Since eIF4G is known to localize in the nucleus to participate in splicing (Das et al., 2014), we hypothesize that the Xrn1/eIF4G complex is formed already in the nucleus and Xrn1 gets exported together with eIF4G to the cytoplasm. Xrn1 will then recruit cellular mRNAs to the endoplasmic reticulum to resume or initiate localized translation. Given that Xrn1 co-precipitates with microtubules (Interthal et al., 1995), an interesting possibility is that Xrn1 drives ER recruitment of translating mRNAs through the microtubule network. In fact, several evidences relate the cytoskeleton with the translation machinery. For instance, the disruption of microtubules in S*accharomyces cerevisiae* slowed protein synthesis approximately 20% (Gross and Kinzy, 2007; Kandl et al., 2002); moreover, microscopy and proteomic studies performed in several eukaryotic systems confirmed the interaction of microtubules with proteins from the translation initiation machinery (reviewed in Chudinova and Nadezhdina, 2018 and Kim and Coulombe, 2010). Once at the ER and after favoring translation for several rounds, Xrn1 will degrade the mRNAs co-translationally. When cells are forced to grow under suboptimal environmental conditions, nuclear Xrn1 promotes transcription of stress-related genes within the first minutes of shock (Braun et al., 2014; our study). In the cytosol, a fraction of Xrn1 is rapidly sequestered to stress-induced aggregates to prevent over-

accumulation of catalytically active Xrn1. The remaining soluble fraction of Xrn1 destroys mRNAs targeted for degradation and activates translation of stress-induced genes that exhibit the same structural traits as found for physiological conditions.

Altogether, the results of our work confirm that Xrn1 functions as a master regulator of gene expression by acting in transcription, translation and degradation processes. In addition, Xrn1 role extends to altered environmental conditions, where it stimulates transcription and translation of stress-related genes by the interconnection of Snf1 and Hog1 signaling pathways. Important aspects which should be approached in future studies are (i) whether the role of Xrn1 in translation is conserved in multicellular organisms such as humans, (ii) whether the function of Xrn1 under osmotic stress is extended to other environmental stresses and (iii) how does Xrn1 modulate the transitions between its functions.

CONCLUSIONS

From the results presented in this thesis, the following conclusions can be drawn:

Xrn1 is a master regulator of gene expression:

- Xrn1 promotes translation of viral BMV RNA2 and a subset of cellular mRNAs with highly structural traits around the translation initiation site.
- Xrn1 interacts with components of the translation initiation machinery through its C-terminal domain and activates translation initiation.
- Xrn1 promotes translation initiation of yeast mRNAs encoding membrane proteins, whose correct localization at the endoplasmic reticulum is also regulated by Xrn1.
- Xrn1 coordinates the three major steps of gene expression by promoting transcription, translation and degradation of the same group of genes.

Xrn1 links the Snf1 and Hog1 pathways to control gene expression upon osmotic stress

- Xrn1 is sequestered to stress-induced aggregates rapidly after osmotic shock.
- Xrn1 sequestration is mediated by its unstructured C-terminal domain and depends on Snf1 AMPK presence.
- Xrn1 sequestration leads to a diminished exonuclease activity.
- Xrn1 modulates cellular transcriptional and translational responses upon hyper-osmotic shock.
- Xrn1-dependent osmo-induced genes are enriched for Hog1 MAPK interactors.
- Xrn1 links the Snf1 and Hog1 pathways to control gene expression upon osmotic stress.

LIST OF ABBREVIATIONS

aa	Amino acid
AID	Auxin-inducible degron
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
BMV	Brome mosaic virus
CDK	Cyclin dependent kinase
CDS	Coding sequence
ChIP	Chromatin immunoprecipitation assay
CHX	cycloheximide
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
ESR	Environmental stress response
FHV	Flock house virus
GDP	Guanosine diphosphate
GFP	Green flurorescent protein
GO	Gene ontology
GRO	Genomic run-on
GTP	Guanosine triphosphate
HLM	Helical leucine-rich motif
HOG	High osmolarity glycerol
HPV	Human papillomavirus
HSE	Heat shock element
HSP	Heat shock protein
HSR	Heat shock response
iESR	Induced environmental stress response

IP	Immunoprecipitation
IRES	Internal ribosome entry site
KD	Knock-down
MAPK	Mitogen-activated protein kinase
MAPKK	Mitogen-activated protein kinase kinase
MAPKKK	Mitogen-activated protein kinase kinase kinase
mRNA	messenger RNA
mRNPs	messenger ribonucleoproteins
NGD	No-go decay
NLS	Nuclear localization sequence
NMD	Nonsense-mediated decay
NSD	Non-stop decay
ORF	Open Reading frame
OSR	Oxidative stress response
PABPs	Poly(A) binding proteins
PARS	Parallel analysis of RNA structure
P-bodies	Processing bodies
PGK	Phosphoglycerate kinase
PIC	Pre-initiation complex
REC	receiver
rESR	Repressed environmental stress response
(+)RNA	Positive-strand RNA
Rluc	Renilla luciferase
RNA	Ribonucleic acid
RNAP II	Ribonucleic acid polymerase II

RNP	ribonucleoprotein
RPE	Retinal pigmented epithelial (cells)
RPF	Ribosome protected fragment
rRNA	ribosomal RNA
RSC	Remodeling the structure of chromatin
SG	Stress granules
SHK	Sensor histidine kinase
SLiMs	Short linear motifs
snoRNAs	Small nucleolar RNAs
SRP	Signal recognition particle
STREs	Stress response elements
TC	Ternary complex
TCA	The citric acid (cycle)
TFs	Transcription factors
TIS	Translation initiation site
TOR	Target of rapamycin
TRs	Transcription rates
tRNA	transfer RNA
TSS	Transcription start site
Ub	Ubiquitin
UTR	Untranslated region
WB	Western blot
WT	Wild-type

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