IV MEETING

RED DE EXCELENCIA TEMÁTICA

BFU2015-71978-REDT/RED2018-102467-T

12-13 Julio 2021

SEVILLA

Monday, 12 July

15:50 RNA Life II: Presentation. José E. Pérez

SESSION 1 (Chairwoman Puri Fortes)

16:00-16:35 José Carlos Reyes

Regulation of enhancers, co-expression domains and splicing efficiency by TGFβ

16:45-17:20 E. Rovira (lab Puri Fortes)

U1A is a positive regulator of the expression of heterologous and cellular genes involved in cell proliferation and migration

17:30-18:05 Sebastián Chávez

Human prefoldin modulates co-transcriptional pre-mRNA splicing

18:15-18:45 COFFEE BREAK

SESSION 2 (Chairman Sergi Puig)

18:45-19:20 Olga Calvo & Araceli González-Jiménez

Functions and regulation of the RNAPII stalk domain

19:30-20:05 Carlos Fernánez-Tornero

Transcriptional regulation and DNA lesion detection by RNA polymerase I

Tuesday, 13 July

SESSION 3 (Chairman Julio Salinas)

9:00-09:15 Rafael Catalá (lab. Julio Salinas)

Exploring the function of the LSM2-8 complex through the characterization of a suppressor of the lsm8 mutation

9:15-09:35 Eduardo Tranque (lab. Julio Salinas)

Differential regulation of stress responses in plants by PAT1 proteins

09:45-10:20 Cristina Moreno-Castro & María Duarte (lab C. Suñé)

Investigating the roles of TCERG1 and PRPF40B in transcription and RNA splicing

10:30-11:00 COFFEE BREAK

SESSION 4 (Chairwoman Xenia Peñate)

11:00-11:35 José E Pérez-Ortín

Study of the cellular component-dependent functions of Xrn1

11:45-12:00 Ana I. Garrido-Godino (lab F. Navarro)

Rpb4 as a key element between the synthesis and degradation of mRNAs

12:00-12:20 Francisco Navarro

Bud27 and RNAs: some results and many questions

12:30-13:00. I.P. Meeting

13:00-15:00 Lunch

SESSION 5 (Chairwoman S. Rodríguez-Navarro)

15:00-15:35 Carme Nuño-Cabanes & Ana Tejada-Colón (lab S. Rodríguez-Navarro)

Mip6 participates at different steps during RNA metabolism

15:45-16:00 Tania Jordá (lab S. Puig)

The lipid composition of yeast cells modulates the response to iron deficiency mediated by the transcriptional factor Aft1

16:00-16:20 Antonia María Romero (lab S. Puig)

The yeast mRNA-binding protein Cth2 regulates gene expression at the transcriptional and post-transcriptional level in iron deficiency.

16:30- 17:05 Sergio Camero & José M. Pérez Cañadillas

NMR studies of the low complexity domain of hnRNP A1: Conformational properties and nucleic acid recognition

17:15-18:00. Coffee-break

SESSION 6 (Chair A. Jordán)

18:00-18:15 José Fernández (lab J. de la Cruz)

Early role of ribosomal protein eL15 during the assembly of 60S ribosomal subunits in Saccharomyces cerevisiae

18:15-18:35 Sara Martín (lab J. de la Cruz)

The RNA helicase Dbp7 is required for the release of the snR190 snoRNA chaperone from early pre-60S ribosomal particles in Saccharomyces cerevisiae

18:45-19:20 Rosario Francisco-Velilla (lab E. Martínez-Salas)

Gemin5, a multifunctional RNA-binding protein involved in translation control

19:30 Debate sobre el estado de la Red ***RNA life*** *y su futuro*

RNA life

ABSTRACTS

SESSION 1

REGULATION OF ENHANCERS, CO-EXPRESSION DOMAINS AND SPLICING EFFICIENCY BY TGFβ.

Jose A. Guerrero-Martínez, Elena Sanchez-Escabias, Elena Gómez-Marín, Laura Basurto-Cayuela, Isabel Pozuelo-Sánchez, María Ceballos-Chávez, and Jose C. Reyes

Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER. Consejo Superior de Investigaciones Científicas-Universidad de Sevilla-Universidad Pablo de Olavide (CSIC-USE-UPO).

TGFβ cytokines have crucial roles in development, proliferation, tissue homeostasis, differentiation and immune regulation. Consequently, alterations in TGFβ signaling underlie numerous diseases, including cancer. TGFβ is one of the most potent inductors of epithelial to mesenchymal transition (EMT) in normal and oncogenic epithelial cells from different origins. EMT and its reversion (MET) are common processes during embryonic development and have attracted considerable interest due to the fact that they seem to be related to tumor cells dissemination and migration, generation of tumor circulating cells, cancer stem cells and metastasis formation. TGFβ causes a large reorganization of gene expression patterns and epigenetic information, that we are only starting to understand. In our group we are investigating how TGFβ causes these reorganization of gene expression patterns.

We have cartographied the genomic transcriptional enhancers that are regulated by TGFβ in a breast epithelial cell line. In fact, TGFβ triggers a fast and widespread increase in chromatin accessibility in about 80% of enhancers, irrespective of whether they are activated, repressed or not regulated by TGFβ. We have also shown that most TGFβ-regulated genes are located around enhancers regulated in the same way, often creating domains of several co-regulated genes that we term TGFβ regulatory domains (TRD). We are currently investigating how the 3D organization of the genome connect the enhancers with the TRDs.

We have also investigated whether regulation co-transcriptional splicing efficiency at the whole gene level is used to regulate gene expression by TGFβ. First we found that the existence of two well-differentiated strategies for co-transcriptional splicing efficiency, at the extremes of a gradient: short genes, that produces high levels of pre-mRNA display a relatively inefficient splicing while long genes with relatively low pre-mRNA levels present efficient splicing. Furthermore, we found that the TGFβ pathway regulates the general co-transcriptional splicing efficiency causing changes in mature mRNA levels. Taken together, our data indicate that co-transcriptional splicing efficiency is a gene-specific characteristic that can be regulated to control gene expression.

Finally, other members of the group are looking for chromatin factors that control gene expression changes caused by TGFβ.

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U1A is a positive regulator of the expression of heterologous and cellular genes involved in cell proliferation and migration

Rovira E[1]., Moreno B[1]., Razquin N[1]., Barrio R[2]., Ule J[3]., Pastor F[1]., Blázquez L[3,4]., Fortes. P[1]

[1.] CIMA / UNAV. Pamplona. Spain; [2.] CICBiogune. Bilbao. Spain; [3.] Francis Crick Institute. London. UK;[4.] Biodonostia. San Sebastian. Spain

Some years ago, we developed a U1snRNP-based inhibitory system termed U1 interference (U1i), which we demonstrated to be synergistic when combined with RNAi both in vivo and in vitro. We now show that such synergism is conserved also in Drosophila. In addition, experiments performed to address the molecular mechanism of synergy lead to the unexpected finding that U1A binding helps to increase target gene expression. This is contrary to previous knowledge showing that U1A inhibits the expression of U1A mRNA and other specific targets. We show by transcriptomic analyses that genome-wide, U1A is more an activator than a repressor of gene expression. Further, many U1A-deregulated genes are bound by U1A according to iCLIP studies. Additionally, we show that U1A-mediated positive regulation can be transferred to a heterologous system for biotechnological purposes. U1A binding sequences positioned at the 3´UTR of the gene of interest lead to an increase in expression of 2-4 fold. Finally, U1A bound genes are enriched for those involved in RNA binding and those playing relevant roles in neurological diseases and cancer. In agreement with this, U1A expression negatively correlates with both disease-free survival and overall survival in many cancer types and U1A levels correlate with those of proto-oncogenes involved in cell proliferation BCL2L12, or MYCBP, among others. In line, U1A depletion leads to decreased expression of these genes and the migration related gene CTGF, causing a strong defect in the expression of EMT targets, cell migration and proliferation. These results made U1A an unexpected therapeutic target for the treatment of several oncological malignancies.

Human prefoldin modulates co-transcriptional pre-mRNA splicing

Laura Payán-Bravo[1,2], Sara Fontalva[1,2], Xenia Peñate[1,2,], Ildefonso Cases[3], José Antonio Guerrero-Martínez[5], Yerma Pareja-Sánchez [1], Yosu Odriozola-Gil[1], Esther Lara[1], Silvia Jimeno-González[2,5], Carles Suñé[4], Mari Cruz Muñoz-Centeno[1,2], José C. Reyes[5] and Sebastián Chávez [1,2,*]*

[1]Instituto de Biomedicina de Sevilla, Universidad de Sevilla-CSIC-Hospital Universitario V. del Rocio, Seville, Spain,
[2]Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain,
[3]Centro Andaluz de Biología del Desarrollo, CSIC-Universidad Pablo de Olavide, Seville, Spain,
[4]Department of Molecular Biology, Institute of Parasitology and Biomedicine "López Neyra" IPBLN-CSIC, PTS, Granada, Spain
[5]Andalusian Center of Molecular Biology and Regenerative Medicine-CABIMER, Junta de Andalucia-University of Pablo de Olavide-University of Seville-CSIC, Seville, Spain

Prefoldin is a heterohexameric complex conserved from archaea to humans that plays a co-chaperone role during the co-translational folding of actin and tubulin monomers. Additional functions of prefoldin have been described, including a positive contribution to transcription elongation and chromatin dynamics in yeast. Here we show that prefoldin perturbations provoked transcriptional alterations across the human genome. Severe pre-mRNA splicing defects were also detected, particularly after serum stimulation. We found impairment of co-transcriptional splicing during transcription elongation, which explains why the induction of long genes with a high number of introns was affected the most. We detected genome-wide prefoldin binding to transcribed genes and found that it correlated with the negative impact of prefoldin depletion on gene expression. Lack of prefoldin caused global decrease in Ser2 and Ser5 phosphorylation of the RNA polymerase II carboxy-terminal domain. It also reduced the recruitment of the CTD kinase CDK9 to transcribed genes, and the association of splicing factors PRP19 and U2AF65 to chromatin, which is known to depend on CTD phosphorylation. Altogether the reported results indicate that human prefoldin is able to act locally on the genome to modulate gene expression by influencing phosphorylation of elongating RNA polymerase II, and thereby regulating co-transcriptional splicing.

SESSION 2

Functions and regulation of the RNAPII stalk domain

Olga Calvo and Araceli González-Jiménez

Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas, Universidad de Salamanca, Calle Zacarías González 2, 37007 Salamanca, Spain.

In eukaryotes, cellular RNAs are produced by three nuclear RNA polymerases (RNAPI, II, and III), which are multisubunit complexes. They share structural and functional features, although they are specialized in the synthesis of specific RNAs. The structure of RNAPII is highly conserved in all eukaryotes, consisting of 12 subunits (Rpb1-12) organized into five structural modules, among which the Rpb4 and Rpb7 subunits form the stalk. Early studies suggested an accessory role for Rpb4, which is required for specific gene transcription pathways. Far from this initial hypothesis, it is now well established that the Rpb4/7 heterodimer plays much wider roles in gene expression regulation. It participates in nuclear and cytosolic processes ranging from transcription to translation and mRNA degradation in a cyclical process. Therefore, the stalk plays a key role in integrating cellular responses to the environment with mRNA synthesis, translation, and decay, and places RNAPII as a critical regulator of the major stages of gene expression. We have discovered new functions to add to the list of stalk functions during transcription (1-4): first, a role in maintaining proper RNAPII phosphorylation levels, and second, a role in the establishment of looped gene architecture in actively transcribed genes. How Rpb4/7 performs so many different functions, spatially and temporally separated, regulating gene expression, is explained partly by its ability to interact with different nuclear and cytosolic complexes. However, how these interactions are regulated and how Rpb4/7 dissociates from the core polymerase at the end of transcription, staying bound to the mRNAs to exit to the cytoplasm, are fundamental unanswered questions. We hypothesize that post-translational modifications, specifically Rpb4 phosphorylation could regulate Rpb4/7 functions. We present here data demonstrating that indeed Rpb4 is phosphorylated *in vivo*, and we show preliminary data suggesting a role for Rpb4 phosphorylation during transcription.

References:

(1) Calvo (2020).RNA polymerase II phosphorylation and gene looping: new roles for the Rpb4/7 heterodimer in regulating gene expression. *Current Genetics.* 66: 927-937.

(2) Allepuz-Fuster et al (2019). RNA polymerase II plays an active role in the formation of gene loops through the Rpb4 subunit. *Nucleic Acids Research.* 47: 8975–8987.

(3) Garavís et al (2017). Sub1 contacts the RNA polymerase II stalk to modulate mRNA synthesis *Nucleic Acids Research.* 45: 2458-71.

(4) Allepuz-Fuster P et al. (2014). Rpb4/7 facilitates RNA polymerase II CTD dephosphorylation. *Nucleic Acids Research.* 42: 13674-13688.

Transcriptional regulation and DNA lesion detection by RNA polymerase I

Marta Sanz-Murillo, Adrián Plaza-Pegueroles, Sonia Huecas, Phong Nguyen, Federico M. Ruiz, Carlos Fernández-Tornero

Centro de Investigaciones Biológicas Margarita Salas, CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

RNA polymerases are central to transcription as they catalyze RNA synthesis using DNA as template. Malfunction of these cellular machines is related to a number of diseases including cancer. Eukaryotes require three different RNA polymerases, each transcribing a specific set of genes. In growing eukaryotic cells, about 60% of the total transcriptional activity involves the synthesis of ribosomal RNA (rRNA) by RNA polymerase I (Pol I), which in yeast is composed of 14 subunits [1]. This enzyme presents unique regulatory features, such as hibernation by self-dimerization and reactivation through Rrn3 binding [2,3]. During rRNA synthesis, Pol I monitors rDNA integrity and influences cell survival, but how this enzyme handles DNA lesions remains largely unknown. We used cryo-EM and *in vitro* transcription tests to investigate Pol I transcriptional stalling by one of the most common UV light-induced lesions, i.e. cyclobutane pyrimidine dimers (CPD) [4]. A two-step mechanism operates in Pol I to firmly stall the enzyme at CPD lesions, whereas RNA polymerase II (Pol II), which transcribes protein-coding genes, is able to bypass such lesions. RNA polymerase stalling constitutes a signal for recruitment of DNA repair factors, including the XPG endonuclease that cleaves the damaged DNA strand for its elimination. The crystal structure of the XPG endonuclease catalytic core in complex with DNA shed light on DNA incision by this enzyme and provided the molecular basis of genetic disorders associated to XPG mutations [5]. Our laboratory aims to further unravel the molecular mechanisms underlying cell endurance to different lesions on DNA and how this affects RNA synthesis.

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2. Torreira E, Louro JA, Pazos I, González-Polo N, Gil-Carton D, Duran AG, Tosi S, Gallego O, Calvo O, Fernández-Tornero C (2017) eLife 6:e20832
3. Fernández-Tornero C (2018) Transcription 9:248-254
4. Sanz-Murillo M, Xu J, Belogurov GA, Calvo O, Gil-Carton D, Moreno-Morcillo M, Wang D, Fernández-Tornero C (2018) Proc. Natl. Acad. Sci. USA 115:8972-8977
5. González-Corrochano R, Ruiz FM, Taylor NMI, Huecas S, Drakulic S, Spínola-Amilibia M, Fernandez-Tornero C (2020) Nucleic Acids Res 48, 9943-9958

SESSION 3

Exploring the function of the LSM2-8 complex through the characterization of a suppressor of the lsm8 mutation

Rafael Catalá, Eduardo Tranque, M. Fernanda Ruiz and Julio Salinas

Centro de Investigaciones Biológicas Margarita Salas-CSIC, Madrid

In the last years, results from several laboratories, including our, have revealed that the modulation of RNA splicing is key for adequate response of plants to environmental stresses. We demonstrated that *Arabidopsis* contains a fully functional LSM2-8 complex with an important role in pre-mRNA splicing. Our data unveiled that this complex displays high functional specificity to shape a correct and specific transcriptome reprogramming when plants are exposed to internal (development) or external (environmental stress) stimuli. To further understand the function of the LSM2-8 complex, we performed a screening of suppressors of the *lsm8* mutation in *Arabidopsis*. We isolated sol8, a mutant with restored wild-type leaf shape and tolerance to freezing temperatures. High-coverage RNA-seq experiments showed that sol8 also suppresses some of the alterations caused by *lsm8* mutation in pre-mRNA splicing. We found that the *sol8* suppressor phenotype is caused by a null mutation in ARIA, a gene encoding an armadillo repeat protein. Although ARIA null mutants do not display any apparent morphological phenotype, they are more sensitive to freezing temperatures than the wild-type plants. Moreover, RNA-seq analysis revealed that ARIA does not seem to regulate pre-mRNA splicing at 4 °C, but positively controls the expression of 71 genes, being 43 % of them cold-induced genes. Taken together, these data indicate that ARIA positively regulates plant tolerance to freezing temperatures by promoting cold-induced gene expression. The possible roles of ARIA in regulating cold-induced gene expression will be discussed.

Differential regulation of stress responses in plants by PAT1 proteins

Eduardo Tranque, M. Fernanda Ruiz, Ema Olate, Rafael Catalá and Julio Salinas

Centro de Investigaciones Biológicas Margarita Salas-CSIC, Madrid

The regulation of mRNA turnover is crucial to ensure an adequate response of plants to their environment. In our lab, we have demonstrated that the complex LSM1-7, a component of the decapping machinery, regulates the adaptation of plants to different abiotic stresses by targeting selected stress-inducible transcripts for decapping and subsequent degradation, depending on the stress conditions. Whether this feature is specific of the LSM1-7 complex or shared with other components of the decapping machinery remains unknown. To address this issue, we focused on the PAT1 proteins, which are, as the LSM1-7 complex, activators of mRNA decapping. Interestingly, the Arabidopsis genome encodes three PAT1 proteins, named PAT1, PAT1H1 and PAT1H2, but, so far, only PAT1 has been shown to participate in mRNA decapping. Moreover, the implication of Arabidopsis PAT1 proteins in plant response to abiotic stress has not yet been studied. Our results have revealed that the expression of the PAT1 genes is differentially regulated in response to low temperature, water deficiency and high salt. Furthermore, the characterization of *pat1*, *pat1h1* and *pat1h2* mutants has shown that PAT1 proteins are involved in regulating Arabidopsis tolerance to abiotic stress, and that each PAT1 controls the expression of specific stress-induced genes, depending on the environmental conditions. All these results suggest that PAT1 proteins could differentially regulate Arabidopsis tolerance to abiotic stresses by promoting selective mRNA decapping.

Investigating the roles of TCERG1 and PRPF40B in transcription and RNA Splicing

Cristina Moreno-Castro[1], María Duarte[1], Noelia Esteban-Rodríguez[1], Cristina Hernández-Munain[2], and Carles Suñé[1]

1Department of Molecular Biology and 2Department of Cell Biology and Immunology, Institute of Parasitology and Biomedicine "López Neyra" (IPBLN-CSIC), PTS, Granada 18016, Spain.

The functional coupling of transcription and alternative splicing is emerging as an essential component of gene expression regulation. Despite considerable efforts, numerous questions remain regarding the functional significance and global impact of this coupling on cellular and organism homeostasis as well as its underlying molecular mechanisms. The hypothesis of our work considers that coupling mechanisms enable the establishment of check-points or quality controls that would promote each step of pre-mRNA synthesis and processing while ensuring the accomplishment of the essential preceding stages. Proteins acting at the interface of these processes would serve as checkpoint factors to regulate co-transcriptional splicing. This work is mainly focused on two WW- and FF-containing proteins, which confer the potential ability to act as scaffolding elements, that have been shown to play dual roles both in transcription and splicing regulation and/or to physically interact with components of both the splicing and transcription machinery. These factors include TCERG1 and PRPF40B, one of the putative mammalian orthologues of the essential yeast splicing factor Prp40. Elucidation of the mechanisms through which these proteins function is objective of our research efforts. Here, we will present our most recent data on the interaction of TCERG1 with NOLC1/Nopp140, a nucleolar phosphoprotein that shuttles between the nucleolus and the Cajal bodies (CBs), in relation to the biogenesis of small nuclear ribonucleoproteins (snRNPs) to form the spliceosome. Regarding PRPF40B, we will show preliminary data that indicate a putative role for PRPF40B through the repressive PRC2 Polycomb complex.

SESSION 4

Study of the cellular component-dependent functions of Xrn1

Jordán-Pla, A[1]; Moreno-García, J[1]; Zhang, Y[2]; de Campos-Mata, L[3].; Choder, M[4].; Díez, J[3].; Pelechano, V[2]. García-Martínez, J[1]. and Pérez-Ortín, J.E[1].

[1]Instituto de Biotecnología y Biomedicina (Biotecmed), Universitat de València, Burjassot, Valencia, 46100, Spain.
[2]SciLifeLab, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna 171 65, Sweden.
[3]Virology Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.
[4]Department of Molecular Microbiology, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel.

The main decay pathway of yeast mRNAs in the cytoplasm uses the 5'-3' exonuclease Xrn1 (1). This protein shuttles from the cytoplasm to the nucleus, where it has a role as transcription factor (2). We have recently demonstrated that importing depends on two nuclear localization sequences (NLS 1 & NLS2) and that exporting depends on its binding (presumably co-transcriptional) to mRNAs (3). It is also known that Xrn1 is able to degrade decapped mRNAs that are still being translated by ribosomes (4). In this work, we analyze the pleiotropic functions of Xrn1 by comparing the phenotypes of yeast strains lacking Xrn1 or its capacity to be imported into the nucleus with those of the substitution of Xrn1 by a cytoplasmic version of the paralogous 5'-3'exonuclease Rat1 (cRat1). We find that most of the global phenotypes of an *xrn1* mutant are partially complemented by cRat1 indicating that this 5'-3'-exonuclease has a similar enzymatic capacity as Xrn1 and that the lack of a cytoplasmatic 5'-3'-exoribonuclease is the cause of the physiological defects of an *xrn1* mutant. The capacity of cRat1 to perform co-translational decay is, however, very limited. The comparison with the strain having a NLS1Δ-NLS2Δ-Xrn1 version shows that it is slightly deficient in 5'-co-translational decay but much more efficient than cRat1. In both strains, cRat1 and NLS1&2Δ-Xrn1, the lack of nuclear Xrn1 has a very minor influence on cell growth. However, in challenging growth conditions it has a determinant role suggesting that Xrn1 shuttling is more important in circumstances of physiological changes than in steady conditions and that co-translational decay has a limited role in yeast physiology.

References:

1.- Johnson AW. 1997. Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. Mol Cell Biol. 17(10):6122-30
2.- Haimovich G, et al. 2013. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. Cell. 153(5):1000-11
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4.- Pelechano V, Wei W, Steinmetz LM. 2015. Widespread Co-translational RNA Decay Reveals Ribosome Dynamics. Cell. 161(6):1400-12

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Rpb4 as a key element between the synthesis and degradation of mRNAs

Ana I. Garrido Godino

University of Jaen

Despite the Central Dogma of Molecular Biology establishes a linear flow of information from DNA to proteins, several groups have demonstrated that it could be considered a circular process coordinating transcription and mRNA decay. This coordination process, called mRNA crosstalk, allows maintaining the mRNA homeostasis. To keep in balance the mRNA levels, transcription machinery could influences mRNA decay process, as well as, mRNA decay elements could influence the transcription process.

We and others have demonstrate that the RNA polymerase II subunits Rpb4 and Rpb7 bind mRNA during transcription and remain associated with it throughout its life by regulating processes such as export, translation and decay. By imprinting mRNA, Rpb4 serves as a key protein to globally modulate mRNA stability and to coordinate transcription and decay under optimal grow conditions. In addition, Rpb4-mediated posttranscriptional regulation also plays a major role in controlling the Environmental Stress Response (ESR) at both the transcriptional and mRNA decay levels. Our data suggest that Rpb4 could cooperate with different RBPs to coordinate synthesis and degradation of specific sets of mRNAs. In agreement, our data demonstrate that Rpb4 cooperate with the RNA binding protein Puf3 regulating the Puf3 mRNA targets, and also, controlling Rpb4 and Puf3 association with the mRNAs and chromatin. According with this hypothesis, Rpb4 influences the stability of Xrn1, Ccr4 and Not1 and their association to chromatin.

Finally, we also propose that defects in RNA polymerase II biogenesis could impact mRNA stability. In agreement, we propose that the Ser5P-CTD phosphatase Rtr1 could influence RNA pol II biogenesis, probably promoting Rpb4 association with the rest of the enzyme (and likely of the Rpb4/7 subassembly complex). Our results demonstrate that *rtr1Δ* mutation provokes RNA pol II assembly defects, leading to chromatin-associated RNA pol II lacking the Rpb4 subunit, which affects Rpb4- mRNA imprinting and mRNA stability.

Taken together these data, we propose Rpb4 as a key element of RNA pol II acting on mRNA crosstalk regulation.

Bud27 and RNAs: some results and many questions

Francisco Navarro.

University of Jaen

Bud27 is a prefolin-like identified as interactor of the common subunit to the three eukaryotic RNA pols, Rpb5. It is a conserved protein in higher eukaryotes, whose orthologue is called URI. Bud27 is necessary for the biogenesis of the three RNA pols in an Rpb5-dependent manner, allowing the correct assembly of these complexes in the cytoplasm before their entry into the nucleus. This prefoldin-like shuttles between the cytoplasm and the nucleus were it participates in transcription. In addition, the role of Bud27 in the TOR pathway suggest its role in the transcription of the three RNA pols by mediating TOR-dependent processes such as ribosome biogenesis.

In this presentation, we will show evidences about the role of Bud27 regulating the transcription of the three RNA pols in *S. cerevisiae*, by mediating the elongation of RNA pol II via its interaction with the chromatin remodeler RSC, as well as the synthesis of transcripts required for ribosome biogenesis. We will raise data and hypotheses about the mechanisms mediating these processes, as well as about the influence of lack of Bud27 on processes such as polyadenylation site selection and chromatin arrangement on genes transcribed by the three RNA pols.

SESSION 5

Mip6 participates at different steps during RNA metabolism

Carme Nuño-Cabanes, Ana Tejada-Colón and Susana Rodríguez-Navarro

Instituto de Biomedicina de Valencia, CSIC

mRNA export is connected to upstream and downstream events during mRNA fate through the action of a repertoire of factors and multiprotein complexes. Among these factors, the yeast export receptor heterodimer **Mex67**-Mtr2 (TAP-p15 in humans). Apart from Mex67-Mtr2, many shuttling adaptor proteins are also recruited as part of the mRNPs. Besides their role in different mRNA processing steps, these adaptors might positively or negatively regulate mRNA export. Indeed some RNA-binding proteins -"*the guard proteins*",- might prevent unwanted Mex67 contacts as a mechanism to block undesirable RNA export by binding pre-mRNA to signal its unprocessed state. This quality control mechanism might also serve to regulate export routes under specific circumstances during cellular growth as for instance stress.

During the last years, the idea that export of specific mRNAs could be regulated emerged. A new view in mRNA export advocates that under normal conditions Mex67 binding to the maturing mRNA is blocked by the co-transcriptional decoration of the immature transcript with the guard proteins. In contrast, Mex67 binds directly to specific stress-responsive transcripts to facilitate their export potentially allowing these transcripts to bypass quality control. As part of our previous project, we discovered **Mip6** –a Mex67 associated factor as novel guard protein (1). Mip6 binds preferentially to heat shock transcripts that depend on *MSN2/4*. *MSN2/4* are zinc finger transcription factors that recognize STRE DNA sequences.

In this talk we will present our last advances in the understanding of Mip6 role in gene expression. We found significant expression changes of trehalose metabolism genes in *mip6*Δ cells through a multi-omic approach (2). Interestingly, Mip6 is also required for upstream transcriptional events as for instance histone acetylation and transcriptional memory under heat shock. Some of these results will be presented for discussion.

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1. Mip6 binds directly to the Mex67 UBA domain to maintain low levels of Msn2/4 stress-dependent mRNAs.
Martín-Expósito M, Gas ME, Mohamad N, **Nuño-Cabanes C, Tejada-Colón A**, Pascual-García P, de la Fuente L, Chaves-Arquero B, Merran J, Corden J, Conesa A, Pérez-Cañadillas JM, Bravo J, **Rodríguez-Navarro S***
EMBO Rep. 2019 Dec 5;20(12):e47964. doi: 10.15252/embr.201947964. Epub 2019 Nov 3.

2. A multi-omics dataset of heat-shock response in the yeast RNA binding protein Mip6.
Nuño-Cabanes C, Ugidos M, Tarazona S, Martín-Expósito M, Ferrer A, **Rodríguez-Navarro S*** and Conesa A*
Sci Data. 2020 Feb 27;7(1):69. doi: 10.1038/s41597-020-0412-z.

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The lipid composition of yeast cells modulates the response to iron deficiency mediated by the transcriptional factor Aft1

Tania Jordá[1], Antonia María Romero[1], Ana Perea-García[1,2], Nicolas Rozès[3], María Teresa Martínez-Pastor[2] and Sergi Puig[1]

[1]Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Paterna, Valencia, Spain.
[2]Departament de Bioquímica i Biologia Molecular, Universitat de València, Burjassot, Valencia, Spain.
[3]Departament de Bioquímica i Biotecnología, Universitat Rovira i Virgili, Tarragona, Spain.

Iron is a vital micronutrient because it participates as a redox cofactor in multiple metabolic pathways, including lipid biosynthesis. In response to iron deficiency, the *Saccharomyces cerevisiae* iron-responsive transcription factor Aft1 accumulates in the nucleus and activates a group of genes, collectively known as the iron regulon, which are involved in increasing the intracellular bioavailability of iron for essential processes. However, yeast cells with genetic defects in unsaturated fatty acids (UFAs) or ergosterol biosynthesis display a defect in the activation of the iron regulon when iron is scarce, which in turn contributes to limiting cell growth. Subcellular localization studies reveal that low levels of both UFAs or ergosterol cause a mislocalization of Aft1 protein to the vacuole under iron deprivation. The expression of an *AFT1* allele that is constitutively nuclear and activates the iron regulon regardless of the cellular iron status (*AFT1-1^{up}*) rescues both the iron regulon activation defect and the growth defect displayed by cells with defective UFA or ergosterol biosynthesis under iron starvation. Thereby, the lipid composition of yeast cells modulates the Aft1-dependent adaptation to iron deficiency.

The yeast mRNA-binding protein Cth2 regulates gene expression at the transcriptional and post-transcriptional level in iron deficiency.

A. M. Romero[1], *A. Garrido*[2], *J. García*[3], *F. Navarro*[2], *J. E. Pérez-Ortín*[3], *M. T. Martínez-Pastor*[3] *and S. Puig*[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Paterna, Valencia.
[2] Departamento de Biología Experimental – Genética, Universidad de Jaén (UJA), Jaén.
[3] Departamento de Bioquímica y Biología Molecular, Universitat de València, Burjassot, Valencia.

In response to iron deficiency, the budding yeast *Saccharomyces cerevisiae* undergoes a metabolic remodeling in order to optimize iron utilization. The tandem zinc finger (TZF)-containing protein Cth2 is specifically expressed in response to iron deficiency where it plays a critical role in adaptation by binding and promoting the decay and the translational repression of multiple mRNAs that contain AU-rich elements (AREs). This post-transcriptional regulation limits iron waste in non-essential pathways and activates essential iron-dependent processes like DNA synthesis and repair when iron is scarce. The molecular mechanism for this post-transcriptional regulation has been partially deciphered. Cth2 protein shuttles between the nucleus and the cytoplasm. Once inside the nucleus, Cth2 cotranscriptionally binds to its target transcripts and stimulates alternative 3' end processing that promotes nuclear mRNA turnover. Alternatively, the Cth2/mRNA-containing complex can be exported to the cytoplasm, where the mRNA is degraded by the 5' to 3' degradation pathway and its translation is inhibited. *In vivo* studies have determined the sequential assembly of mRNA decay factors with Cth2 during shuttling. Cth2 associates to the RNA helicase Dhh1 and the deadenylase Pop2/Caf1 complex before binding to its target mRNAs. However, the association between Cth2 and Xrn1 exonuclease and Cth2 nuclear export requires interaction with mRNA. In this work, we explore a potential role for the mRNA-binding protein Cth2 in regulating the transcription of its target mRNAs.

NMR studies of the low complexity domain of hnRNP A1: Conformational properties and nucleic acid recognition

Sergio Camero and José M. Pérez Cañadillas

Instituto de Química-Física "Rocasolano". CSIC

RNA binding proteins are often composed by folded domains (i.e. KH, RRM, etc.) embedded in long low complexity domains. These proteins have different RNA binding specificities, depending on the class and number of folded domains, and are frequently involved in Liquid Liquid Phase Separation (LLPS) phenomena. Both aspects are fundamental to understand their function. The hnRNP A1 is an abundant heteronuclear ribonucleoprotein with different functions on the cell including: mRNA packaging, splite-site selection, miRNA processing, etc. HnRNP A1 gene products contain two RRM in tandem in the N-terminal and a C-terminal low complexity domain (LCD) of variable length between the different isoforms. Here we have studied the LCD of the longest hnRNP A1 isoforms (372 residues for full-length protien) that represents about 20% population of hnRNP A1 proteins in vivo. This LCD have a RGG rich region followed by a YGG-rich region. We have obtained the full NMR assignment of the spectra of this LCD and analyzed the LLPS behavior of the wild type an several mutants. The hnRNP A1 LCD domain has low nucleic acids affinity compared with the RRM tandems. However, we have found that it has some structural selectivity for different nucleic acids. These results will be presented and discussed.

SESSION 6

Early role of ribosomal protein eL15 during the assembly of 60S ribosomal subunits in *Saccharomyces cerevisiae*

José Fernández-Fernández[1,2], *Jorge Pérez-Fernández*[3] *and Jesús de la Cruz*[1,2]

[1]Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío / CSIC / Universidad de Sevilla, Spain, [2] Departamento de Genética, Universidad de Sevilla, Spain, [3]Departamento de Biología Experimental-Genética, Universidad de Jaén, Spain.

Ribosome synthesis is a multistep process that includes the concomitant processing of the precursors of ribosomal RNAs and the assembly of ribosomal proteins. In eukaryotes, this complex process takes place successively in the nucleolus, the nucleoplasm and the cytoplasm. In the yeast *Saccharomyces cerevisiae*, it involves about 300 trans-acting factors and the 79 ribosomal proteins. The role of most ribosomal proteins in the biogenesis of each ribosomal subunit has been analysed and the timing of their in vivo assembly has been investigated. However, few ribosomal proteins still await functional characterization. Herein, we have analysed the contribution of ribosomal protein eL15 to ribosome biogenesis. We show that depletion of eL15 results in a severe shortage of 60S ribosomal subunits. Northern blotting, primer extension and pulse chase analyses indicate that processing of 27SA to 27SB pre-rRNAs as well as processing of 27SB to mature rRNAs is impaired upon the depletion of eL15. As a result, export of pre-60S particles from the nucleus to the cytoplasm is blocked. These phenotypes most likely appear as the direct consequence of the reduced pre-60S particle association not only of eL15 upon its depletion but also of a subset of neighbouring ribosomal proteins (e.g. eL13, eL36) and trans-acting factors either involved in the processing of 27SB pre-rRNA (e.g. Spb4, Nug1, Erb1, Spb1 or Has1) or 27SA3 pre-rRNA (e.g. Erb1, Nop7). These factors have likely not a direct role in the pre-rRNA processing reactions but a structural role in the formation of nucleolar pre-60S intermediates.

The RNA helicase Dbp7 is required for the release of the snR190 snoRNA chaperone from early pre-60S ribosomal particles in Saccharomyces cerevisiae

Sara Martín Villanueva[3], Mariam Jaafar[1,2], Julia Contreras[3,4], Carine Dominique[1], Régine Capeyrou[1], Patrice Vitali[1], Olga Rodríguez-Galán[3,4], Carmen Velasco[3,5], Odile Humbert[1], Nicholas J. Watkins[6], Eduardo Villalobo[3,5], Yves Henry[1], Raghida Abou Merhi[2], Jesús de la Cruz[3,4] and Anthony K. Henras[1]

[1]Laboratoire de Biologie Moléculaire Eucaryote, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UPS, 31000 France. [2]Genomic Stability and Biotherapy (GSBT) Laboratory, Faculty of Sciences, Rafik Hariri Campus, Lebanese University, Lebanon. [3]Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain. [4]Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain.[5]Departamento de Microbiología, Facultad de Biología, Universidad de Sevilla, Seville, Spain. [6]Institute for Cell and Molecular Biosciences, The Medical School, Newcastle University, Newcastle upon Tyne, UK.

Synthesis of eukaryotic ribosomal subunits involves the assembly and maturation of complex precursor particles (pre-ribosomal particles) containing ribosomal RNA (rRNA) precursors, ribosomal proteins and a plethora of assembly and maturation factors (AMFs). Formation of the earliest particle precursor to the large ribosomal subunit is among the least understood stages of ribosome biogenesis in eukaryotes. We have previously characterized the Npa1 complex as a protein module composed of five AMFs suggested to play a key role in the compaction of the central RNA core of the 25S rRNA within the earliest pre-60S r-particles in *Saccharomyces cerevisiae*. Most members of this complex display genetic interactions with the DEAD-box protein Dbp7, which is involved in the maturation of early pre-60S r-particles, but whose precise molecular function remains elusive. Here, we demonstrate a novel functional link between Dbp7 and snR190 box C/D snoRNA, which interacts predominantly with the Npa1 complex. This snoRNA has long been predicted to act as a methylation guide targeting a nucleotide of the Peptidyl Transferase Center (PTC) of the 25S rRNA, although the target methylation has not been identified yet. We show that snR190 is required for optimal yeast proliferation and efficient maturation of early pre-60S r-particles. We propose that snR190 functions as a novel snoRNA chaperone, which cooperates with the Npa1 complex to promote the compaction of the pre- rRNA in early pre-60S r-particles through two evolutionarily conserved antisense elements. We further show that deletion of DBP7 leads to an aberrant retention within pre-60S r-particles of snR190 and several modification guide snoRNAs targeting the PTC region of the 25S rRNA. In addition, loss-of-function of snR190 partially alleviates the growth defect of a strain lacking Dbp7 and restores early pre-60S r-particle maturation to some extent. We propose that Dbp7 regulates the dynamic base-pairing between snR190 and the pre-rRNA within the earliest pre-60S r- particles, thereby participating in the structuring of the PTC region of the large ribosomal subunit.

Gemin5, a multifunctional RNA-binding protein involved in translation control

Rosario Francisco-Velilla, Azman Embarc-Buh and Encarnación Martínez-Salas

Centro de Biología Molecular Severo Ochoa, Nicolás Cabrera 1, 28049, Madrid

RNA-binding proteins (RBPs) regulate all steps of RNA metabolism including protein synthesis. Gemin5 is an RBP involved in the assembly of splicing machinery, gene expression reprogramming, and translation control. This protein is organized in structural and functional domains that interact with distinct cellular targets. The N-terminal region comprises 14 WD repeat motifs (residues 1-739) that recognizes small nuclear RNAs, and also binds to the ribosome.

The C-terminal end bears a bipartite RNA-binding site, designated as RBS1 and RBS2 (residues 1287-1508). RBS1 is a non-canonical RNA-binding domain (RBD) that recognizes a sequence located within the coding region of Gemin5 mRNA, termed H12, consisting of two stem-loops, SL1 and SL2. Remarkably, Gemin5 stimulates its own translation through direct interaction between RBS1 and H12, providing a regulatory feedback loop that allows to fine-tune its cellular levels. An RNA-protein coevolution study predicted coevolving pairs between H12 and RBS1 showing all coevolving nucleotides within SL1. Biochemical and functional assays revealed that the PXSS motif in RBS1 is involved in the recognition of H12 through SL1. This study paves the way for the recognition of non-canonical RBDs carrying similar motifs.

We have also identified a dimerization domain within the central region of the protein. The crystal structure of this domain showed a tetratricopeptide (TPR) (residues 845-1097) that self-assembles into a compact canoe-shaped dimer and acts as a protein-protein interaction platform. Despite the tight association of this dimer, a single point mutation (A951E) at the closest inter-subunit distance was sufficient to destabilize it. We have proven that p85, a viral cleavage fragment that includes the TPR and the RBS domains, recruits the endogenous Gemin5 interfering with the role of Gemin5 in translation control.

The presence of distinct RBDs in Gemin5 facilitates the interaction with a wide variety of cellular mRNAs. In order to identify the mRNAs selectively translated by Gemin5, we performed a genome-wide analysis of mRNAs associated to polysomes in Gemin5-depleted cells relative to control cells. Among the transcripts displaying enhanced association to polysomes, there are mRNAs encoding for ribosomal proteins, histones, mitochondrial ribosomal proteins, proteins of cytochrome P450, and Sm and like-Sm proteins. Remarkably, we have found that Gemin5 stimulates translation of the TOP mRNA family, which includes all ribosomal protein transcripts.