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PO Box 117
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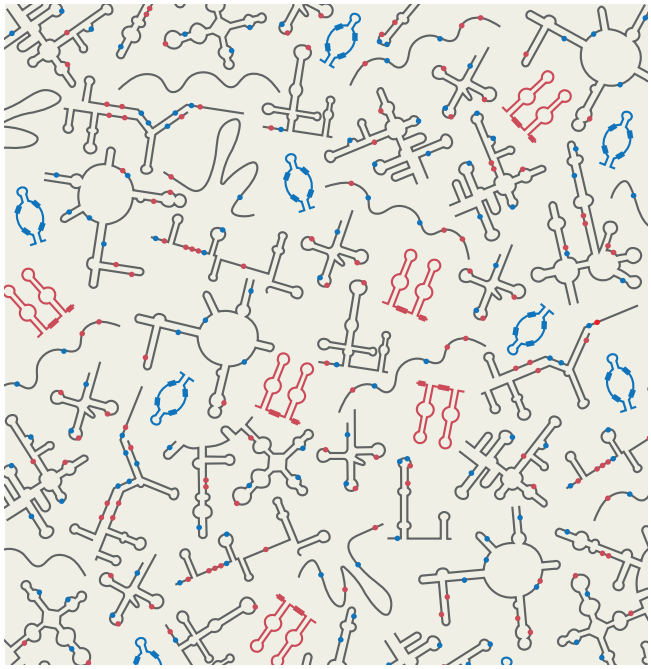
+46 46-222 00 00

RNA modifications and post-transcriptional control in cancer and stem cells

GIULIA BENEVENTI
DIVISION OF MOLECULAR HEMATOLOGY | FACULTY OF MEDICINE | LUND UNIVERSITY



RNA modifications are evolutionarily conserved hallmarks of gene expression regulation. Here is represented a multitude of RNA species modified with pseudouridine (red dots) and 2'-O-methylation (blue dots) guided by snoRNAs, drawn in red and blue.

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RNA modifications and post-transcriptional control in cancer and stem cells

RNA modifications and post-transcriptional control in cancer and stem cells

Giulia Beneventi

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended on December 8th 2021 at 9:00 in Segerfalksalen, BMC A10, Lund.

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Associate professor Jean-Yves Roignant

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Abstract

Splicing and translation are two of the key steps of post-transcriptional regulation of gene expression. Their tight regulation is essential for development, whereas their deregulation is involved in cancer pathogenesis. Nevertheless, many of the molecular mechanisms controlling these processes are still unknown. Hence, the main aim of this thesis is to elucidate novel regulatory mechanisms that affect splicing and translation in stem and cancer cells.

An emerging layer of regulation is represented by RNA modifications, evolutionarily conserved hallmarks of coding and non-coding RNA. Indeed, small nuclear RNA (snRNA) and ribosomal RNA (rRNA), the RNA components of the spliceosome and ribosome, are decorated with pseudouridines (Ψ) and 2'-O-methyl groups (2'OMe) within key functional regions. These modifications are introduced by RNA-dependent small ribonucleoproteins (snoRNPs), guided by snoRNAs and scaRNAs. In *Paper I* I identified the role of the SCARNA15-guided U2 snRNA-Ψ in driving alternative splicing events affecting the pivotal tumor suppressor p53 and redox homeostasis in cancer cells. In *Paper II* I unraveled the importance of the rRNA pseudouridylation machinery for the homeostasis of the hematopoietic system and the reconstitution capacity of HSCs *in vivo*. In *Paper III* I discovered a developmentally regulated 28S rRNA-2'OMe guided by SNORD123. The loss of this modification affected hESCs differentiation and caused translation defects perturbing the resistance to A-site specific antibiotics in fibroblasts. In *Paper IV* I highlighted a novel interplay between splicing and translation. Here, I uncovered a translationally regulated splicing factor, SF3A3, upon oncogenic stress which affects splicing of genes contributing to mitochondrial homeostasis and metabolism and that influences tumorigenesis of MYC-driven breast cancer.

In sum, this doctoral thesis explores novel post-transcriptional regulatory mechanisms, especially involving RNA modification modulation and dysregulation, with the aim to broaden the knowledge on stem and cancer cells functioning and to contribute to the discovery of future clinical implications.

Key words: RNA modifications, pseudouridine, 2'-O-methylation, splicing, translation, non-coding RNAs, cancer, stem cells, differentiation, stress response

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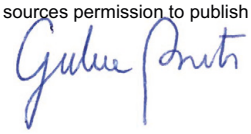
Signature  Date: 2021-10-25

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Giulia Beneventi

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“If you experiment, you have to fail. By definition, experimenting means going to territory where you’ve never been, where failure is very possible. How can you know you’re going to succeed? Having the courage to face the unknown is so important.”

Marina Abramović - Walk through walls

To my family

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Beneventi G, Munita R, Ngoc PCT, Madej M, Cieśla M, Muthukumar S, Krogh N, Nielsen H, Swaminathan V and Bellodi C.
NAR Cancer, 2021, Volume 3, Issue 3, zcab026

Paper II
Gar1 directs site-specific rRNA pseudouridylation and impacts self-renewal and survival of hematopoietic stem and progenitor cells
Beneventi G, Chikne V, Cieśla M, Ngoc PCT, Nir R, Soyris C, Vanhee S, Schwartz S, Yuan J and Bellodi C.
Manuscript in preparation, 2021

Paper III
The non-coding RNA SNORD123 drives a site specific rRNA 2'-O-methylation that is developmentally regulated and affects stem cell fate
Munita R*, **Beneventi G***, Ngoc PCT, Guzzi N, Cieśla M, Muthukumar S, Yudovich D, Larsson J and Bellodi C.
*Equal contribution
Manuscript in preparation, 2021

Paper IV
Oncogenic translation directs spliceosome dynamics revealing an integral role for SF3A3 in breast cancer
Cieśla M, Ngoc PCT, Cordero E, Sejas Martinez A, Morsing M, Muthukumar S, **Beneventi G**, Madej M, Munita R, Jönsson T, Lövgren K, Ebbesson A, Nodin B, Hedenfalk I, Jirstrom, Vallon-Christersson J, Honeth G, Staaf J, Incarnato D, Pietras K, Bosch A and Bellodi C.
Molecular Cell, 2021, Volume 81, Issue 7, 1453–1468

Articles not included in this thesis

The X-Linked DDX3X RNA Helicase Dictates Translation Reprogramming and Metastasis in Melanoma
Phung B*, Cieśla M*, Sanna A, Guzzi N, **Beneventi G**, Ngoc PCT, Lauss M, Cabrita R, Cordero E, Bosch A, Rosengren F, Häkkinen J, Griewank K, Paschen A, Harbst K, Olsson H, Ingvar C, Carniero A, Tsao H, Schadendorf D, Pietras K, Bellodi C° and Jönsson G°.
*° Equal contribution
Cell Reports, 2019, Volume 27, Issue 12, 3573-3586

Lin28b controls a neonatal to adult switch in B cell positive selection
Vanhee S, Åkerstrand H, Kristiansen TA, Datta S, Montano G, Vergani S, Lang S, Ungerbäck J, Doyle A, Olsson K, **Beneventi G**, Jensen CT, Bellodi C, Soneji S, Sigvarsson M, Jaensson Gyllenbäck E and Yuan J.
Science Immunology, 2019, Volume 4, Issue 39, eaax4453

Preface

Since the moment I started studying biology, I always had a fascination for the complexity of biological systems and for the billions of processes that simultaneously happen in all the tiny cells of our body to make life possible. They might seem extremely chaotic to our eye but, once you start to dig deep, they reveal how tightly regulated and magnificently programmed they are. I pursued my doctoral studies driven by the curiosity to understand this biological order.

During these years I focused my attention on the world of non-coding RNAs and RNA modifications with the aim to better understand their regulation and, when things go wrong, how and if they have a role to play in cancer development. My work, together with the others in the field, is adding an extra layer of complexity to the already complex picture we have of biological processes and increasing our knowledge of RNA biology. The hope is that the basic knowledge I gained now will contribute, even if just in a very small part, to more specialized and targeted therapies in the future.

Abstract

Splicing and translation are two of the key steps of post-transcriptional regulation of gene expression. Their tight regulation is essential for development, whereas their deregulation is involved in cancer pathogenesis. Nevertheless, many of the molecular mechanisms controlling these processes are still unknown. Hence, the main aim of this thesis is to elucidate novel regulatory mechanisms that affect splicing and translation in stem and cancer cells.

An emerging layer of regulation is represented by RNA modifications, evolutionarily conserved hallmarks of coding and non-coding RNA. Indeed, small nuclear RNA (snRNA) and ribosomal RNA (rRNA), the RNA components of the spliceosome and ribosome, are decorated with pseudouridines (Ψ) and 2'-O-methyl groups (2'OMe) within key functional regions. These modifications are introduced by RNA-dependent small ribonucleoproteins (snoRNPs), guided by snoRNAs and scaRNAs. In *Paper I* I identified the role of the SCARNA15-guided U2 snRNA-Ψ in driving alternative splicing events affecting the pivotal tumor suppressor p53 and redox homeostasis in cancer cells. In *Paper II* I unraveled the importance of the rRNA pseudouridylation machinery for the homeostasis of the hematopoietic system and the reconstitution capacity of HSCs *in vivo*. In *Paper III* I discovered a developmentally regulated 28S rRNA-2'OMe guided by SNORD123. The loss of this modification affected hESCs differentiation and caused translation defects perturbing the resistance to A-site specific antibiotics in fibroblasts. In *Paper IV* I highlighted a novel interplay between splicing and translation. Here, I uncovered a translationally regulated splicing factor, SF3A3, upon oncogenic stress which affects splicing of genes contributing to mitochondrial homeostasis and metabolism and that influences tumorigenesis of MYC-driven breast cancer.

In sum, this doctoral thesis explores novel post-transcriptional regulatory mechanisms, especially involving RNA modification modulation and dysregulation, with the aim to broaden the knowledge on stem and cancer cells functioning and to contribute to the discovery of future clinical implications.

List of abbreviations

Ψ	Pseudouridine
2'OMe	2'-O-methylation
3'ss	3' splice site
5'ss	5' splice site
AML	Acute myeloid leukemia
ASE	Alternative splicing event
CMCT	N-Cyclohexyl-N′-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate
DKC1	Dyskerin
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EBs	Embryoid bodies
FBL	Fibrillarin
G	Guanine
hESC	Human embryonic stem cell
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cell
IRES	Internal ribosome entry site
KO	Knock-out
LSU	Large subunit
m^6A	N^6-methyladenosine
MDS	Myelodysplastic syndrome

MEFs	Mouse embryonic fibroblasts
MPP	Multipotent progenitor
mRNA	Messenger RNA
ncRNA	Non-coding RNA
PRF	Programmed ribosomal frameshifting
PUS	Pseudouridine synthase
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
rRNA	Ribosomal RNA
scaRNA	Small Cajal body-specific RNA
SF	Splicing factor
snoRNA	Small nucleolar RNA
snoRNP	Small nucleolar ribonucleoprotein
snRNA	Small nuclear RNA
snRNP	Small nuclear ribonucleoprotein
SSU	Small subunit
TNBC	Triple negative breast cancer
tRNA	Transfer RNA
U	Uridine
UTR	Untranslated region
WT	Wild type
X-DC	X-linked Dyskeratosis Congenita

Introduction

RNA modifications

The central dogma is one of the fundamental principles of molecular biology that describes the flow of the genetic information encoded in the DNA, which is transcribed into RNA and finally translated into proteins. The linearity of this process is juxtaposed to an intricate network consisting of a multitude of regulatory layers that tightly control the molecular machineries involved at each critical step of DNA replication, RNA transcription and translation. Accumulating evidence illustrated that the addition of chemical modifications is pivotal for the accurate function of DNA, RNA, and proteins. The most studied, so far, are the modifications of DNA and histone proteins, collectively named epigenetics, which regulate the expression and transcription of genes (Allis and Jenuwein, 2016). Post-translational modification of proteins is also very well studied and can represent a fast way to inhibit, enhance or modify the activity of a given protein (Mann and Jensen, 2003). Similarly, it has long been known that non-coding and coding RNAs are heavily decorated with a collection of modifications, giving rise to a new research field recently named the "epitranscriptome" (Roundtree et al., 2017; Saletore et al., 2012). Among around 170 different RNA modifications (Boccaletto et al., 2018), I will focus my work on pseudouridine (Ψ) and 2'-O-methylation (2'OMe), which are some of the first modifications discovered and are very abundant on regulatory RNAs, such as ribosomal RNA (rRNA) and small nuclear RNA (snRNA), and how their dynamics regulate splicing and translation to coordinate gene expression.

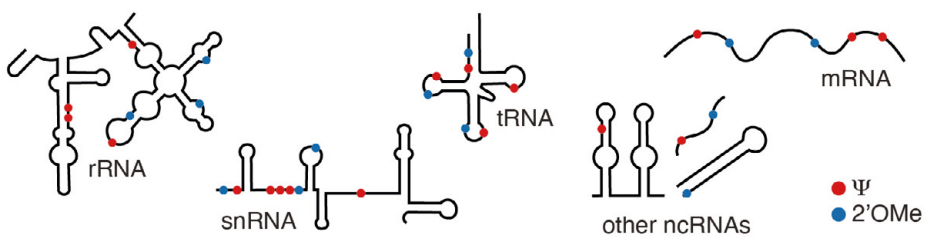

Figure 1: Schematic representation of the target RNAs modified with Ψ and 2'OMe.

snoRNAs and scaRNAs

Recent advances in high-throughput RNA sequencing approaches revealed that the majority of our genome encodes for a great variety of non-coding RNAs (ncRNAs) that play important roles in essentially every biological process that impacts cell fate (Morris and Mattick, 2014). An evolutionarily conserved class of ncRNAs consists of the abundant small nucleolar RNAs (snoRNAs), small RNAs of approximately 60-200 nucleotides length. In cells, snoRNAs are primarily assembled with proteins within small nucleolar ribonucleoprotein (snoRNP) complexes that are responsible for post-transcriptional modification of rRNA, snRNA, and also mRNA and other ncRNAs (Figure 1) (Carlile et al., 2014; Kiss, 2001; Schwartz et al., 2014). Interestingly, the vast majority of snoRNAs are encoded from the intronic regions of host genes; however, a minority is transcribed from an independent promoter (Tollervey and Kiss, 1997).

The role of snoRNAs is to provide the base complementarity to the target RNA and guide the snoRNP to the site of modification. They are classified in two main categories depending on the presence of evolutionarily conserved sequence motifs (Kiss, 2001). The first class is represented by H/ACA box snoRNAs, which guide Ψ modifications and are characterized by the presence of the conserved motifs known as box H (ANANNA) and box ACA (ACA), and two hairpin structural elements (Figure 2) (Kiss et al., 2010). The second class consists of C/D box snoRNAs, involved in 2'OMe, which possess distinct conserved sequence elements: the C (UGAUGA) and D (CUGA) boxes (Figure 2) (Kiss-Laszlo et al., 1998). Importantly, snoRNAs guiding modification of snRNA are considered a separate sub-class and are named small Cajal body-specific RNAs (scaRNAs). scaRNAs share a specific sequence (CAB box, with a consensus of UGAG) to promote their accumulation into the Cajal bodies, the major site of spliceosome assembly (Figure 2) (Kiss et al., 2010). They can belong to both the H/ACA and C/D box categories and sometimes they exist even as composite H/ACA-C/D scaRNAs (Kiss et al., 2010). Notably, snoRNAs and scaRNAs are frequently altered in cancers (Gong et al., 2017; Mannoor et al., 2012) such as hematological malignancies (Ronchetti et al., 2013; Ronchetti et al., 2012; Teittinen et al., 2013), solid tumors (Gao et al., 2015), and in the cancer-susceptibility syndrome X-linked Dyskeratosis Congenita (X-DC) (Bellodi et al., 2013).

A special category of snoRNAs enlists a multitude of so-called "orphan" snoRNAs, still harboring H/ACA or C/D box domains, but lacking a defined target and function yet (Dupuis-Sandoval et al., 2015). Recent data show that specific orphan

snoRNAs are deregulated in human diseases and cancer, and a non-canonical biological mechanism has been proposed for some members of this class in alternative splicing, cell growth and oncogenic signaling (Chu et al., 2012; Kishore et al., 2010; Kishore and Stamm, 2006; Siprashvili et al., 2016; Valleron et al., 2012). For instance, a processed version of SNORD115 regulates the alternative splicing of the serotonin receptor 5c affecting its expression and contributing to the pathogenesis of Prader-Willi syndrome (Kishore et al., 2010; Kishore and Stamm, 2006). SNORD50A and 50B are frequently deleted in cancer and their loss determines activation of K-Ras and the downstream MAPK pathway (Siprashvili et al., 2016). Nevertheless, the biological role of most of the orphan snoRNAs remains completely unknown. Interestingly, a recent study showed that some snoRNAs, including some orphan snoRNAs, have a tissue specific expression profile (Jorjani et al., 2016), indicating the possibility of specialized functions for these small RNAs.

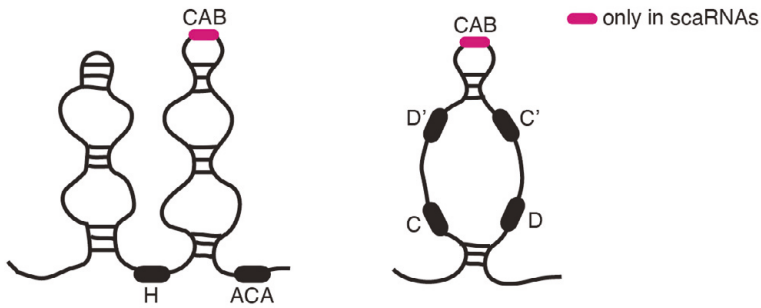

Figure 2: Representation of the two categories of snoRNAs with the respective sequence motifs: H/ACA box on the left and C/D box on the right. The CAB box is only present in scaRNAs.

Pseudouridine

The conversion of uridine into its isomer pseudouridine (Ψ) is the most abundant single-nucleotide RNA modification in living organisms (Kiss et al., 2010). Ψ possesses unique chemical properties determined by the formation of a C-C bond between the sugar and the base and the presence if an additional hydrogen bond donor site (Figure 3), which enhances the rigidity of the RNA backbone and increases base stacking (Charette and Gray, 2000). Ψ modifications are enriched on regulatory RNAs such as rRNA, snRNA and tRNAs, within clusters located in conserved important structural and functional regions. These include sequences involved in RNA-RNA and RNA-protein interaction regions of snRNA, the

peptidyltransferase and decoding center of rRNA and the anticodon stem and loop of tRNA (Charette and Gray, 2000). Recent advances in high-throughput Ψ sequencing methods also highlighted the presence of the modification in mRNA and other ncRNAs, expanding on the epitranscriptome (Carlile et al., 2014; Li et al., 2015; Lovejoy et al., 2014; Schwartz et al., 2014).

Pseudouridylation can be catalyzed via RNA-dependent or RNA-independent mechanisms. The RNA-dependent mechanism is driven by the snoRNP complex composed of an H/ACA snoRNAs and four evolutionarily conserved proteins: DKC1, which is the catalytic component, NOP10, NHP2 and GAR1 (Figure 3) (Kiss et al., 2010). Notably, the human telomerase RNA (hTERC) harbors the H/ACA domain at the 3' and assembles into a pre-RNP with H/ACA proteins NAF1, DKC1, NOP10, and NHP2, with a structural role that does not seem to involve catalysis of Ψ (Mitchell et al., 1999a). The RNA-independent mechanism is instead represented by the pseudouridine synthase (PUS) family of enzymes, which recognize the target RNA region using a consensus sequence or structural elements (Hamma and Ferre-D'Amare, 2006). The work of this thesis will focus on RNA-dependent complexes, while the PUS enzymes are investigated in other projects of our group.

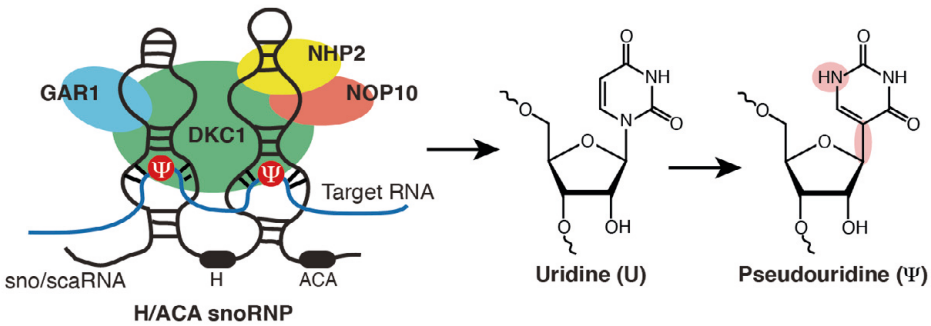

Figure 3: Pseudouridylation performed via the RNA-dependent mechanism. On the left the H/ACA snoRNP is represented with the target RNA in position for modification. On the right, highlighted in pink, the C-C bond and the extra hydrogen bond donor, which give Ψ its special chemical properties.

Pseudouridine has proven more challenging to detect and quantify in comparison to other RNA modifications. This is because of several reasons: Ψ has the same mass and creates the same Watson and Crick base pairings as uridine; it is "silent" in RNA sequencing since it does not introduce specific errors during reverse transcription as it happens, for instance, with inosine (Oakes et al., 2017); and there

are currently no antibodies that can recognize it specifically, as for example for N^6-methyladenosine (m^6A) (Saletore et al., 2012). Most of the currently used techniques for Ψ detection are based on the chemical CMCT, that binds Ψ with higher affinity compared to other nucleotides, U and G, from which it can be removed by mild alkali treatment (Ofengand et al., 2001). This binding has been exploited, since it causes the stop of reverse transcription reactions, for primer extension (Bakin and Ofengand, 1993) and was recently adapted for high-throughput sequencing (Carlile et al., 2014; Li et al., 2015; Lovejoy et al., 2014; Schwartz et al., 2014). These CMCT-based techniques have been used in *Paper I* and *II* to analyze snRNA and rRNA modifications. However, these assays are limited in their quantitative properties and the difference in sequencing and Ψ site calling methods between groups makes results more variable for the discovery of new sites in low abundance RNAs (Zaringhalam and Papavasiliou, 2016). Other techniques for Ψ detection are instead based on RNase H site specific cleavage of 2'-O-methyl RNA and DNA hybrids and the ability to distinguish Ψ from U in a thin layer chromatography (TLC) (Liu et al., 2013; Zhao and Yu, 2004). These methods, though, are labor-intensive, inefficient, and highly dependent on the design of a good RNase H oligo, which is mostly influenced by the sequence surrounding the modification. Very recently a study showed the possibility to detect Ψ by sequencing native RNA directly with Nanopore technology (Begik et al., 2021), opening new prospects for more reliable and direct Ψ identification with single molecule resolution.

2'-O-methylation

2'-O-methylation (2'OMe) is an evolutionarily conserved RNA modification consisting in the addition of a methyl group to the 2' hydroxyl of the ribose of a nucleoside (Figure 4) and it can stabilize single base pairs or hydrogen bonds and strengthen or alter RNA folds (Watkins and Bohnsack, 2012). As described for Ψ, 2'OMe is common in rRNA, snRNA and tRNA, and was also recently found in internal sites of mRNA (Dai et al., 2017). Moreover, 2'OMe sites location often corresponds with significant structural and functional regions of the target RNA. The effect of 2'OMe modification on structure and biogenesis has been predominantly studied in the context of rRNA and tRNAs, ranging from bacteria to yeast and vertebrates. On the other hand, the function of 2'OMe on mRNA, with few evidence showing an effect on translation, still remains largely unknown (Ayadi

et al., 2019). I will further expand on the function of 2'OMe modification in snRNA and rRNA in the following chapters.

2'-O-methylation is catalyzed via an RNA-dependent mechanism that involves C/D box snoRNAs assembled within snoRNPs together with four evolutionarily conserved protein components: FBL, which is the catalytic component, NOP56, NOP58 and SNU13 (Figure 4) (Watkins and Bohnsack, 2012).

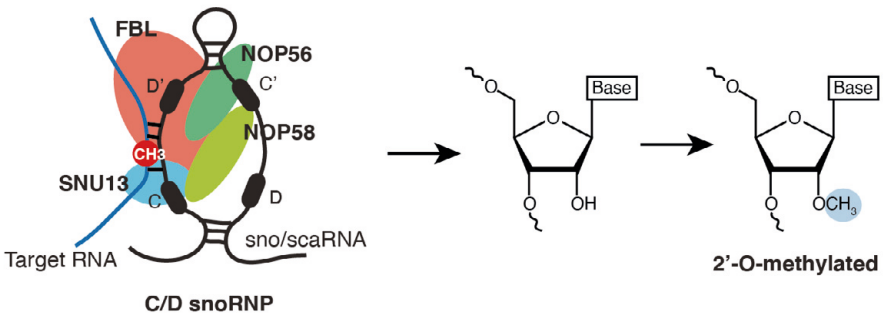

Figure 4: 2'-O-methylation performed by the C/D box snoRNP, shown on the left with the target RNA in position for modification. On the right the methyl group (CH_3) added to the nucleotide ribose is highlighted in light blue.

Several methods are available to detect and quantify 2'OMe, both in a site-specific and high-throughput manner, and they are based on three different properties of this RNA modification. The first class of techniques is based on the resistance of 2'OMe nucleotides to alkaline fragmentation. This principle is exploited in RiboMeth-seq (Birkedal et al., 2015; Marchand et al., 2016), which identifies a methylated nucleotide by the absence or less representation of read-ends at that specific site. This method quantitatively identifies sub-stoichiometric modification sites, even though the resolution is reduced with sites modified at low levels (below 30%). Another class of techniques is based on the resistance of 2'OMe nucleotides to RNase H site specific cleavage of 2'-O-methyl RNA and DNA hybrids, which I used in *Paper III* coupled with RT-qPCR quantification (Yu et al., 1997). Although this method is not high-throughput and does not enable discovery of new sites, it is quite robust in site-specific analysis, with the only limitation given by the quality of the RNase H oligo, which, as mentioned for Ψ detection techniques, is highly dependent on the sequence surrounding the modification. The last category of methods is based on the discovery that 2'OMe stops reverse transcription when

performed with low dNTP concentration. This approach was first used for simple primer extension (Maden et al., 1995) or coupled with qPCR quantification (used in *Paper III*) (Dong et al., 2012) for site-specific detection and, more recently, was adapted for sequencing (Incarnato et al., 2017).

RNA modifications and cancer

Emerging evidence has revealed that RNA modification pathways are frequently altered in human disease and cancer and the most well studied modifications in this context have been so far rRNA and tRNA modifications (Barbieri and Kouzarides, 2020; Janin et al., 2020). Mutations of the pseudouridine synthase DKC1 have been associated with X-linked Dyskeratosis Congenita (X-DC). X-DC is characterized by bone marrow failure, skin abnormalities, and high cancer susceptibility, including hematological malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (Heiss et al., 1998; Mitchell et al., 1999b; Montanaro, 2010). Defects in DKC1 and rRNA-Ψ have been associated with aberrant translation fidelity (Jack et al., 2011) and IRES-mediated translation leading to impairments in specific mRNAs encoding tumor suppressors, such as p53 (Bellodi et al., 2010a; Montanaro et al., 2010) and p27 (Bellodi et al., 2010b; Yoon et al., 2006), in X-DC and solid tumors. Moreover, dysregulation of individual snoRNAs have also been reported in cancer (Gong et al., 2017; Mannoor et al., 2012). For example, depletion of SNORA24, which guides two rRNA-Ψ sites, is correlated with reduced translation accuracy and development of liver cancer in mouse, and poor prognosis in human hepatocellular carcinoma (McMahon et al., 2019). Additionally, data from our group showed that PUS7-dependent pseudouridylation of specific tRNA-derived fragments has an impact on global protein synthesis and HSCs function and is reduced in specific subtypes of MDS with high risk of transformation to AML (Guzzi et al., 2018). Another recent study reported that inhibition of PUS7-dependent tRNA pseudouridylation affects the tumorigenic potential of glioblastoma stem cells (Cui et al., 2021).

Altered 2'OMe has also been linked to cancer (Janin et al., 2020). For instance, high levels of the methyltransferase FBL in breast cancer cause alteration of the rRNA 2'OMe pattern, which leads to defects in translation fidelity and IRES translation contributing to tumorigenesis and representing a poor prognostic factor (Marcel et al., 2013). Similarly, it was also shown by another group that FBL levels and snoRNA biogenesis are frequently increased in breast and prostate cancer, with important roles for the tumorigenic potential *in vitro* and *in vivo* (Su et al., 2014).

Subsequently, rRNA 2'OMe was also associated with leukemia by the identification of a link between the fusion protein AML1-ETO and enhanced snoRNAs functionality. The study showed how abrogation of specific rRNA methylation sites affected the clonogenic potential *in vitro* and leukemogenesis *in vivo*, without however showing the direct effects on translation (Zhou et al., 2017).

A wealth of studies has highlighted a key role for m^6A, another very common mRNA modification, in cancer pathogenesis. Important works by different groups have identified a connection between defects m^6A writers and erasers in leukemia and solid tumors, with examples of both mRNA and rRNA modification (Barbieri and Kouzarides, 2020; Shen et al., 2020; Vu et al., 2019). However, I will not discuss the details by which m^6A contributes to tumorigenesis in my thesis. Taken together, this body of evidence strengthens the importance of RNA modifications in cancer biology, opening new avenues for developing prognostic and therapeutic possibilities. One recent example is the pharmacological inhibition of the methyltransferase METTL3, which has been tested as therapeutic strategy for AML in cell lines and mouse models (Yankova et al., 2021). Nevertheless, very much is still unknown, especially regarding defects of snRNA modifications, which have been largely overlooked so far and that I will expand more on with the work of this thesis.

Splicing

Splicing is a central cellular process that enables removal of intronic sequences from pre-mRNAs and it governs the flow of the genetic information by directing the maturation of the mRNA that will be finally translated into proteins. Splicing was first observed in the 1970s in adenoviruses by Philip Sharp and Richard Roberts, which were awarded the Nobel Prize for their discovery (Berget et al., 1977; Chow et al., 1977). Splicing is a complex and energy demanding process that involves a highly dynamic multi-subunit machinery and undergoes multiple layers of regulation, many of which are still unknown. In this thesis, I will primarily focus on snRNA modification dynamics and splicing factor abundance as novel splicing regulatory mechanisms, which can be hijacked by cancer cells to improve their survival and increase tumorigenic potential.

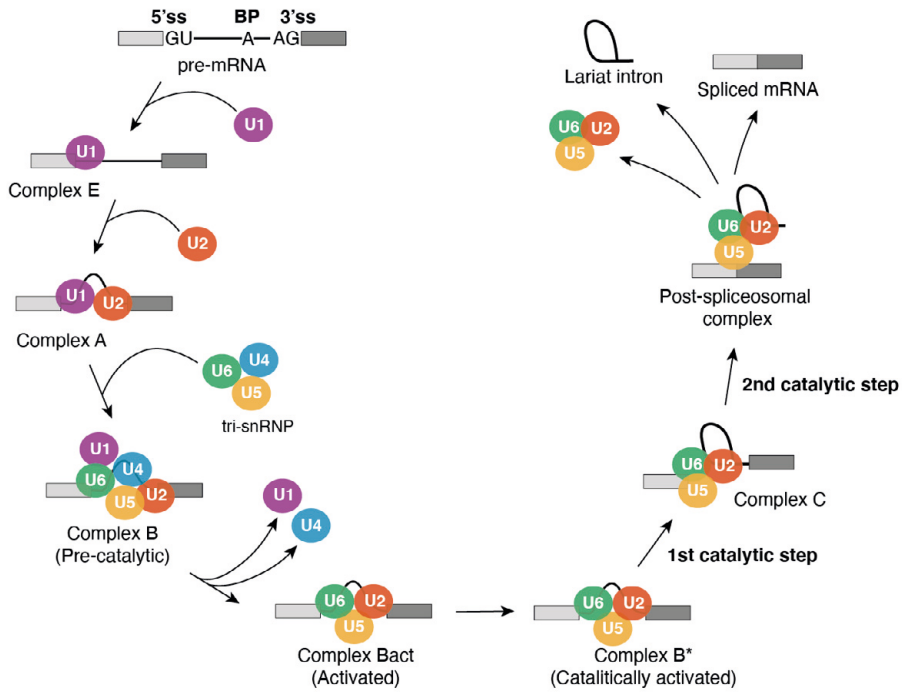

Figure 5: Simplified schematic of the main steps of the splicing process showing the formation of the different dynamic complexes. On the top left, the intron is represented highlighting the 5'ss, branchpoint (BP) and 3'ss signals with the respective sequence elements. Figure adapted from Will and Luhrmann, 2011.

The spliceosome and splicing

The spliceosome is a dynamic macromolecular machinery consisting of five small nuclear ribonucleoproteins (snRNPs), each with a core snRNA backbone bound to several core and associated splicing factors (Will and Luhrmann, 2011). Two unique spliceosomes coexist in eukaryotic cells, including humans: the major (or U2-dependent) spliceosome, which is composed of U1, U2, U4, U5 and U6 snRNPs, and the minor (or U12-dependent) spliceosome, which is composed of U11, U12, U4atac, U5 and U6atac snRNPs (Will and Luhrmann, 2011). The major spliceosome catalyzes the removal of U2-type introns, which represent most of the introns, while the less abundant minor spliceosome splices the rare U12-type class of introns. U12-type introns are mainly present in genes related to processes such as DNA replication and repair, transcription, RNA processing, and translation (Turunen et al., 2013).

The primary signals that define the boundaries between exons and introns are the 5' splice site (5'ss) at the beginning of the intron, the 3' splice site (3'ss) at the end of the intron, and the branch point, which is usually optimally located around 15-50 nucleotides upstream the 3'ss (Figure 5). These primary signals are also accompanied by several *cis*-acting regulatory elements, which can enhance or reduce the splicing of specific introns (Matera and Wang, 2014). During the splicing reaction, introns are removed from the pre-mRNA by two consecutive transesterification reactions. The first reaction is the nucleophilic attach from the 2'OH of the branch point adenosine on the 5'ss, which leads to the cleavage at the 5'ss and to the formation of the lariat structure. This is followed by a second reaction where the 3'ss is attached by the 3'OH group of 5' exon, enabling the ligation of the 5' and 3' exons and the release of the intron. The execution of these two chemical reactions is made possible by a complicated orchestrated process guided by the snRNPs and many co-factors that dynamically assemble and dis-assemble forming different complexes (Figure 5) (Matera and Wang, 2014; Will and Luhrmann, 2011). The process of spliceosome assembly is very well described in yeast, but the main steps are shared in humans. Briefly, the first to be formed is complex E, where the U1 snRNP binds the 5'ss of the intron. Then, the U2 snRNP binds the branch point sequence and the 3'ss and interacts with the U1 snRNP forming the pre-splicing complex A. At this point, the pre-catalytic complex B is formed by the addition of the pre-assembled tri-snRNP U4/U6.U5. In the next step, the U1 and U4 snRNPs are released and U6 snRNA substitutes U1 in the binding of the 5'ss to form the activated B complex. Subsequently, the B* catalytically activated complex performs the first transesterification reaction, and the complex C is formed, where

the 5’ exon end is free, and the lariat structure is bound to the 3’ exon. The complex C performs the second catalytic step, leaving a post-splicing complex from which the lariat, the spliced mRNA and the snRNP will be released (Figure 5) (Matera and Wang, 2014; Will and Luhrmann, 2011). snRNAs are key for most of the steps and allow the recognition of specific structures in the pre-mRNA introns, for instance U1 snRNA with the 5’ss or U2 snRNA with the branch point (Will and Luhrmann, 2011). Interestingly, snRNAs, and not the proteins, are the elements that yield the catalytic activity during the splicing process (Valadkhan et al., 2009; Valadkhan et al., 2007).

snRNA modifications

snRNAs are heavily modified molecules harboring several Ψ and 2’OMe sites clustered in key functional regions critical for snRNA biogenesis, structure, and function, that involve intramolecular snRNPs interactions and pre-mRNA binding (Ge and Yu, 2013; Karijolich and Yu, 2010). Some examples are the U2 region involved in branchpoint recognition, the interaction areas between U4 and U6, and the region of U1 which contacts the 5’ splice site on the pre-mRNA (Ge and Yu, 2013; Karijolich and Yu, 2010). Given the location of most modifications along the snRNA secondary and tertiary structure, it has been suggested that they might be implicated in structure maintenance as well as in the splicing function of snRNAs (Karijolich and Yu, 2010). Indeed, it has been shown that U2 snRNA Ψ and particularly those located close to the branchpoint recognition site are essential for U2 snRNP assembly and proper pre-mRNA splicing in *Xenopus* (Yu et al., 1998; Zhao et al., 2002). Moreover, 2’OMe at the 5’ of U2 snRNA were shown to be individually required for pre-mRNA splicing in HeLa cells (Donmez et al., 2004). Although snRNA modifications were long considered static and constitutive, this assumption has been challenged by recent data showing that some U2 and U6 pseudouridylations are instead inducible in yeast, especially upon stress conditions such as nutrient deprivation and filamentous growth (Basak and Query, 2014; Wu et al., 2016; Wu et al., 2011). Another study recently indicated that specific snRNA 2’OMe sites are sub-stoichiometric upon T cell activation and in a T cell leukemia cell line (Krogh et al., 2017).

Seminal work in yeast cells and other organisms has unraveled the central role of snRNA modifications within the splicing machinery; however, the contribution and the dynamics of snRNA modifications in humans and disease remain not fully understood. Very recent evidence started to highlight the importance of snRNA

modifications for splicing fidelity in development, for example U6 2'OMe in connection to a developmental syndrome in humans (Hasler et al., 2020) and to spermatogenesis in mouse (Wang et al., 2020), as well as a SCARNA1-driven U2-Ψ for heart development (Nagasawa et al., 2020; Patil et al., 2015). Nevertheless, dysregulation and dynamics of snRNA modifications in tumorigenesis remain an outstanding question, which I explored in *Paper I* and will further discuss in the next chapters.

Alternative splicing

Alternative splicing is the process that enables the formation of multiple mature mRNAs from the same pre-mRNA, thus expanding the coding capacity of the genome. There are several types of alternative splicing events: cassette-exon alternative splicing, which is the most common type and consists in the exclusion or inclusion of a single exon; alternative 5' and 3' splice sites; intron retention; and, lastly, mutually exclusive exons (Figure 6) (Blencowe, 2006). It is now known that the vast majority of human genes, around 90-95%, undergoes alternative splicing (Pan et al., 2008; Wang et al., 2008), thus making very important to understand the final functional outcome of such events. Alternative splicing can generate different protein isoforms from the same gene, thus expanding the cell's coding capacity. A notable example is the gene Bcl-x that can produce two different protein isoforms by alternative splicing, Bcl-xL and Bcl-xS, which have opposite effect on apoptosis (Boise et al., 1993). Another option is that the alternative protein isoforms contain new regions that determine an increase or decrease of protein stability, as we show in *Paper IV* for a critical regulator of mitochondrial dynamics, namely DRP1 (Ciesla et al., 2021). It is also possible that alternative splicing directly affects the stability and the localization of the mRNA, by altering both translated and untranslated regions (Baralle and Giudice, 2017; Blencowe, 2006). Although it was recently shown that most of the alternatively spliced mRNAs with exon skipping events and high-medium abundance are associated to polysomes (Weatheritt et al., 2016), yet not all the products of alternative splicing are translated. A big part of the events, around 30%, determine the introduction of an in frame premature termination codon (PTC) which might target the mRNA for non-sense mediated decay (NMD) (Lewis et al., 2003) and define a way to control gene expression of specific genes. There is still a debate in the field on how much these PTCs directly yield mRNA decay as it was shown that these aberrant variants occur with very low frequency (Pan et al., 2006).

Alternative splicing was shown to be key for development and tissue specification in various organs and cell types, for example brain, muscle, pancreas, liver, and the hematopoietic system (Baralle and Giudice, 2017). Moreover, it was also shown that alternative splicing is affected in several human diseases and cancers by genetic mutations of splicing signals (Cartegni et al., 2002). The potential role of splicing factors (SFs) and snRNAs as determinants of alternative splicing variability remains an open question, which will be further discussed in the context of cancer in the next chapter and that has been one of the main focuses of this thesis work.

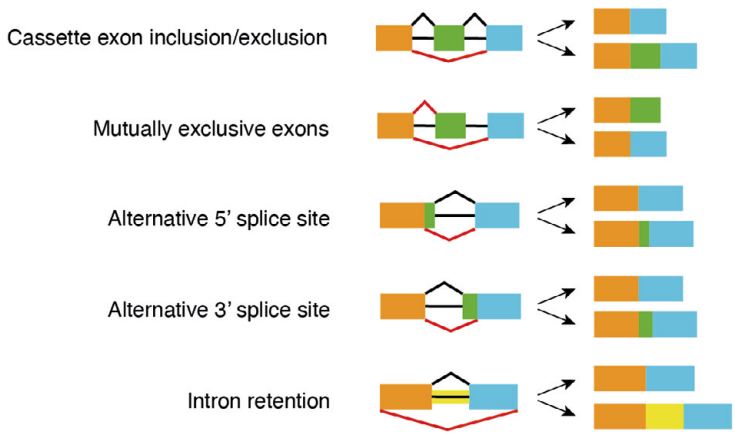

Figure 6: Representation of the main categories of alternative splicing events. With black and red lines are shown the two different combinations for each type of event, which can give rise the two products depicted on the right.

Splicing and cancer

There is a growing realization that splicing is frequently altered in many cancer types and that alternative splicing affects genes involved in all the hallmarks of cancer (Oltean and Bates, 2014; Sveen et al., 2016). The recent discovery of splicing factors mutations in a large number of hematological cancers and solid tumors has highlighted a direct role for splicing in disease pathogenesis (Dvinge et al., 2016). One notable example is the mutation of the splicing factor SF3B1, which affects selection of 3' splice sites and induces aberrant splicing and decay of many mRNA targets (Darman et al., 2015). However, it has been shown that splicing alterations can occur even in absence of splicing factor mutations (Danan-Gotthold et al., 2015; Dvinge and Bradley, 2015; Simon et al., 2014). For example, a way to alter the splicing patterns in cancer could be via regulation of the abundance of specific

splicing factors. Interestingly, recent studies showed how the oncogene MYC regulates the levels of PRMT5, BUD31 and SRSF1 splicing factors to ensure accurate splicing and cancer cell survival and growth (Das et al., 2012; Hsu et al., 2015; Koh et al., 2015). Nevertheless, the mechanisms whereby oncogenes hijack the spliceosome to promote tumorigenesis remain poorly understood. With our work in *Paper IV*, we identified a translation-based program downstream MYC that controls the levels of SF3A3 splicing factor to ensure accurate splicing of genes important for survival and tumorigenesis in breast cancer cells (Ciesla et al., 2021). Another element that could contribute to the spliceosome regulation is the catalytic snRNA component. A recent study showed how mutations of U1 snRNA can function as cancer drivers (Shuai et al., 2019), but other involvements of snRNA in cancer alternative splicing remain largely unexplored. Based on the evidence that scaRNAs are frequently altered in cancer (Gong et al., 2017; Ronchetti et al., 2013; Ronchetti et al., 2012; Teittinen et al., 2013), we hypothesized a relevant contribution of snRNA modification dynamics in tumorigenesis, which so far has been overlooked. Following up on this hypothesis, in *Paper I* I uncovered the role of SCARNA15 in regulation of specific U2 snRNA-Ψ and cancer promoting splicing events, that promote the survival through regulation of the cellular stress response (Beneventi et al., 2021). In conclusion, all these studies show the central role of splicing in tumorigenesis and highlight splicing as a cancer prognostic factor and therapeutic vulnerability to be further exploited.

Translation

Translation is the process that decodes the information contained in the mature mRNA into proteins and it is one of the most critical and energy demanding cellular processes. It requires tight regulation by coordination of several players such as ribosomes, mRNA, tRNAs and a multitude of additional and regulatory factors, which can be represented by proteins or RNAs. It is not surprising that defects in multiple steps of translation regulation are often observed in human diseases ranging from, for example, genetic ribosomopathies to cancer (Tahmasebi et al., 2018; Vaklavas et al., 2017). Because of the complexity of the translation process, still many layers of regulation remain uncharacterized and especially their biological role is still largely unexplored. *Paper II* and *III* are centered on ribosome function and translation control mediated by changes in rRNA modifications, specifically in the context of embryonic and hematopoietic stem cell biology.

The ribosome and translation

The ribosome is the machinery that performs translation of spliced mRNAs into proteins. The eukaryotic ribosome, also known as 80S ribosome, is made of two subunits both composed of rRNA and several proteins. The large subunit (LSU), or 60S subunit, is composed of the RNA scaffolds 28S, 5S and 5.8S rRNA together with 47 proteins, while the small subunit (SSU), or 40S subunit, has only 18S rRNA as scaffold together with 33 proteins (Ben-Shem et al., 2011; Khatter et al., 2015). The LSU contains the peptidyltransferase center, where the peptide bond is formed, while the SSU is mostly responsible for the decoding process and the proper binding between the mRNA codon and the tRNA anticodon (Sloan et al., 2017). The "old school" assumption of ribosomes as static machineries has been challenged over the past years by several studies supporting the notion that ribosomes are heterogeneous with respect to their composition and function (Gay et al., 2021; Genuth and Barna, 2018). Potential sources of heterogeneity are the tissue specific expression of ribosomal protein paralogs or different rRNA alleles, the presence of sub-stoichiometric levels of core ribosomal proteins or the addition of several ribosome associated proteins, post-translational modifications of ribosomal proteins and rRNA modifications (Gay et al., 2021; Genuth and Barna, 2018). Although the exact function of all these heterogeneous ribosomes is mostly uncharacterized, this idea opens great possibilities for additional layers of translation regulation. In *Paper III*,

I studied rRNA modifications as a potential source of heterogeneity and regulation of specific ribosome properties during development.

The process of mRNA translation is divided into four main stages: initiation, elongation, termination, and ribosome recycling (Figure 7). In this section, I will briefly describe all the steps focusing on initiation and elongation, which are relevant for this thesis. Translation initiation involves several eukaryotic translation initiation factors (eIFs) and consists of multiple phases leading to the formation of an 80S ribosome competent for elongation, with the Met-tRNA$_i^{Met}$ anticodon bound to the start codon of the mRNA in the P site (Jackson et al., 2010; Sonenberg and Hinnebusch, 2009). The main mechanism for initiation involves the binding of the 43S pre-initiation complex (PIC) close to the 5'cap of the mRNA through the binding with the eIF4F cap-complex. The eIF4F cap-complex is formed by eIF4E, the cap-binding protein, eIF4G, that acts as a scaffold, and eIF4A, an RNA helicase that unwinds the secondary structures present in the 5' UTR. The PIC scans the 5' UTR to find the AUG start codon and enable the pairing of the Met-tRNA$_i^{Met}$ anticodon to form the 48S complex. Then, the 60S subunit is bound, the eIFs are released and the 80S is formed (Figure 7) (Jackson et al., 2010; Sonenberg and Hinnebusch, 2009). This process is called cap-dependent translation initiation.

Although the cap-dependent mRNA activation is mainly reliant on the eIF4F cap-complex and other factors, it was recently shown that cap-dependent initiation can also be stimulated by the eIF3 complex by binding internal secondary structures of the mRNA 5' UTR and with eIF3D as the cap-binding protein (Figure 8) (Lee et al., 2015; Lee et al., 2016). In *Paper IV* we found that the latter mechanism regulates translation of the splicing factor SF3A3 levels in response to the oncogene MYC (Ciesla et al., 2021). There are other non-canonical initiation mechanisms including internal ribosomal entry sites (IRES), structural motifs present in the 5' UTR which enable ribosome recruitment bypassing the cap recognition complexes (Figure 8) (Cullen, 2009). IRES elements were first discovered in picornaviruses (Jang et al., 1988; Pelletier and Sonenberg, 1988) and, subsequently, in hepatitis C virus (HCV), which recruits the 43S pre-initiation complex with only a few initiation factors (Pestova et al., 1998) or even directly the translating 80S ribosome (Yokoyama et al., 2019), and in cricket paralysis virus (CrPV), which directly recruits the 40S small subunit (Pestova and Hellen, 2003; Wilson et al., 2000). Interestingly, IRES elements are also present in cellular mRNAs; however, much less is known about their exact structure and regulatory functions. Cellular IRES-mediated translation is important during stress conditions when canonical cap-dependent translation is reduced or inefficient (Cullen, 2009; Schuster and Hsieh, 2019). As previously

described, IRES translation is affected and contributes to tumorigenesis in the disease X-DC, where rRNA-Ψ modifications are reduced (Bellodi et al., 2010a; Bellodi et al., 2010b; Yoon et al., 2006).

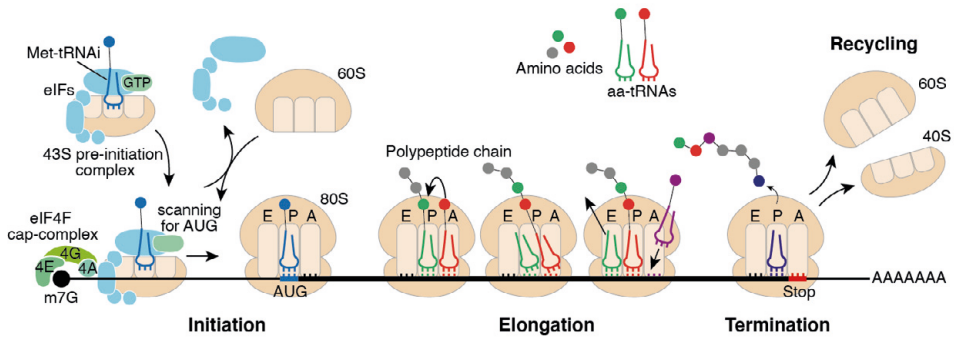

Figure 7: Simplified schematic of the main phases of translation. In the initiation phase, the 43S pre-initiation complex binds close to the 5'cap through the eIF4F cap-complex and starts to scan for the AUG start codon. Once the start codon is found, the 60S subunit joins and the eIFs are released. For the elongation phase the ribosomes are represented in different states: with P- and A-site occupied by tRNAs to form the peptidyl bond (arrow); in the "hybrid" state, where tRNAs occupy different states on the two subunits, before the translocation; with the unloaded tRNA in the E-site about to exit the ribosome and the new aa-tRNA that needs to bind the A-site to restart the cycle. Then, also the termination and recycling phase are shown. Figure adapted from Schuller and Green, 2018 and Saba et al., 2021.

Translation elongation is characterized by the 80S ribosome moving along the mRNA, three nucleotides at the time, encoding the mRNA codons into a polypeptide chain using aminoacyl-tRNAs (aa-tRNAs), which are the tRNA molecules charged with the amino acid correspondent to the anticodon. During elongation, tRNAs transition between three important ribosomal domains: the aminoacyl (A) site, the peptidyl (P) site and the exit (E) site. The A-site accommodates and binds the incoming aa-tRNAs, the P-site accommodates the tRNA with attached the nascent polypeptide chain and the E-site contains the uncharged tRNA that will then exit the ribosome (Figure 7) (Schuller and Green, 2018). The process is regulated by elongation factors (eEFs) including eEF1A and eEF2, which regulate the tRNA binding to the A-site and the transition state, or "hybrid" state, where tRNAs are partially positioned in two sites and need to translocate to enable the formation of the next peptide bond (Schuller and Green, 2018). During the elongation, the ribosome may encounter some obstacles that slow down the process. These include specific amino acid combinations or rare cognate tRNAs, which might stall the

ribosome, and strong secondary structures, which might stop elongation (Schuller and Green, 2018). For example, –1 programmed ribosomal frameshifting (PRF) is a well-established event affecting elongation that is promoted by secondary structures consisting of pseudoknots and slippery sequences (Figure 8). These were originally identified in viruses to induce elongating ribosomes to slip one-base in the 5' direction to a –1 coding frame, and so enabling the virus to encode multiple genes in a small genome and to produce the proteins with a precise ratio (Jacks and Varmus, 1985). Moreover, –1 PRF signals have also been found in cellular mRNAs, where they seem to have more a function in mRNA stability rather than new protein production (Dinman, 2012). It was shown that defects in rRNA pseudouridylation affect –1 PRF rates and might also contribute to translation impairments underlying X-DC pathogenesis (Jack et al., 2011).

Lastly, translation termination consists in the recognition of the stop codon at the end of the open reading frame and the release of the nascent polypeptide chain from the ribosome. At the end of translation, the post-termination 80S ribosome is recycled into 40S and 60S subunits, which can be used again for the initiation phase (Figure 7) (Schuller and Green, 2018).

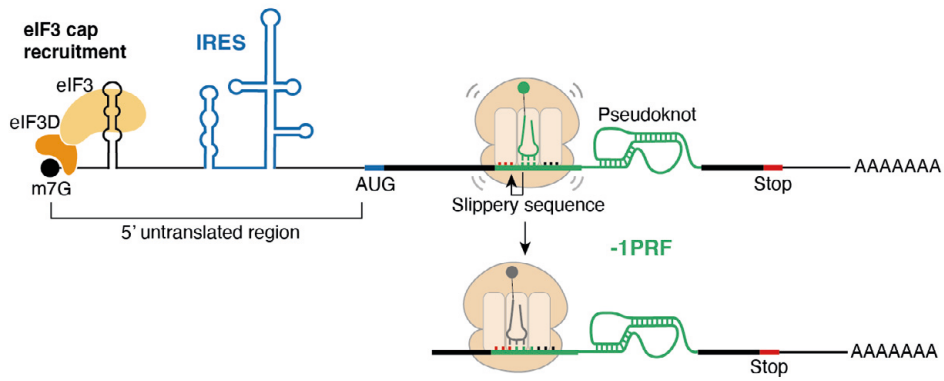

Figure 8: Representation of some regulatory elements of translation initiation and elongation. The non-canonical cap recruitment via the eIF3 complex and the non-canonical initiation via IRES element are shown in the 5' UTR region. The –1 PRF event is shown in the coding region with the slippery sequence and the pseudoknot which make the ribosome shift one nucleotide back towards the 5', thus changing the frame of translation.

rRNA modifications

As previously mentioned for snRNA, also rRNA is heavily pseudouridylated and 2'-O-methylated. Interestingly, the modifications are progressively increasing in number with evolution ranging from, for example, ten Ψ sites and four 2'OMe in *E. Coli*, to 45 Ψ and 55 2'OMe in *S. cerevisiae* and to around 100 sites for each modification in humans (Bakin and Ofengand, 1993; Birkedal et al., 2015; Piekna-Przybylska et al., 2008; Taoka et al., 2016). In bacteria most of the modifications are catalyzed by stand-alone enzymes, while in eukaryotes both RNA-dependent and independent mechanisms are involved, consistently with the evolutionarily expansion of the rRNA modification repertoire (Decatur and Fournier, 2002). The majority of the modifications occur co-transcriptionally during the early steps of ribosome biogenesis (Kos and Tollervey, 2010; Turowski and Tollervey, 2015), although in humans some occur at later stages of rRNA maturation with the contribution of RNA helicases (Sloan et al., 2015). Notably, rRNA modifications cluster within key evolutionarily conserved functional regions such as the peptidyltranferase center (PTC), the regions of tRNA and mRNA binding represented by the A-, P- and E-sites, and the intersubunit regions, suggesting their importance for ribosome function. (Decatur and Fournier, 2002). Several studies have highlighted the importance of single or clusters of rRNA modifications for ribosome structure, biogenesis, activity, and fidelity in different model systems (Sloan et al., 2017). For example, loss of a few Ψ sites within the PTC caused reduction in translation in yeast (King et al., 2003). Similar reductions were observed upon depletion of several modifications in the intersubunit bridge, which were also associated with increased stop codon read-through (Liang et al., 2007). Depletion of a cluster of modifications in the decoding center affected stop codon termination and maintenance of the frame (Baudin-Baillieu et al., 2009). As previously described in the chapter on the role of RNA modifications in cancer, alterations of rRNA Ψ and 2'OMe are associated with functional defects of the ribosome, in particular translation fidelity and IRES-mediated translation of specific mRNAs, including some tumor-suppressor genes (Bellodi et al., 2010a; Bellodi et al., 2010b; Chaudhuri et al., 2007; Erales et al., 2017; Jack et al., 2011; Marcel et al., 2013; Montanaro et al., 2010; Yoon et al., 2006).

Interestingly, it has been proposed that rRNA modifications may occur at sub-stoichiometric levels (Andersen et al., 2004; Erales et al., 2017; Krogh et al., 2016; Taoka et al., 2016). Even though these observations highlight rRNA modifications as a possible source of ribosome heterogeneity, still very much is unknown about the dynamic changes of rRNA modifications in mammals and humans. In line with

this hypothesis, in the work of *Paper III,* we unraveled a previously undetected 28S rRNA 2'OMe at position U2044 that is sub-stoichiometric and upregulated during development. Importantly, we found that this 2'OMe site affects the sensitivity to A-site specific translation inhibitors and impacts ribosomal frameshifting rates. In conclusion, rRNA Ψ and 2'OMe are conserved features important for the functionality of the ribosome and potentially involved in many cellular systems and in the pathogenesis of disease.

Translation regulation in stem cells

Tight regulation of protein synthesis, which can be achieved through several mechanisms involving the ribosome and translation regulatory factors, is emerging as a key element for ESCs self-renewal and differentiation (Buszczak et al., 2014; Gabut et al., 2020; Saba et al., 2021). It has been shown that there are significant differences in bulk protein levels between stem cells and their progeny, with ESCs maintaining low translation which then increases during embryoid bodies (EBs) differentiation (Ingolia et al., 2011; Sampath et al., 2008). Recent work from our lab showed how increased translation, caused by the depletion of the pseudouridine synthase PUS7 and the consequent reduction of pseudouridylated tRNA fragments, alters hESCs differentiation with specific defects in the mesoderm lineage (Guzzi et al., 2018). This study highlights the role of RNA modifications as a mean to control and maintain proper levels of protein synthesis in stem cells. Other studies showed how loss of specific rRNA m^6A causes impaired mouse ESCs differentiation and global as well as specific translation defects (Ignatova et al., 2020; Xing et al., 2020), strengthening the link between RNA modifications and translation regulation in ESCs. Nevertheless, still very much remains unknown about the contribution of RNA modification, especially rRNA Ψ and 2'OMe, to translation control in stem cells. Very recent studies showed that specific rRNA 2'OMe are regulated during development in zebrafish and mouse (Hebras et al., 2020; Ramachandran et al., 2020), but without exploring their functional effect on ribosomes and translation or their biological role. In *Paper III,* we showed that the developmentally regulated modification Um2044 of 28S rRNA affects specific ribosomal properties and is essential for accurate hESCs differentiation and proper endoderm germ layer formation, highlighting the connection between rRNA modifications, ribosome function and stem cell biology.

As for ESCs, tight regulation of translation is also important for adult stem cells, for example hematopoietic stem cells (HSCs) (Buszczak et al., 2014). Maintenance of

appropriate levels of protein synthesis was shown to be important for HSCs self-renewal and differentiation potential (Guzzi et al., 2018; Signer et al., 2014; Signer et al., 2016). The study from Signer and colleagues showed that both lower and higher protein synthesis caused impairment in HSC function (Signer et al., 2014). Considering the observations in ESCs, it is interesting to explore whether rRNA modifications could also play a role in translation regulation in HSCs. It is known that defects in rRNA pseudouridylation, caused by mutations of DKC1 in X-DC, lead to bone marrow failure and impairment of HSCs differentiation (Bellodi et al., 2013). Nevertheless, the direct contribution of rRNA pseudouridylation defects to translation defects and HSCs biology *in vivo* remains largely unknown. In *Paper II*, I explored how depletion of Gar1, one of the H/ACA snoRNP components, impairs rRNA pseudouridylation, decreases global protein synthesis and affects hematopoiesis in mice. *Gar1*-KO HSCs showed defects in reconstitution capacity, again highlighting the link between accurate translation levels and stem cell functionality, which is consistent with previous results in *Rpl24* mutants characterized by reduced protein synthesis (Signer et al., 2014). However, while *Rpl24* mutant mice are viable and smaller in size (Signer et al., 2014), the full body KO of Gar1 is not compatible with life and we could only employ conditional KO models in the hematopoietic system.

Aims of the thesis

The overarching aim of my thesis is to unravel post-transcriptional mechanisms that regulate gene expression and impact cancer and stem cells. Specifically, I focused on the regulation of two important steps during the flow of the genetic information from DNA to proteins: splicing and translation. The main outstanding question is whether and how Ψ and 2'OMe RNA modifications, among other factors, can act as key regulators of both splicing and translation during tumorigenesis, development and stem cells differentiation. To answer this question, I took several approaches, employing cancer cells, mice models and embryonic stem cells, to understand how spliceosome and ribosome dynamic changes may act as an extra layer of gene expression regulation, which has been mostly overlooked so far.

Specific aims:

- Investigate how changes in snRNA modifications and splicing factors (SFs) abundance affect spliceosome function and impact on cancer-promoting splicing programs (*Paper I* and *IV*)
- Study the effects of impaired rRNA pseudouridylation on translation and hematopoiesis *in vivo* (*Paper II*)
- Explore the role of orphan snoRNAs in stem cell regulation and fate determination (*Paper III*)

Summary of results

Paper I

The starting point for this work was the realization of a missing link between the dysregulation of snRNA modifications and the effect they might have on transformation and cancer development. Splicing is very frequently altered in cancer (Oltean and Bates, 2014; Sveen et al., 2016), but the contribution of snRNAs has been mostly overlooked in favor of research on SFs. Indeed, several studies showed that scaRNA expression is altered in solid and hematological cancers (Gong et al., 2017; Ronchetti et al., 2013; Ronchetti et al., 2012; Teittinen et al., 2013), leading to the possibility of a connection between snRNA modifications and malignancies. Nevertheless, how scaRNA levels can affect specific splicing patterns and play a role in tumorigenesis is still an open question.

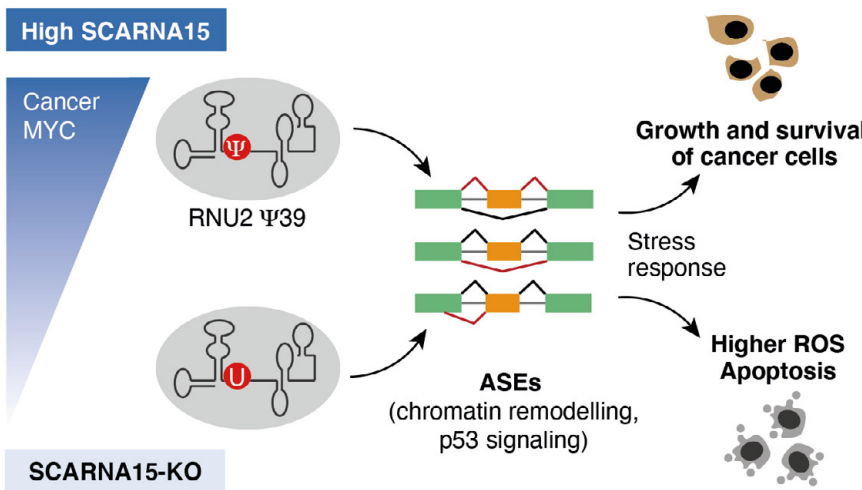

Figure 9: Graphical abstract of *Paper I*. Figure adapted from Beneventi et al., 2021.

In *Paper I*, we uncovered the role of SCARNA15 which, guiding a specific pseudouridylation of U2 snRNA, can affect alternative splicing events important for

stress response of cancer cells (Figure 9). Briefly, we first found that SCARNA15 was the most upregulated scaRNA upon oncogenic stress after MYC and AKT over-expression and it was highly expressed in cancer cells compared to their normal counterpart. These data showed a potential specificity of SCARNA15 expression in cancer and upon MYC oncogenic stress. By developing the first SCARNA15-KO cell line in HEK293T, we showed that SCARNA15 guides a specific pseudouridylation of U2 snRNA at position 39, which is proximal to the functional region of the snRNA that binds the pre-mRNA during splicing. Moreover, we identified the specific alternative splicing program dependent on SCARNA15 and U2-Ψ39, with an enrichment for genes related to chromatic organization and p53 signaling. In line with these splicing changes, SCARNA15-KO cells hyperactivated the p53 pathway, indicating a role of SCARNA15 in the regulation of this tumor suppressive pathway. Importantly, we could show a direct connection between the U2-modifying function of SCARNA15 and the observed molecular effects since the validated splicing events and the p53 pathway could not be rescued by a mutant version of SCARNA15 which is not able to guide U2-Ψ39. Furthermore, directed by the categories of genes that were transcriptionally affected in SCARNA15-KO cells, we uncovered profound defects in anchorage-independent growth and wound closure, which were accompanied by an accumulation of reactive oxygen species (ROS) and increased H_2O_2-dependent cell death. Similarly, we observed that SCARNA15-KO leukemia cell lines showed defects in colony-forming capacity, increased cell death and ROS accumulation. These phenotypes suggested a function of SCARNA15 and U2-Ψ39 in supporting growth and survival of cancer cells in stress conditions with an involvement in some of the major cancer hallmarks. In sum, the results included in *Paper I* revealed a key role for SCARNA15 and U2-Ψ39 in alternative splicing, mostly affecting chromatin remodeling and p53 signaling, which influenced the oxidative stress response and survival of cancer cells.

Paper II

Defects in rRNA pseudouridylation have been associated with translation defects and HSC dysfunction in the context of X-DC, which is characterized by bone marrow failure and high predisposition to cancer, especially of the hematopoietic system (Bellodi et al., 2010a; Bellodi et al., 2010b; Bellodi et al., 2013; Jack et al., 2011; Yoon et al., 2006). Yet, how snoRNP defects impact HSC function and

whether this is uncoupled from the telomere maintenance, remains poorly understood. Moreover, the embryonic lethality of *Dkc1*-KO mice (He et al., 2002) made very challenging to study the consequences of snoRNP impairment *in vivo*.

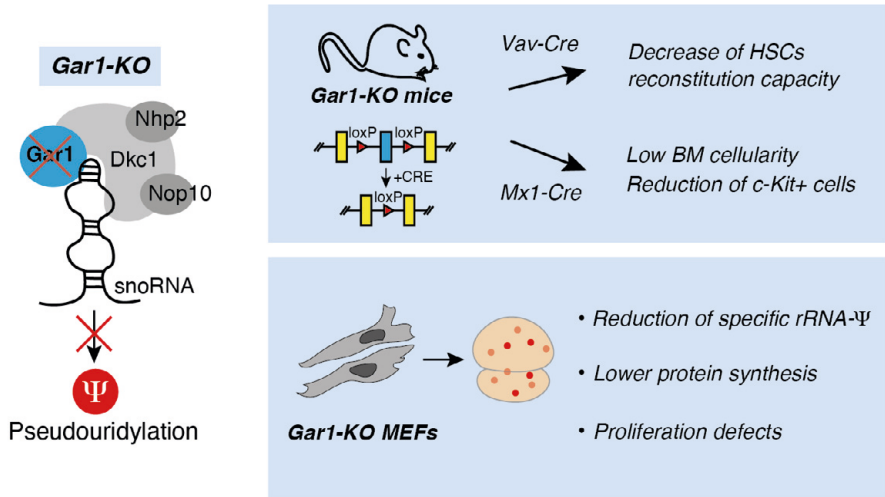

Figure 10: Graphical abstract of *Paper II*.

In *Paper II*, we developed a novel mouse model KO for *Gar1*, one of the components of the H/ACA snoRNP, which affects the efficiency of pseudouridylation without being involved in telomere maintenance. Employing this mouse model, we unraveled the importance of Gar1 and rRNA modifications for hematopoiesis *in vivo*, especially for the hematopoietic stem and progenitor cell (HSPC) populations. Additionally, we showed that loss of Gar1 reduced specific rRNA-Ψ sites and affected global protein synthesis rate with effects on cell proliferation (Figure 10). Briefly, in mice with conditional Gar1 loss in the hematopoietic system (Vav-Cre; *Gar1*-cKO), we observed a reduced frequency of multipotent progenitors (MPPs) but not of HSCs in the bone marrow. However, when challenged in transplantation experiments, these *Gar1*-KO HSCs showed reduced reconstitution capacity, indicating a key role of rRNA-Ψ for the functionality and self-renewal of HSCs. Interestingly, we noticed that Gar1 depleted cells were outcompeted *in vivo* in *Gar1*-cKO mice, especially in HSPC populations, suggesting a potential disadvantage of cells with defects in rRNA modifications. We then used an inducible *Gar1*-KO mouse model (Mx1-Cre) to analyze the response to acute Gar1 depletion and observed a deleterious effect on hematopoiesis. Gar1 loss determined a drastic reduction of white blood cells and platelets in the

peripheral blood, a general reduction of bone marrow cellularity as well as an almost complete loss of the c-Kit+ compartment in the bone marrow. These results indicated that Gar1 function is key for maintenance of the hematopoietic system homeostasis. Ultimately, we employed *Gar1*-KO mouse embryonic fibroblasts (MEFs) to assess the molecular consequences of Gar1 loss on ribosome and translation. Here, differently from what shown with defects of Dkc1, we surprisingly detected a small reduction in the levels of both 18S and 28S rRNA levels and lower total protein synthesis. Using Ψ-seq, we also determined the reduction of specific rRNA-Ψ sites in *Gar1*-KO MEFs, indicating that some modifications might be more sensitive than others to Gar1 depletion. Moreover, we observed that loss of Gar1 and rRNA-Ψ affected the phenotype of the cells, which had defects in cell cycle and proliferation rate. In sum, the results of *Paper II* highlight the importance of the rRNA modifying machinery for hematopoiesis and specifically for the HSC and HSPC populations.

Paper III

Although several orphan snoRNAs have been implicated in human syndromes and cancer with non-canonical functions (Chu et al., 2012; Kishore et al., 2010; Kishore and Stamm, 2006; Siprashvili et al., 2016; Valleron et al., 2012), the biological role of these small RNAs remains mostly unknown. Further inspired by the evidence that some orphan snoRNAs have a tissue specific expression (Jorjani et al., 2016), we hypothesized they might possess specialized roles in different cell types and during cell fate determination.

In *Paper III* we delineated a new function for the orphan snoRNA SNORD123 in rRNA 2'OMe during embryonic development. We showed that SNORD123 and Um2044 are critical for accurate hESCs differentiation and, functionally, affect specific ribosomal properties (Figure 11). Briefly, we first identified SNORD123 as one the most differentially expressed orphan snoRNAs among different cell types, showing increasing expression from hESCs to differentiated cells. We then uncovered the function of this orphan snoRNA as a canonical C/D box snoRNA by confirming that it modifies a predicted 2'OMe site on rRNA, 28S U2044, previously undetected. In line with the expression levels of SNORD123, we also showed that the levels of Um2044 progressively increase during hESCs differentiation, with a different distribution of the modification levels between the three germ layers. Interestingly, Um2044 was always present at sub-stoichiometric levels in all the cell

types tested, indicating the potential presence of heterogenous ribosomes within these cells or within the populations. Next, by developing a SNORD123-KO hESC line, we observed that while SNORD123 depletion had no effect on pluripotency, it instead caused profound defects during spontaneous differentiation with an increase towards the endoderm lineage. These results indicate a novel role for a rRNA 2'OMe in directing stem cell fate and lineage specification. Lastly, we sought to determine the effect Um2044 loss on ribosome function and found no defects in global protein synthesis or IRES-mediated translation. By analyzing the three-dimensional structure surrounding U2044, our models indicated direct binding to key amino acids of RPL3 involved in the regulation A-site accessibility. Consistently with this observation, we showed that SNORD123-KD fibroblasts were resistant to the A-site specific translation inhibitor anisomycin and had reduced −1 PRF, indicating that Um2044 may affect specific properties of the ribosome. In conclusion, the work from *Paper III* illustrates a developmentally regulated rRNA modification and creates a potential link between heterogeneously modified ribosomes and specific ribosomal functions, which might impact on hESCs differentiation and proper tissue specification.

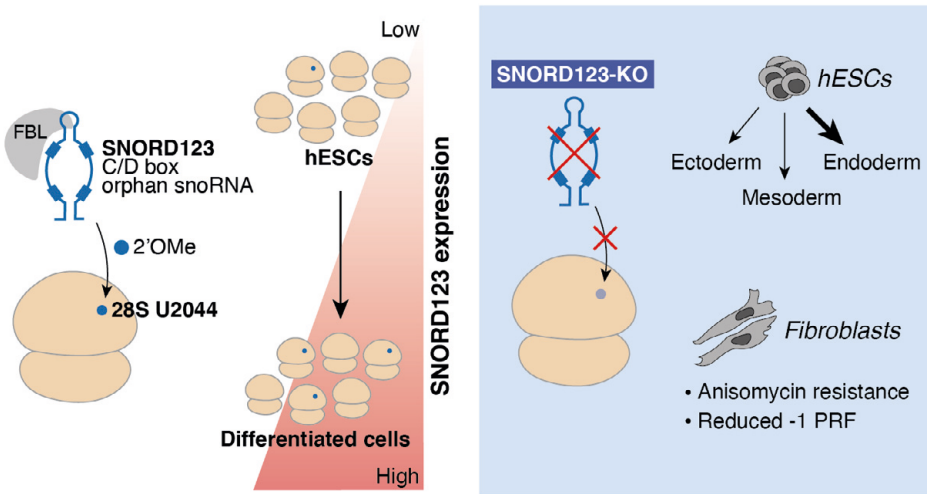

Figure 11: Graphical abstract of *Paper III*.

Paper IV

The work of *Paper IV* investigates the mechanism by which individual SFs are hijacked to steer alternative splicing in cancer cells. Findings that splicing defects in cancer can occur also in the absence on SF mutations (Danan-Gotthold et al., 2015; Dvinge and Bradley, 2015; Simon et al., 2014), suggested that dysregulation of SF levels might be achieved also through alternative methods, such as copy number variation or expression regulation via transcription or translation.

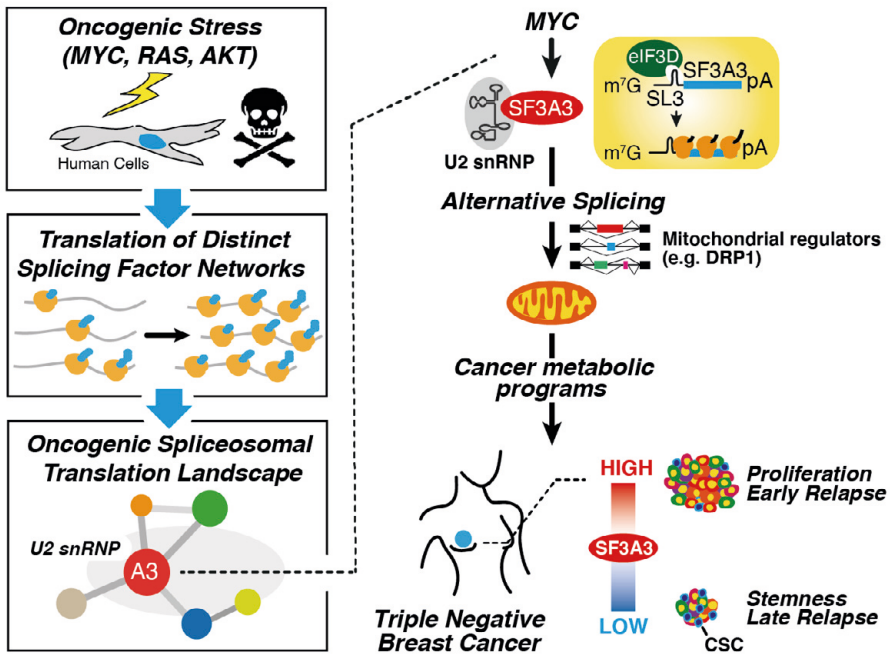

Figure 12: Graphical abstract of *Paper IV*. Figure from Ciesla et al., 2021.

In *Paper IV* we uncovered a translation regulatory program that governs the levels of specific SFs during oncogenic stress. Specifically, levels of the core factor SF3A3 affect splicing of mitochondrial related genes, which impact on metabolism and stem-cell properties of MYC-driven breast cancer and are fundamental for its tumorigenesis *in vivo* (Figure 12). Briefly, we first uncovered a network of splicing factors which are translationally regulated in response to the major oncogenes MYC, RAS and AKT, of which SF3A3 was one of the few regulated by all three oncogenes. We determined that translation regulation of SF3A3 in response to MYC

occurs in a cap-dependent manner via eIF3D and the binding to a stem loop structure (SL3) in the 5' UTR of the SF3A3 mRNA. Hampering SF3A3 up-regulation in the context of MYC hyperactivation caused several alternative splicing changes with enrichment for mRNAs related to mitochondria and apoptosis. A notable example is the gene DRP1, a central regulator of mitochondrial fission/fusion process (Kalia et al., 2018). These changes in splicing led to defects in mitochondrial biogenesis and respiration, showing the importance of SF3A3 for the establishment of the metabolic program during MYC transformation. Interestingly, the alternative splicing and transcriptional changes of SF3A3-KD cells were associated to breast cancer gene signatures, suggesting a potential connection between SF3A3 and MYC-driven breast cancer. Indeed, we showed that low levels of SF3A3 determined a boost of the tumorigenic potential of breast cancer cell lines *in vivo*. This was consistent with the observation of a switch to a more stem cell-like cancer phenotype that promotes tumor initiation. Lastly, SF3A3 gene signature was able to stratify triple negative breast cancer patients depending on their clinical outcome in aggressive breast cancer, having increased late relapse risk correlating with lower SF3A3 gene signature and stemness properties. In sum, findings from *Paper IV* reveal a connection between the translation and splicing programs during oncogenic stress and in cancer, which is key to maintain accurate levels of metabolic genes and sustain MYC-driven tumorigenesis.

Discussion and future perspectives

In this thesis I worked on post-transcriptional mechanisms of gene expression control focusing on two main systems: splicing and translation. I investigated how dynamic changes in snRNA modifications and crosstalk between the translation and splicing programs can be used as a mean for oncogenes to hijack the spliceosome and promote cancer. Moreover, I studied the contribution of specific and global differences in rRNA modification for the maintenance and differentiation of embryonic stem cells and adult hematopoietic stem cells.

More specifically, I identified the role of the SCARNA15-driven snRNA modification, U2-Ψ39, in driving splicing events that help cells cope with p53 signaling and redox stress response, sustaining the survival and growth of cancer cells (*Paper I*). This work represents one of the first reports illustrating the potential contribution of scaRNAs, snRNA-Ψ and the consequently regulated splicing events to tumorigenesis. It inserts within the recent literature showing the involvement of U6-2'OMe in maintenance of specific splicing programs related, in this case, to developmental defects (Hasler et al., 2020; Wang et al., 2020) instead of cancer. While we analyzed the effect of the loss of a single scaRNA on snRNA modifications, these studies showed the involvement of an RNA-binding protein in regulating multiple modifications simultaneously to determine alterations in splicing and the consequent phenotype. We focused on the SCARNA15-guided modification, but several other snoRNAs and scaRNAs were affected by MYC over-expression and higher in transformed cells, for example SCARNA9, guiding U2-2'OMe, and SNORA79, guiding U6-Ψ. Interestingly, it was shown that modifications have a hierarchy of biogenesis (Deryusheva and Gall, 2018), so the absence or presence of one modification can affect others. We, therefore, cannot exclude the involvement of a modification network in the maintenance of a cancerous splicing program, which might be more extended that what we show just by the loss of U2-Ψ39. Further work will be necessary to explore this possibility. Additionally, our study remains incomplete in explaining the exact molecular effects that the presence or absence of U2-Ψ39 has on the activity of the U2 snRNP and the spliceosome. Although we performed an analysis of the features

contributing to alternative exon usage, which gave us some hints on why certain exons might be more reliant or sensitive to U2-Ψ39, still an in-depth investigation needs to be carried out. Furthermore, it would be interesting to expand the work and perform a broad inspection of different cancers to evaluate SCARNA15 expression and U2-Ψ39 status and test whether loss of SCARNA15 would affect the same pathways of p53 regulation, ROS response management, and migration and, consequently, affect the tumorigenic potential *in vitro* and *in vivo*.

Continuing with the objective of understanding the regulation of the spliceosome in cancer, I moved my attention from snRNAs to SFs. I contributed to the identification of a translational program downstream of the oncogene MYC that regulates the level of specific SFs, including SF3A3. This work highlighted a new connection between translation and splicing in cancer. Maintenance of proper SF3A3 levels is key to ensure accurate splicing of genes important for mitochondria and metabolism and for stem cell-like properties involved in MYC-driven breast cancer tumorigenesis (*Paper IV*). Some mechanistic questions remain open, for instance, regarding the direct connection between different levels of SF3A3 and the effects on the spliceosome structure and activity. We showed that SF3A3 binding to the U2 snRNP upon MYC overexpression is not affected and we supplied evidence of increased binding of specific SFs around the exons affected by SF3A3 loss; however, the molecular effect of SF3A3 absence or presence on the spliceosome still needs to be further explored. A possibility can be, indeed, the involvement of accessory splicing factors, the binding of which might be increased or decreased by the presence of SF3A3. Another option is that SF3A3 containing spliceosomes might be necessary to maintain the efficiency of certain splicing events, maybe more difficult to splice, in limiting conditions like oncogenic stress. Moreover, our initial screening revealed a network of several SFs that are translationally regulated by oncogenes and especially upon MYC overexpression. We focused our attention on SF3A3, but it would be extremely interesting to dig deeper in the possibility of a concerted regulation of multiple SFs and the crosstalk between their activity in response to oncogenic stress.

The second part of the thesis was directed towards understanding the biological role of rRNA modifications (*Paper II* and *III*). Although the modifications have already been shown to be involved in disease and cancer (Barbieri and Kouzarides, 2020; Janin et al., 2020), their contribution to stem cell biology is mostly unknown. In *Paper III* I highlighted the role of the previously uncharacterized and developmentally regulated SNORD123-guided rRNA 2'OMe in embryonic stem

cell differentiation. Unlike previous works only showing differences in rRNA 2'OMe during development (Hebras et al., 2020; Ramachandran et al., 2020), in our study we started to unravel the translation defects downstream of the affected rRNA modification. Nevertheless, many questions remain unanswered in this regard. For instance, one question concerns the mechanistic explanation of why reduction of that specific modification determined those effects on translation. The analysis of the position of the SNORD123-guided modification, 28S Um2044, gave us an idea on the potentially implicated region of the ribosome, the A-site, but we did not uncover whether or how exactly the modification affects the ribosome conformation and, consequently, its function. Yeast mutants of the same amino acids interacting with U2044 show phenotypes very similar to what we observed in SNORD123-KD cells, including anisomycin resistance and defects in −1 PRF, but also different affinity for eEF1A-aa-tRNA and eEF2, which we have not fully explored yet (Mailliot et al., 2016; Meskauskas and Dinman, 2007; Meskauskas et al., 2005). The hypothesis might be that, by changing the conformation of the nucleotide sugar, the 2'OMe of U2044 could affect the interaction with the RPL3 amino acids. This would produce conformational changes of RPL3 similar to the ones observed in yeast mutants, thus affecting the A-site. All together, these observations point towards potential defects during the elongation process. It would be interesting to determine whether specific mRNAs with features that affect elongation (fox example specific codons or combinations of codons) are differentially translated in the presence or absence of Um2044. Additionally, defects in −1 PRF might indicate difficulty in frame maintenance and decrease fidelity (Jack et al., 2011), which could lead to mistranslation of certain mRNAs and even read-through events (Mohler and Ibba, 2017; Zaher and Green, 2009). Elucidation of such specific translation defects might help us explain the phenotype of SNORD123-KO ESCs. Future work will be necessary to explore these hypotheses and clarify the mechanism.

Lastly, in *Paper II,* I unraveled that depletion of Gar1, one of the proteins responsible for rRNA pseudouridylation, has a strong impact on the homeostasis of the hematopoietic system and on the reconstitution capacity of HSCs. We also observed a reduced rate of global protein synthesis and a small reduction of rRNA levels. It had already been shown that post-transcriptional regulation of gene expression via control of ribosome biogenesis proteins is particularly important for HSCs (Zaro et al., 2020) and that proper levels of protein synthesis are key for maintenance and differentiation of HSCs (Guzzi et al., 2018; Signer et al., 2014; Signer et al., 2016). In addition, a recent study revealed that the quantity of available ribosomes is key in dictating HSCs commitment to specific lineages but, in this case,

via translation regulation of specific mRNAs (Khajuria et al., 2018). My work further strengthens this connection between translation regulation and HSPCs, by adding rRNA pseudouridylation as an extra factor to maintain protein synthesis balance. Though, the effects on ribosomes and translation of *Gar1*-KO were unexpectedly different from the ones of Dkc1 mutants, where only specific defects in IRES and fidelity were shown without global translation defects (Bellodi et al., 2010a; Bellodi et al., 2010b; Jack et al., 2011; Yoon et al., 2006). We still need to further evaluate whether these specific translation changes are also happening upon loss of Gar1. Indeed, there is only a very small overlap between the rRNA modifications affected by Gar1 depletion and Dkc1 mutants, which might explain some of the differences. Since Gar1 loss affects the efficiency of pseudouridylation (Duan et al., 2009), it is possible that only some sites, maybe structurally more difficult to modify, are more sensitive to it and, thus, would affect and slow ribosome biogenesis. Nevertheless, this hypothesis was not explored yet and we could also not recapitulate, so far, why the loss of certain modifications determined global defects of the ribosome. Future work will be necessary to clarify these possibilities. Additionally, a few more points would be interesting to expand as future perspectives of *Paper II*. Firstly, I have some preliminary data indicating a correlation between Gar1 and Myc expression levels. Myc is known to affect translation and ribosome biogenesis in cancer cells (Ruggero, 2009) as well as to have an important role for HSCs self-renewal and differentiation with implications for AML stem cells (Bahr et al., 2018; Wilson et al., 2004). Since our results indicate an important function of Gar1 in the hematopoietic system and a potential correlation to Myc, it would be interesting to investigate whether Gar1 and rRNA pseudouridylation might play a role or represent a vulnerability in Myc-dependent hematological malignancies, for instance lymphomas. Secondly, I have not examined the effect of Gar1 loss on snRNA pseudouridylation, which could also be affected and lead to defects in alternative splicing important for the hematopoietic system. This aspect has long been overlooked also in previous studies focused on understanding the contribution of pseudouridylation defects in disease, described in the introduction of the thesis, and it would be attractive to expand on it.

Overall, with this thesis I tried to elucidate novel layers of post-transcriptional regulation, controlling specific aspects of splicing and translation, to advance the knowledge on their contribution in stem cell and cancer cell biology. This work converges on several outstanding research questions in the field which I will expand here below.

Specialized spliceosomes and ribosomes

The concept of heterogeneity of ribosomes and spliceosomes entails the presence of differentially composed ribonucleoprotein complexes, each of them potentially with a specialized function. Diversity of ribosome composition was already considered since the discovery of the machinery in the 1950s, with the initial hypothesis of "one gene-one ribosome-one protein", strongly supported by Francis Crick (Crick, 1958). This theory was quickly dismissed, though, and for many years the ribosome was considered a static entity. As previously discussed in the introduction of this thesis, in recent years the heterogeneity of ribosomes has risen to attention again with the evidence of several components that might bring diversity into the ribosome composition, including rRNA modifications (Gay et al., 2021; Genuth and Barna, 2018). The discovery of the developmentally regulated Um2044 guided by SNORD123 on 28S rRNA (*Paper III*) is contributing towards this effort by illustrating the possibility of heterogenous ribosomes between stem cells and their progeny. The same concept is likewise expanding to the spliceosome (Basak and Query, 2014; Krogh et al., 2017; Wu et al., 2016; Wu et al., 2011), partially also through the contribution of this thesis work (*Paper I* and *IV*). Nevertheless, the existence of heterogenous and specialized ribosomes and spliceosomes remains challenging to ascertain definitively. For instance, regarding RNA modifications, the presence of sub-stoichiometric levels was observed (Andersen et al., 2004; Erales et al., 2017; Krogh et al., 2016; Krogh et al., 2017; Taoka et al., 2016); however, the complete modification status of each rRNA or snRNA molecule is not known, making it difficult to assess how many combinations might be present and in which conditions. Moreover, in most cases, the function of all these potentially heterogenous ribosomes or spliceosomes has not been elucidated yet, as well as whether there might be several specialized machineries within the same cell or among cell populations.

One intriguing hypothesis could be represented by 'situation-specific' specialized machineries, which would consist of differentially composed ribosomes or spliceosomes limited to a certain condition, such as cancer, differentiation, or stress, that help fulfil the gene expression program important for that process. A stimulus able to determine such specialization might be, for example, the oncogene MYC, which is already known to be involved in translation by regulating genes involved in protein synthesis and ribosome biogenesis (Dang, 2013; Eilers and Eisenman, 2008; Ruggero, 2009), as well as in splicing by regulating some SFs (Das et al., 2012; Hsu et al., 2015; Koh et al., 2015). With the work of this thesis, I expanded

the knowledge in this direction by showing that SCARNA15 and the consequent snRNA modification, as well as the SF SF3A3 are regulated downstream MYC to sustain tumorigenesis through specific splicing events. It would be extremely interesting to study how MYC globally affects the composition of the ribosome and spliceosome, both in terms of protein and RNA components, to create a 'MYC cancerous machinery' to hijack the systems and pursue its function in cancer development. Another context of heterogeneity explored in this thesis is stem cells differentiation, where I identified differentially modified ribosomes in a specific position dependent from SNORD123 (*Paper III*). This result is in line with recent studies showing that some rRNA 2'OMe sites are regulated during zebrafish and mouse development (Hebras et al., 2020; Ramachandran et al., 2020) and that expression of some H/ACA snoRNA is dynamic during mouse ESC differentiation, reporting also differences in one rRNA-Ψ (McCann et al., 2020). To expand on this idea, it would be attractive to perform a global analysis of the modification status of the ribosome during differentiation, even potentially upon specific differentiation protocols, to assess whether there are specialized ribosomes that translate genes important for the specification of that cell type. The same could be done, of course, for the spliceosome and alternative splicing since the process has already been shown to be important in differentiation and cell specification (Baralle and Giudice, 2017). These important research questions still face the reality of our limitations in terms of available tools to analyze the ribosome and spliceosome differences molecule by molecule and at single cell levels. In an ideal situation, it would be possible to combine structural analysis including cryo-EM, with mass spectrometry, snRNA and rRNA sequencing and RNA modification detection techniques, but this is currently a far-fetched option. Moreover, improvement of the techniques to determine the functional effects of these specialized machineries on translation and splicing needs to be achieved. In the meantime, the effort is focused on unraveling heterogeneity in specific situations and one element at the time, with the hope of converging them together in the future.

Splicing as a therapeutic target for cancer

As I showed throughout this thesis, splicing is frequently altered in cancer, and this has made it an intriguing therapeutic target to pursue (Lee and Abdel-Wahab, 2016; Zhang et al., 2021). There are several therapeutic strategies currently under study and a lot of them aim at a general inhibition of splicing, such as SF3B-targeting

compounds and SR protein phosphorylation regulators. It seems like modulation of splicing might be tolerated by normal cells, while certain types of cancers are more susceptible to it. Into this category fall cancers with SF mutations, which are heterozygous and mutually exclusive between SFs. This suggests the mutations might most likely cause a gain of function and the cells rely only on the normal allele to sustain regular splicing, hence the susceptibility to splicing modulation (Lee and Abdel-Wahab, 2016). Another category of sensitive malignancies are MYC-driven cancers, which show high dependency from splicing (Lee and Abdel-Wahab, 2016), as illustrated also by the results of *Paper I* and *IV*. Still a lot is unknown about the exact modulation levels to achieve, specificity, and therapeutic windows when it comes to these therapies that act globally on the splicing process.

With the work of *Paper IV*, we highlighted the susceptibility of MYC-driven breast cancer to SF3A3, which represents an interesting target to follow up since its translation regulation is strictly related to MYC-dependent responsive elements. RNA therapies such as RNA interference and antisense oligos (ASOs) have already been explored to regulate gene expression via RNA cleavage and translation (Kole et al., 2012; McClorey and Wood, 2015) and it could be intriguing to analyze their potential use in regulating eIF3-dependent translation of SF3A3 to affect cancer. Moreover, ASOs and, specifically, splice-switching oligonucleotides (SSOs) have been used to influence splicing of specific genes and as a potential treatment for diseases like Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA) (Lee and Abdel-Wahab, 2016; Zhang et al., 2021). The use of similar strategies is in discussion for cancer and the specific splicing events we identified downstream of SF3A3 or SCARNA15 in support of cancer cells (*Paper I* and *IV*) might represent future targets of interest. However, while in diseases like DMD or SMA the event to target is only one, in cancer there would most likely be several events affected simultaneously, which might make this strategy more complicated to achieve. Alternatively, SSOs might be used to target a certain category of splicing events affected by the alteration of the same spliceosomal component (Lee and Abdel-Wahab, 2016), which can be represented by either one SF or one snRNA modification. For this reason, more studies like the ones performed in this thesis might be beneficial to determine specific splicing events to target in cancer. These splicing events might not only be directly targeted by ASOs to affect protein expression, but they might also help identifying unique epitopes introduced by alternative splicing in cancer cells to be used as immunotherapy targets (Zhang et al., 2021). Another potential future application of our and similar studies could be to identify the novel vulnerabilities determined by the defects of a spliceosome

component. For instance, dysregulation of certain spliceosomal elements and the consequent ASEs might determine different sensitivity to existing drugs, as showed by the higher sensitivity to stausporine of TNBC cells with down-regulation of SF3A3 (*Paper IV*). Another example might be the possibility to have indications on the relapse probability, in correlation to the expression levels of a SF (*Paper IV*). In conclusion, the more knowledge we obtain on spliceosome defects and alternative splicing patterns of specific cancers, the more therapeutic avenues may be potentially available for the development of precise cancer treatment and this thesis work is hopefully inserted into this long-term effort.

Popular summary

The “central dogma” is one of the fundamental principles of molecular biology. It describes the flow of the genetic information, which is encoded in the DNA, then transcribed into a messenger RNA (mRNA) molecule, and finally translated into proteins. Two key steps along this journey are splicing and translation, which are the focus of my thesis work. Splicing helps transform a precursor of the mRNA (pre-mRNA) into its mature form by cutting away certain parts of the sequence and patching together the ones that compose the mature mRNA. Most mRNAs are subjected to alternative splicing, a process that creates different mature mRNAs from the same pre-mRNA by patching together alternative combinations of sequences, thus increasing the number of possible final products. The mature mRNA is then exported from the cell nucleus into the cytoplasm, where it is translated. Translation converts the information present within the mRNA sequence into proteins, the ultimate building blocks of the cell. Splicing and translation are complicated processes run by dynamic macromolecular complexes, the spliceosome and the ribosome, composed of both RNA and proteins. Both splicing and translation are finely executed through several regulatory steps, some of which are still not fully known.

It is fascinating to notice that only a small part of the RNAs that are transcribed from DNA are mRNAs carrying the information for the creation of proteins. The majority of the RNAs in the cell do not actually code for proteins and are therefore called non-coding RNAs (ncRNAs). These ncRNAs have many different regulatory functions and are involved in almost all cellular processes, including splicing and translation. Indeed, the very molecules that compose the RNA portion of the spliceosome and the ribosome are ncRNAs, known as small nuclear RNA (snRNA) and ribosomal RNA (rRNA) respectively, and they play a crucial role both structurally and functionally in the macromolecular complexes. The snRNA and rRNA are vastly decorated with two chemical modifications, pseudouridine (Ψ) and 2’-O-methylation (2’OMe). These modifications are guided by yet another class of ncRNAs, composed of small nucleolar RNAs (snoRNAs) and small Cajal body-specific RNAs (scaRNAs). The Ψ and 2’OMe modifications are important for the

structure, the biogenesis, and the function on snRNA and rRNA and have recently emerged to be less static than previously thought. Nevertheless, the biological role of these dynamic modifications, especially in cancer and development, remains largely unknown.

The aim of my thesis is to explore new regulatory mechanisms of splicing and translation, especially involving RNA modification modulation and dysregulation, to better understand cancer development and stem cells maintenance and differentiation.

Briefly, in *Paper I* and *IV* I focused on the regulation of the spliceosome in cancer. I found that the loss of a single snRNA-Ψ affects specific splicing events important for stress response and survival of cancer cells (*Paper I*). Furthermore, I observed that maintaining specific levels of one of the protein components of the spliceosome is essential for accurate splicing of genes involved in cell metabolism and for the tumorigenic potential of breast cancer cells (*Paper IV*). In *Paper II* I analyzed the impact of the dysregulation of rRNA-Ψ on the hematopoietic system, which is the system involved in blood cell production. My results show that loss of these modifications in mice is detrimental for hematopoietic stem cells (HSCs), which display a reduced capacity of restoring the whole hematopoietic system. In *Paper III* I discovered a previously uncharacterized rRNA-2'OMe, which I found to be present in different amounts between the ribosomes of human embryonic stem cells (hESCs) and the cells differentiated from those hESCs. The loss of this single modification affects specific properties of the ribosome and alters hESCs differentiation.

In conclusion, with the work of my thesis I help to shed some light on novel layers of gene expression regulation, which have been mostly overlooked so far. My goal with this doctoral work has been to extend the knowledge on cancer and stem cell biology, and to contribute to pave the way for future clinical applications.

Riassunto

Il “dogma centrale” è uno dei principi fondamentali della biologia molecolare e descrive il flusso che porta l’informazione genetica contenuta nel DNA ad essere trascritta in molecole di RNA messaggero (mRNA) e, infine, tradotta in proteine. Due passaggi fondamentali durante questo viaggio dell’informazione genetica sono lo splicing e la traduzione, che sono il fulcro del lavoro della mia tesi. Lo splicing aiuta a trasformare il precursore dell’mRNA (pre-mRNA) nella sua forma matura attraverso il taglio di determinate porzioni della sequenza ribonucleica, e la successiva unione delle rimanenti porzioni a formare l’mRNA maturo. La maggior parte degli mRNA sono sottoposti allo splicing alternativo, un processo che permettere la creazione di mRNA maturi diversi a partire dallo stesso pre-mRNA attraverso l’unione di combinazioni alternative di sequenze, aumentando in questo modo il numero di prodotti finali possibili. L’mRNA maturo viene poi esportato dal nucleo cellulare al citoplasma, dove viene tradotto. La traduzione permette di convertire in proteine, che sono i mattoni che costituiscono le cellule, l’informazione presente nella sequenza dell’mRNA. Lo splicing e la traduzione sono processi complicati che vengono eseguiti da complessi macromolecolari dinamici, lo spliceosoma e il ribosoma, che sono composti sia da RNA che proteine. Sia lo splicing che la traduzione sono finemente eseguiti attraverso molteplici passaggi di regolazione, alcuni dei quali sono ancora sconosciuti.

È affascinante notare come solo una piccola parte degli RNA che vengono trascritti dal DNA siano degli mRNA contenenti l’informazione per creare le proteine. La maggioranza degli RNA presenti nelle cellule, infatti, non codificano per proteine e vengono per questo chiamati RNA non-codificanti (ncRNA). Questi ncRNA hanno svariate funzioni di regolazione e sono coinvolti in quasi tutti i processi cellulari, inclusi lo splicing e la traduzione. Le stesse molecole di RNA che costituiscono lo spliceosoma e il ribosoma sono dei ncRNA, chiamati rispettivamente piccolo RNA nucleare (snRNA) e RNA ribosomale (rRNA), e svolgono un ruolo importante sia per la struttura che per la funzione di questi complessi macromolecolari. Gli snRNA e rRNA sono ampiamente decorati da due modificazioni chimiche, la pseudouridina (Ψ) la 2’-O-metilazione (2’OMe). Queste modificazioni sono guidate da una

ulteriore classe di ncRNA, composta dai piccoli RNA nucleolari (snoRNA) e dai piccoli RNA specifici dei Cajal bodies (scaRNA). Le modificazioni Ψ e 2'OMe sono importanti per la struttura, la biogenesi e la funzione degli snRNA e rRNA ed è recentemente emerso che siano meno statiche di quanto si pensasse in precedenza. Nonostante ciò, il ruolo biologico svolto dai cambiamenti dinamici di queste modificazioni rimane largamente sconosciuto, specialmente in cancro e durante lo sviluppo.

Lo scopo della mia tesi è di esplorare nuovi meccanismi di regolazione dello splicing e della traduzione, specialmente riguardanti la modulazione e la deregolazione delle modificazioni dell'RNA, per capire meglio lo sviluppo del cancro e il mantenimento e il differenziamento delle cellule staminali.

Negli *Articoli I* e *IV* mi sono focalizzata sulla regolazione dello spliceosoma nel cancro. Ho scoperto che la perdita di una singola modificazione Ψ del snRNA altera specifici eventi di splicing importanti per la risposta allo stress e la sopravvivenza delle cellule tumorali (*Articolo I*). Inoltre, ho osservato che il mantenimento di specifici livelli di una delle proteine che sono parte dello spliceosoma è essenziale per l'accurato svolgimento dello splicing di geni coinvolti nel metabolismo cellulare e nel potenziale tumorigenico delle cellule di cancro alla mammella (*Articolo IV*). Nel *Articolo II* ho analizzato l'impatto della deregolazione delle modificazioni Ψ del rRNA sul sistema emopoietico, che è il sistema coinvolto nella produzione delle cellule del sangue. I miei risultati mostrano che la perdita di tali modificazioni nei topi è dannosa per le cellule staminali emopoietiche, che di conseguenza manifestano una ridotta capacità di ricreare l'intero sistema emopoietico. Nel *Articolo III* ho scoperto una modificazione 2'OMe del rRNA che non era stata precedentemente caratterizzata e che ho trovato essere presente a livelli diversi nei ribosomi delle cellule staminali embrionali umane (hESC) rispetto a quelli delle cellule differenziate derivate da quelle stesse hESC. La perdita di tale singola modificazione altera specifiche proprietà dei ribosomi e il differenziamento delle hESC.

In conclusione, con il lavoro della mia tesi ho aiutato a chiarire nuovi livelli della regolazione dell'espressione genica, che erano stati finora sottovalutati. Lo scopo del lavoro di questo dottorato è stato quello di accrescere la conoscenza della biologia delle cellule tumorali e staminali e contribuire ad aprire la strada verso future applicazioni cliniche.

Populärvetenskaplig sammanfattning

Det “centrala dogmat” är en av de mest fundamentala principerna inom molekylärbiologi. Det beskriver hur den lagrade, genetiska informationen som finns kodat i DNA omsätts till funktion genom att först transkriberas till ett ”messenger RNA” (mRNA) som sedan kodar för ett protein. ”Splicing” och ”translation” är två kritiska skeenden för att reglera detta flöde från DNA till protein, och utgör fokuset för min avhandling. Splicing omvandlar ett förstadie (”pre-mRNA”) till mRNA genom att trimma ned vissa delar av RNA-sekvensen och sedan sammanfoga de kvarvarande. Det allra flesta mRNA kan splicas och sättas ihop på olika sätt och därmed ökar komplexiteten av möjliga slutgiltiga protein från ett och samma mRNA, en process som kallas ”alternativ splicing”. För att sedan bilda protein förflyttas splic:at mRNA från cellkärnan ut i cytoplasman, där det translateras. Translation omvandlar informationen som finns i mRNA-sekvensen till ett protein, som är den ultimata byggnadsstenen för cellen. Splicing och translation är komplicerade processer som regleras av dynamiska, makromolekylära sammansättningar (”spliceosomen” och ”ribosomen”, för splicing respektive translation), och är sammansättningar av både RNA och protein. De utför sina respektive biologiska funktioner genom ett flertal regulatoriska steg, varav somliga inte är fullständigt förstådda inom forskningen.

Eftersom protein är den ultimata byggstenen i cellen så är det fascinerande att endast en liten del av allt RNA som produceras faktiskt är mRNA och kodar för ett protein. I stället så utgörs majoriteten utav en subgrupp som kallas ”icke-kodande RNA” (non-coding RNA, ncRNA), som alltså inte kodar för protein alls. I stället har dessa ncRNA många olika regulatoriska funktioner och är involverade i nästan alla cellulära processer, inklusive splicing och translation. Faktiskt så är det ncRNA som utgör kritiska delar av spliceosomen och ribosomen. De kallas ”small nuclear RNA” (snRNA) och ”ribosomalt RNA” (rRNA). Dessa RNA modifieras med hjälp av två kemiska föreningar: pseudouridine (Ψ) och 2’-O-metylering (2’OMe). Modifieringarna fastställs med hjälp av ytterliggare en typ av ncRNA som kallas ”small nucleolar RNA” (snoRNA) samt ”small Cajal body-specific RNA” (scaRNA). Ψ och 2’OME modifieringarna är viktiga för strukturen, utvecklingen,

och funktionen hos snRNA och rRNA och har nyligen fått uppmärksamhet då studier har visat att de är dynamiskt reglerade i stället för statiskt uttryckta som man tidigare trott. Men vad som är den biologiska rollen för deras dynamiska reglering, särskilt för cancer och embryonal utveckling, är fortfarande i stort sett okänt.

Målet med min avhandling är att utforska nya regulatoriska mekanismer för splicing och translation, med ett fokus på RNA-modifieringar, för att bättre förstå cancerutveckling och stamcellers normala biologi.

Sammanfattningsvis, i *publikation I* och *IV* fokuserar vi på regleringen av spliceosomen i cancer. Jag identifierade att en felaktig Ψ modifiering ensamt påverkar splicing på ett sätt som är viktigt för stressrespons och överlevnaden för cancercellerna (*publikation I*). Vidare så observerade jag att vidbehållandet av en specifik proteinkomponent i spliceosomen är essentiellt för riktig splicing av gener som är involverade i cellens metabolism och tumörbildning från bröstcancerceller (*publikation IV*). I *publikation II* analyserade jag konsekvensen av felaktig Ψ-rRNA för det ”hematopoietiska systemet”, alltså det som bildar allt kroppens blod. Mina resultat påvisar en nedsättning i musens hematopoietiska stamcellerna (HSC), som har en nedsatt kapacitet för att producera mogna blodceller. I *publikation III* identifierade jag en tidigare okänd rRNA-2’OMe modifikation, vilket uttrycks olika mycket i den humana embryonala stamcellen (hESC) och de celler som den bildar. Genetisk defekt i den här modifikationen påverkar specifika egenskaper hos ribosomen och resulterar i felaktig mognadspotential hos hESC.

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References

Allis, C.D., and Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. Nature reviews. Genetics *17*, 487-500. 10.1038/nrg.2016.59.

Andersen, T.E., Porse, B.T., and Kirpekar, F. (2004). A novel partial modification at C2501 in Escherichia coli 23S ribosomal RNA. RNA *10*, 907-913. 10.1261/rna.5259404.

Ayadi, L., Galvanin, A., Pichot, F., Marchand, V., and Motorin, Y. (2019). RNA ribose methylation (2'-O-methylation): Occurrence, biosynthesis and biological functions. Biochim Biophys Acta Gene Regul Mech *1862*, 253-269. 10.1016/j.bbagrm.2018.11.009.

Bahr, C., von Paleske, L., Uslu, V.V., Remeseiro, S., Takayama, N., Ng, S.W., Murison, A., Langenfeld, K., Petretich, M., Scognamiglio, R., et al. (2018). A Myc enhancer cluster regulates normal and leukaemic haematopoietic stem cell hierarchies. Nature *553*, 515-520. 10.1038/nature25193.

Bakin, A., and Ofengand, J. (1993). Four newly located pseudouridylate residues in Escherichia coli 23S ribosomal RNA are all at the peptidyltransferase center: analysis by the application of a new sequencing technique. Biochemistry *32*, 9754-9762. 10.1021/bi00088a030.

Baralle, F.E., and Giudice, J. (2017). Alternative splicing as a regulator of development and tissue identity. Nat Rev Mol Cell Biol *18*, 437-451. 10.1038/nrm.2017.27.

Barbieri, I., and Kouzarides, T. (2020). Role of RNA modifications in cancer. Nat Rev Cancer *20*, 303-322. 10.1038/s41568-020-0253-2.

Basak, A., and Query, C.C. (2014). A pseudouridine residue in the spliceosome core is part of the filamentous growth program in yeast. Cell Rep *8*, 966-973. 10.1016/j.celrep.2014.07.004.

Baudin-Baillieu, A., Fabret, C., Liang, X.H., Piekna-Przybylska, D., Fournier, M.J., and Rousset, J.P. (2009). Nucleotide modifications in three functionally important regions of the Saccharomyces cerevisiae ribosome affect translation accuracy. Nucleic Acids Res *37*, 7665-7677. 10.1093/nar/gkp816.

Begik, O., Lucas, M.C., Pryszcz, L.P., Ramirez, J.M., Medina, R., Milenkovic, I., Cruciani, S., Liu, H., Vieira, H.G.S., Sas-Chen, A., et al. (2021). Quantitative

profiling of pseudouridylation dynamics in native RNAs with nanopore sequencing. Nat Biotechnol. 10.1038/s41587-021-00915-6.

Bellodi, C., Kopmar, N., and Ruggero, D. (2010a). Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. Embo J *29*, 1865-1876. 10.1038/emboj.2010.83.

Bellodi, C., Krasnykh, O., Haynes, N., Theodoropoulou, M., Peng, G., Montanaro, L., and Ruggero, D. (2010b). Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. Cancer research *70*, 6026-6035. 0008-5472.CAN-09-4730 [pii]

10.1158/0008-5472.CAN-09-4730.

Bellodi, C., McMahon, M., Contreras, A., Juliano, D., Kopmar, N., Nakamura, T., Maltby, D., Burlingame, A., Savage, S.A., Shimamura, A., and Ruggero, D. (2013). H/ACA Small RNA Dysfunctions in Disease Reveal Key Roles for Noncoding RNA Modifications in Hematopoietic Stem Cell Differentiation. Cell reports *3*, 1493-1502. 10.1016/j.celrep.2013.04.030.

Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenner, L., Yusupova, G., and Yusupov, M. (2011). The structure of the eukaryotic ribosome at 3.0 A resolution. Science *334*, 1524-1529. 10.1126/science.1212642.

Beneventi, G., Munita, R., Cao Thi Ngoc, P., Madej, M., Ciesla, M., Muthukumar, S., Krogh, N., Nielsen, H., Swaminathan, V., and Bellodi, C. (2021). The small Cajal body-specific RNA 15 (SCARNA15) directs p53 and redox homeostasis via selective splicing in cancer cells. NAR Cancer *3*, zcab026. 10.1093/narcan/zcab026.

Berget, S.M., Moore, C., and Sharp, P.A. (1977). Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proceedings of the National Academy of Sciences of the United States of America *74*, 3171-3175. 10.1073/pnas.74.8.3171.

Birkedal, U., Christensen-Dalsgaard, M., Krogh, N., Sabarinathan, R., Gorodkin, J., and Nielsen, H. (2015). Profiling of ribose methylations in RNA by high-throughput sequencing. Angew Chem Int Ed Engl *54*, 451-455. 10.1002/anie.201408362.

Blencowe, B.J. (2006). Alternative splicing: new insights from global analyses. Cell *126*, 37-47. 10.1016/j.cell.2006.06.023.

Boccaletto, P., Machnicka, M.A., Purta, E., Piatkowski, P., Baginski, B., Wirecki, T.K., de Crecy-Lagard, V., Ross, R., Limbach, P.A., Kotter, A., et al. (2018). MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res *46*, D303-D307. 10.1093/nar/gkx1030.

Boise, L.H., Gonzalez-Garcia, M., Postema, C.E., Ding, L., Lindsten, T., Turka, L.A., Mao, X., Nunez, G., and Thompson, C.B. (1993). bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell *74*, 597-608. 10.1016/0092-8674(93)90508-n.

Buszczak, M., Signer, R.A., and Morrison, S.J. (2014). Cellular differences in protein synthesis regulate tissue homeostasis. Cell *159*, 242-251. 10.1016/j.cell.2014.09.016.

Carlile, T.M., Rojas-Duran, M.F., Zinshteyn, B., Shin, H., Bartoli, K.M., and Gilbert, W.V. (2014). Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature *515*, 143-146. 10.1038/nature13802.

Cartegni, L., Chew, S.L., and Krainer, A.R. (2002). Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nature reviews. Genetics *3*, 285-298. 10.1038/nrg775.

Charette, M., and Gray, M.W. (2000). Pseudouridine in RNA: what, where, how, and why. IUBMB Life *49*, 341-351. 10.1080/152165400410182.

Chaudhuri, S., Vyas, K., Kapasi, P., Komar, A.A., Dinman, J.D., Barik, S., and Mazumder, B. (2007). Human ribosomal protein L13a is dispensable for canonical ribosome function but indispensable for efficient rRNA methylation. RNA *13*, 2224-2237. rna.694007 [pii]

10.1261/rna.694007.

Chow, L.T., Gelinas, R.E., Broker, T.R., and Roberts, R.J. (1977). An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. Cell *12*, 1-8. 10.1016/0092-8674(77)90180-5.

Chu, L., Su, M.Y., Maggi, L.B., Jr., Lu, L., Mullins, C., Crosby, S., Huang, G., Chng, W.J., Vij, R., and Tomasson, M.H. (2012). Multiple myeloma-associated chromosomal translocation activates orphan snoRNA ACA11 to suppress oxidative stress. J Clin Invest *122*, 2793-2806. 10.1172/JCI63051.

Ciesla, M., Ngoc, P.C.T., Cordero, E., Martinez, A.S., Morsing, M., Muthukumar, S., Beneventi, G., Madej, M., Munita, R., Jonsson, T., et al. (2021). Oncogenic translation directs spliceosome dynamics revealing an integral role for SF3A3 in breast cancer. Mol Cell *81*, 1453-1468 e1412. 10.1016/j.molcel.2021.01.034.

Crick, F.H. (1958). On protein synthesis. Symp Soc Exp Biol *12*, 138-163.

Cui, Q., Yin, K., Zhang, X., Ye, P., Chen, X., Chao, J., Meng, H., Wei, J., Roeth, D., Li, L., et al. (2021). Targeting PUS7 suppresses tRNA pseudouridylation and glioblastoma tumorigenesis. Nature Cancer *2*, 932-949. 10.1038/s43018-021-00238-0.

Cullen, B.R. (2009). Viral RNAs: lessons from the enemy. Cell *136*, 592-597. 10.1016/j.cell.2009.01.048.

Dai, Q., Moshitch-Moshkovitz, S., Han, D., Kol, N., Amariglio, N., Rechavi, G., Dominissini, D., and He, C. (2017). Nm-seq maps 2'-O-methylation sites in human mRNA with base precision. Nat Methods *14*, 695-698. 10.1038/nmeth.4294.

Danan-Gotthold, M., Golan-Gerstl, R., Eisenberg, E., Meir, K., Karni, R., and Levanon, E.Y. (2015). Identification of recurrent regulated alternative splicing events across human solid tumors. Nucleic Acids Res *43*, 5130-5144. 10.1093/nar/gkv210.

Dang, C.V. (2013). MYC, metabolism, cell growth, and tumorigenesis. Cold Spring Harb Perspect Med *3*. 10.1101/cshperspect.a014217.

Darman, R.B., Seiler, M., Agrawal, A.A., Lim, K.H., Peng, S., Aird, D., Bailey, S.L., Bhavsar, E.B., Chan, B., Colla, S., et al. (2015). Cancer-Associated SF3B1 Hotspot Mutations Induce Cryptic 3' Splice Site Selection through Use of a Different Branch Point. Cell Rep *13*, 1033-1045. 10.1016/j.celrep.2015.09.053.

Das, S., Anczukow, O., Akerman, M., and Krainer, A.R. (2012). Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. Cell Rep *1*, 110-117. 10.1016/j.celrep.2011.12.001.

Decatur, W.A., and Fournier, M.J. (2002). rRNA modifications and ribosome function. Trends in biochemical sciences *27*, 344-351. 10.1016/s0968-0004(02)02109-6.

Deryusheva, S., and Gall, J.G. (2018). Orchestrated positioning of post-transcriptional modifications at the branch point recognition region of U2 snRNA. RNA *24*, 30-42. 10.1261/rna.063842.117.

Dinman, J.D. (2012). Mechanisms and implications of programmed translational frameshifting. Wiley interdisciplinary reviews. RNA *3*, 661-673. 10.1002/wrna.1126.

Dong, Z.W., Shao, P., Diao, L.T., Zhou, H., Yu, C.H., and Qu, L.H. (2012). RTL-P: a sensitive approach for detecting sites of 2'-O-methylation in RNA molecules. Nucleic Acids Res *40*, e157. 10.1093/nar/gks698.

Donmez, G., Hartmuth, K., and Luhrmann, R. (2004). Modified nucleotides at the 5' end of human U2 snRNA are required for spliceosomal E-complex formation. RNA *10*, 1925-1933. 10.1261/rna.7186504.

Duan, J., Li, L., Lu, J., Wang, W., and Ye, K. (2009). Structural mechanism of substrate RNA recruitment in H/ACA RNA-guided pseudouridine synthase. Mol Cell *34*, 427-439. 10.1016/j.molcel.2009.05.005.

Dupuis-Sandoval, F., Poirier, M., and Scott, M.S. (2015). The emerging landscape of small nucleolar RNAs in cell biology. Wiley interdisciplinary reviews. RNA *6*, 381-397. 10.1002/wrna.1284.

Dvinge, H., and Bradley, R.K. (2015). Widespread intron retention diversifies most cancer transcriptomes. Genome Med *7*, 45. 10.1186/s13073-015-0168-9.

Dvinge, H., Kim, E., Abdel-Wahab, O., and Bradley, R.K. (2016). RNA splicing factors as oncoproteins and tumour suppressors. Nat Rev Cancer *16*, 413-430. 10.1038/nrc.2016.51.

Eilers, M., and Eisenman, R.N. (2008). Myc's broad reach. Genes & development *22*, 2755-2766. 10.1101/gad.1712408.

Erales, J., Marchand, V., Panthu, B., Gillot, S., Belin, S., Ghayad, S.E., Garcia, M., Laforets, F., Marcel, V., Baudin-Baillieu, A., et al. (2017). Evidence for rRNA 2'-O-methylation plasticity: Control of intrinsic translational capabilities of human ribosomes. Proceedings of the National Academy of Sciences of the United States of America *114*, 12934-12939. 10.1073/pnas.1707674114.

Gabut, M., Bourdelais, F., and Durand, S. (2020). Ribosome and Translational Control in Stem Cells. Cells *9*. 10.3390/cells9020497.

Gao, L., Ma, J., Mannoor, K., Guarnera, M.A., Shetty, A., Zhan, M., Xing, L., Stass, S.A., and Jiang, F. (2015). Genome-wide small nucleolar RNA expression analysis of lung cancer by next-generation deep sequencing. International journal of cancer. Journal international du cancer *136*, E623-629. 10.1002/ijc.29169.

Gay, D.M., Lund, A.H., and Jansson, M.D. (2021). Translational control through ribosome heterogeneity and functional specialization. Trends in biochemical sciences. 10.1016/j.tibs.2021.07.001.

Ge, J., and Yu, Y.T. (2013). RNA pseudouridylation: new insights into an old modification. Trends in biochemical sciences *38*, 210-218. 10.1016/j.tibs.2013.01.002.

Genuth, N.R., and Barna, M. (2018). Heterogeneity and specialized functions of translation machinery: from genes to organisms. Nature reviews. Genetics *19*, 431-452. 10.1038/s41576-018-0008-z.

Gong, J., Li, Y., Liu, C.J., Xiang, Y., Li, C., Ye, Y., Zhang, Z., Hawke, D.H., Park, P.K., Diao, L., et al. (2017). A Pan-cancer Analysis of the Expression and Clinical Relevance of Small Nucleolar RNAs in Human Cancer. Cell Rep *21*, 1968-1981. 10.1016/j.celrep.2017.10.070.

Guzzi, N., Ciesla, M., Ngoc, P.C.T., Lang, S., Arora, S., Dimitriou, M., Pimkova, K., Sommarin, M.N.E., Munita, R., Lubas, M., et al. (2018). Pseudouridylation of tRNA-Derived Fragments Steers Translational Control in Stem Cells. Cell *173*, 1204-1216 e1226. 10.1016/j.cell.2018.03.008.

Hamma, T., and Ferre-D'Amare, A.R. (2006). Pseudouridine synthases. Chem Biol *13*, 1125-1135. 10.1016/j.chembiol.2006.09.009.

Hasler, D., Meduri, R., Bak, M., Lehmann, G., Heizinger, L., Wang, X., Li, Z.T., Sement, F.M., Bruckmann, A., Dock-Bregeon, A.C., et al. (2020). The Alazami Syndrome-Associated Protein LARP7 Guides U6 Small Nuclear RNA Modification and Contributes to Splicing Robustness. Mol Cell *77*, 1014-1031 e1013. 10.1016/j.molcel.2020.01.001.

He, J., Navarrete, S., Jasinski, M., Vulliamy, T., Dokal, I., Bessler, M., and Mason, P.J. (2002). Targeted disruption of Dkc1, the gene mutated in X-linked dyskeratosis congenita, causes embryonic lethality in mice. Oncogene *21*, 7740-7744. 10.1038/sj.onc.1205969.

Hebras, J., Krogh, N., Marty, V., Nielsen, H., and Cavaille, J. (2020). Developmental changes of rRNA ribose methylations in the mouse. RNA Biol *17*, 150-164. 10.1080/15476286.2019.1670598.

Heiss, N.S., Knight, S.W., Vulliamy, T.J., Klauck, S.M., Wiemann, S., Mason, P.J., Poustka, A., and Dokal, I. (1998). X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. Nat Genet *19*, 32-38. 10.1038/ng0598-32.

Hsu, T.Y., Simon, L.M., Neill, N.J., Marcotte, R., Sayad, A., Bland, C.S., Echeverria, G.V., Sun, T., Kurley, S.J., Tyagi, S., et al. (2015). The spliceosome is a therapeutic vulnerability in MYC-driven cancer. Nature *525*, 384-388. 10.1038/nature14985.

Ignatova, V.V., Stolz, P., Kaiser, S., Gustafsson, T.H., Lastres, P.R., Sanz-Moreno, A., Cho, Y.L., Amarie, O.V., Aguilar-Pimentel, A., Klein-Rodewald, T., et al. (2020). The rRNA m(6)A methyltransferase METTL5 is involved in pluripotency and developmental programs. Genes & development *34*, 715-729. 10.1101/gad.333369.119.

Incarnato, D., Anselmi, F., Morandi, E., Neri, F., Maldotti, M., Rapelli, S., Parlato, C., Basile, G., and Oliviero, S. (2017). High-throughput single-base resolution mapping of RNA 2-O-methylated residues. Nucleic Acids Res *45*, 1433-1441. 10.1093/nar/gkw810.

Ingolia, N.T., Lareau, L.F., and Weissman, J.S. (2011). Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. Cell *147*, 789-802. 10.1016/j.cell.2011.10.002.

Jack, K., Bellodi, C., Landry, D.M., Niederer, R.O., Meskauskas, A., Musalgaonkar, S., Kopmar, N., Krasnykh, O., Dean, A.M., Thompson, S.R., et al. (2011). rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. Molecular cell *44*, 660-666. 10.1016/j.molcel.2011.09.017.

Jacks, T., and Varmus, H.E. (1985). Expression of the Rous sarcoma virus pol gene by ribosomal frameshifting. Science *230*, 1237-1242.

Jackson, R.J., Hellen, C.U., and Pestova, T.V. (2010). The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol *11*, 113-127. 10.1038/nrm2838.

Jang, S.K., Krausslich, H.G., Nicklin, M.J., Duke, G.M., Palmenberg, A.C., and Wimmer, E. (1988). A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. J Virol *62*, 2636-2643. 10.1128/JVI.62.8.2636-2643.1988.

Janin, M., Coll-SanMartin, L., and Esteller, M. (2020). Disruption of the RNA modifications that target the ribosome translation machinery in human cancer. Molecular cancer *19*, 70. 10.1186/s12943-020-01192-8.

Jorjani, H., Kehr, S., Jedlinski, D.J., Gumienny, R., Hertel, J., Stadler, P.F., Zavolan, M., and Gruber, A.R. (2016). An updated human snoRNAome. Nucleic Acids Res *44*, 5068-5082. 10.1093/nar/gkw386.

Kalia, R., Wang, R.Y., Yusuf, A., Thomas, P.V., Agard, D.A., Shaw, J.M., and Frost, A. (2018). Structural basis of mitochondrial receptor binding and constriction by DRP1. Nature *558*, 401-405. 10.1038/s41586-018-0211-2.

Karijolich, J., and Yu, Y.T. (2010). Spliceosomal snRNA modifications and their function. RNA Biol *7*, 192-204.

Khajuria, R.K., Munschauer, M., Ulirsch, J.C., Fiorini, C., Ludwig, L.S., McFarland, S.K., Abdulhay, N.J., Specht, H., Keshishian, H., Mani, D.R., et al. (2018). Ribosome Levels Selectively Regulate Translation and Lineage Commitment in Human Hematopoiesis. Cell *173*, 90-103 e119. 10.1016/j.cell.2018.02.036.

Khatter, H., Myasnikov, A.G., Natchiar, S.K., and Klaholz, B.P. (2015). Structure of the human 80S ribosome. Nature *520*, 640-645. 10.1038/nature14427.

King, T.H., Liu, B., McCully, R.R., and Fournier, M.J. (2003). Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. Mol Cell *11*, 425-435. 10.1016/s1097-2765(03)00040-6.

Kishore, S., Khanna, A., Zhang, Z., Hui, J., Balwierz, P.J., Stefan, M., Beach, C., Nicholls, R.D., Zavolan, M., and Stamm, S. (2010). The snoRNA MBII-52 (SNORD 115) is processed into smaller RNAs and regulates alternative splicing. Hum Mol Genet *19*, 1153-1164. 10.1093/hmg/ddp585.

Kishore, S., and Stamm, S. (2006). The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science *311*, 230-232. 10.1126/science.1118265.

Kiss, T. (2001). Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs. Embo J *20*, 3617-3622. 10.1093/emboj/20.14.3617.

Kiss, T., Fayet-Lebaron, E., and Jady, B.E. (2010). Box H/ACA small ribonucleoproteins. Mol Cell *37*, 597-606. 10.1016/j.molcel.2010.01.032.

Kiss-Laszlo, Z., Henry, Y., and Kiss, T. (1998). Sequence and structural elements of methylation guide snoRNAs essential for site-specific ribose methylation of pre-rRNA. Embo J *17*, 797-807. 10.1093/emboj/17.3.797.

Koh, C.M., Bezzi, M., Low, D.H., Ang, W.X., Teo, S.X., Gay, F.P., Al-Haddawi, M., Tan, S.Y., Osato, M., Sabo, A., et al. (2015). MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. Nature *523*, 96-100. 10.1038/nature14351.

Kole, R., Krainer, A.R., and Altman, S. (2012). RNA therapeutics: beyond RNA interference and antisense oligonucleotides. Nature reviews. Drug discovery *11*, 125-140. 10.1038/nrd3625.

Kos, M., and Tollervey, D. (2010). Yeast pre-rRNA processing and modification occur cotranscriptionally. Mol Cell *37*, 809-820. 10.1016/j.molcel.2010.02.024.

Krogh, N., Jansson, M.D., Hafner, S.J., Tehler, D., Birkedal, U., Christensen-Dalsgaard, M., Lund, A.H., and Nielsen, H. (2016). Profiling of 2'-O-Me in human rRNA reveals a subset of fractionally modified positions and provides evidence for ribosome heterogeneity. Nucleic Acids Res *44*, 7884-7895. 10.1093/nar/gkw482.

Krogh, N., Kongsbak-Wismann, M., Geisler, C., and Nielsen, H. (2017). Substoichiometric ribose methylations in spliceosomal snRNAs. Org Biomol Chem *15*, 8872-8876. 10.1039/c7ob02317k.

Lee, A.S., Kranzusch, P.J., and Cate, J.H. (2015). eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. Nature *522*, 111-114. 10.1038/nature14267.

Lee, A.S., Kranzusch, P.J., Doudna, J.A., and Cate, J.H. (2016). eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation. Nature *536*, 96-99. 10.1038/nature18954.

Lee, S.C., and Abdel-Wahab, O. (2016). Therapeutic targeting of splicing in cancer. Nat Med *22*, 976-986. 10.1038/nm.4165.

Lewis, B.P., Green, R.E., and Brenner, S.E. (2003). Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. Proceedings of the National Academy of Sciences of the United States of America *100*, 189-192. 10.1073/pnas.0136770100.

Li, X., Zhu, P., Ma, S., Song, J., Bai, J., Sun, F., and Yi, C. (2015). Chemical pulldown reveals dynamic pseudouridylation of the mammalian transcriptome. Nat Chem Biol *11*, 592-597. 10.1038/nchembio.1836.

Liang, X.H., Liu, Q., and Fournier, M.J. (2007). rRNA modifications in an intersubunit bridge of the ribosome strongly affect both ribosome biogenesis and activity. Mol Cell *28*, 965-977. 10.1016/j.molcel.2007.10.012.

Liu, N., Parisien, M., Dai, Q., Zheng, G., He, C., and Pan, T. (2013). Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. RNA *19*, 1848-1856. 10.1261/rna.041178.113.

Lovejoy, A.F., Riordan, D.P., and Brown, P.O. (2014). Transcriptome-wide mapping of pseudouridines: pseudouridine synthases modify specific mRNAs in S. cerevisiae. PloS one *9*, e110799. 10.1371/journal.pone.0110799.

Maden, B.E., Corbett, M.E., Heeney, P.A., Pugh, K., and Ajuh, P.M. (1995). Classical and novel approaches to the detection and localization of the numerous modified nucleotides in eukaryotic ribosomal RNA. Biochimie *77*, 22-29. 10.1016/0300-9084(96)88100-4.

Mailliot, J., Garreau de Loubresse, N., Yusupova, G., Meskauskas, A., Dinman, J.D., and Yusupov, M. (2016). Crystal Structures of the uL3 Mutant Ribosome: Illustration of the Importance of Ribosomal Proteins for Translation Efficiency. J Mol Biol *428*, 2195-2202. 10.1016/j.jmb.2016.02.013.

Mann, M., and Jensen, O.N. (2003). Proteomic analysis of post-translational modifications. Nat Biotechnol *21*, 255-261. 10.1038/nbt0303-255.

Mannoor, K., Liao, J., and Jiang, F. (2012). Small nucleolar RNAs in cancer. Biochimica et biophysica acta *1826*, 121-128. 10.1016/j.bbcan.2012.03.005.

Marcel, V., Ghayad, S.E., Belin, S., Therizols, G., Morel, A.P., Solano-Gonzalez, E., Vendrell, J.A., Hacot, S., Mertani, H.C., Albaret, M.A., et al. (2013). p53 acts as a safeguard of translational control by regulating fibrillarin and rRNA methylation in cancer. Cancer Cell *24*, 318-330. 10.1016/j.ccr.2013.08.013.

Marchand, V., Blanloeil-Oillo, F., Helm, M., and Motorin, Y. (2016). Illumina-based RiboMethSeq approach for mapping of 2'-O-Me residues in RNA. Nucleic Acids Res *44*, e135. 10.1093/nar/gkw547.

Matera, A.G., and Wang, Z. (2014). A day in the life of the spliceosome. Nat Rev Mol Cell Biol *15*, 108-121. 10.1038/nrm3742.

McCann, K.L., Kavari, S.L., Burkholder, A.B., Phillips, B.T., and Hall, T.M.T. (2020). H/ACA snoRNA levels are regulated during stem cell differentiation. Nucleic Acids Res *48*, 8686-8703. 10.1093/nar/gkaa612.

McClorey, G., and Wood, M.J. (2015). An overview of the clinical application of antisense oligonucleotides for RNA-targeting therapies. Curr Opin Pharmacol *24*, 52-58. 10.1016/j.coph.2015.07.005.

McMahon, M., Contreras, A., Holm, M., Uechi, T., Forester, C.M., Pang, X., Jackson, C., Calvert, M.E., Chen, B., Quigley, D.A., et al. (2019). A single H/ACA small nucleolar RNA mediates tumor suppression downstream of oncogenic RAS. Elife *8*. 10.7554/eLife.48847.

Meskauskas, A., and Dinman, J.D. (2007). Ribosomal protein L3: gatekeeper to the A site. Mol Cell *25*, 877-888. 10.1016/j.molcel.2007.02.015.

Meskauskas, A., Petrov, A.N., and Dinman, J.D. (2005). Identification of functionally important amino acids of ribosomal protein L3 by saturation mutagenesis. Mol Cell Biol *25*, 10863-10874. 10.1128/MCB.25.24.10863-10874.2005.

Mitchell, J.R., Cheng, J., and Collins, K. (1999a). A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. Mol Cell Biol *19*, 567-576. 10.1128/MCB.19.1.567.

Mitchell, J.R., Wood, E., and Collins, K. (1999b). A telomerase component is defective in the human disease dyskeratosis congenita. Nature *402*, 551-555. 10.1038/990141.

Mohler, K., and Ibba, M. (2017). Translational fidelity and mistranslation in the cellular response to stress. Nat Microbiol *2*, 17117. 10.1038/nmicrobiol.2017.117.

Montanaro, L. (2010). Dyskerin and cancer: more than telomerase. The defect in mRNA translation helps in explaining how a proliferative defect leads to cancer. J Pathol *222*, 345-349. 10.1002/path.2777.

Montanaro, L., Calienni, M., Bertoni, S., Rocchi, L., Sansone, P., Storci, G., Santini, D., Ceccarelli, C., Taffurelli, M., Carnicelli, D., et al. (2010). Novel dyskerin-mediated mechanism of p53 inactivation through defective mRNA translation. Cancer research *70*, 4767-4777. 10.1158/0008-5472.CAN-09-4024.

Morris, K.V., and Mattick, J.S. (2014). The rise of regulatory RNA. Nature reviews. Genetics *15*, 423-437. 10.1038/nrg3722.

Nagasawa, C.K., Kibiryeva, N., Marshall, J., O'Brien, J.E., Jr., and Bittel, D.C. (2020). scaRNA1 Levels Alter Pseudouridylation in Spliceosomal RNA U2 Affecting Alternative mRNA Splicing and Embryonic Development. Pediatr Cardiol *41*, 341-349. 10.1007/s00246-019-02263-4.

Oakes, E., Vadlamani, P., and Hundley, H.A. (2017). Methods for the Detection of Adenosine-to-Inosine Editing Events in Cellular RNA. Methods Mol Biol *1648*, 103-127. 10.1007/978-1-4939-7204-3_9.

Ofengand, J., Del Campo, M., and Kaya, Y. (2001). Mapping pseudouridines in RNA molecules. Methods *25*, 365-373. 10.1006/meth.2001.1249.

Oltean, S., and Bates, D.O. (2014). Hallmarks of alternative splicing in cancer. Oncogene *33*, 5311-5318. 10.1038/onc.2013.533.

Pan, Q., Saltzman, A.L., Kim, Y.K., Misquitta, C., Shai, O., Maquat, L.E., Frey, B.J., and Blencowe, B.J. (2006). Quantitative microarray profiling provides evidence against widespread coupling of alternative splicing with nonsense-mediated mRNA decay to control gene expression. Genes & development *20*, 153-158. 10.1101/gad.1382806.

Pan, Q., Shai, O., Lee, L.J., Frey, B.J., and Blencowe, B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat Genet *40*, 1413-1415. 10.1038/ng.259.

Patil, P., Kibiryeva, N., Uechi, T., Marshall, J., O'Brien, J.E., Jr., Artman, M., Kenmochi, N., and Bittel, D.C. (2015). scaRNAs regulate splicing and vertebrate heart development. Biochimica et biophysica acta *1852*, 1619-1629. 10.1016/j.bbadis.2015.04.016.

Pelletier, J., and Sonenberg, N. (1988). Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature *334*, 320-325. 10.1038/334320a0.

Pestova, T.V., and Hellen, C.U. (2003). Translation elongation after assembly of ribosomes on the Cricket paralysis virus internal ribosomal entry site without initiation factors or initiator tRNA. Genes & development *17*, 181-186. 10.1101/gad.1040803.

Pestova, T.V., Shatsky, I.N., Fletcher, S.P., Jackson, R.J., and Hellen, C.U. (1998). A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. Genes & development *12*, 67-83. 10.1101/gad.12.1.67.

Piekna-Przybylska, D., Decatur, W.A., and Fournier, M.J. (2008). The 3D rRNA modification maps database: with interactive tools for ribosome analysis. Nucleic Acids Res *36*, D178-183. 10.1093/nar/gkm855.

Ramachandran, S., Krogh, N., Jorgensen, T.E., Johansen, S.D., Nielsen, H., and Babiak, I. (2020). The shift from early to late types of ribosomes in zebrafish development involves changes at a subset of rRNA 2'-O-Me sites. RNA *26*, 1919-1934. 10.1261/rna.076760.120.

Ronchetti, D., Mosca, L., Cutrona, G., Tuana, G., Gentile, M., Fabris, S., Agnelli, L., Ciceri, G., Matis, S., Massucco, C., et al. (2013). Small nucleolar RNAs as new biomarkers in chronic lymphocytic leukemia. BMC Med Genomics *6*, 27. 10.1186/1755-8794-6-27.

Ronchetti, D., Todoerti, K., Tuana, G., Agnelli, L., Mosca, L., Lionetti, M., Fabris, S., Colapietro, P., Miozzo, M., Ferrarini, M., et al. (2012). The expression pattern

of small nucleolar and small Cajal body-specific RNAs characterizes distinct molecular subtypes of multiple myeloma. Blood Cancer J *2*, e96. 10.1038/bcj.2012.41.

Roundtree, I.A., Evans, M.E., Pan, T., and He, C. (2017). Dynamic RNA Modifications in Gene Expression Regulation. Cell *169*, 1187-1200. 10.1016/j.cell.2017.05.045.

Ruggero, D. (2009). The role of Myc-induced protein synthesis in cancer. Cancer research *69*, 8839-8843. 10.1158/0008-5472.CAN-09-1970.

Saba, J.A., Liakath-Ali, K., Green, R., and Watt, F.M. (2021). Translational control of stem cell function. Nat Rev Mol Cell Biol. 10.1038/s41580-021-00386-2.

Saletore, Y., Meyer, K., Korlach, J., Vilfan, I.D., Jaffrey, S., and Mason, C.E. (2012). The birth of the Epitranscriptome: deciphering the function of RNA modifications. Genome Biol *13*, 175. 10.1186/gb-2012-13-10-175.

Sampath, P., Pritchard, D.K., Pabon, L., Reinecke, H., Schwartz, S.M., Morris, D.R., and Murry, C.E. (2008). A hierarchical network controls protein translation during murine embryonic stem cell self-renewal and differentiation. Cell Stem Cell *2*, 448-460. 10.1016/j.stem.2008.03.013.

Schuller, A.P., and Green, R. (2018). Roadblocks and resolutions in eukaryotic translation. Nat Rev Mol Cell Biol *19*, 526-541. 10.1038/s41580-018-0011-4.

Schuster, S.L., and Hsieh, A.C. (2019). The Untranslated Regions of mRNAs in Cancer. Trends Cancer *5*, 245-262. 10.1016/j.trecan.2019.02.011.

Schwartz, S., Bernstein, D.A., Mumbach, M.R., Jovanovic, M., Herbst, R.H., Leon-Ricardo, B.X., Engreitz, J.M., Guttman, M., Satija, R., Lander, E.S., et al. (2014). Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell *159*, 148-162. 10.1016/j.cell.2014.08.028.

Shen, C., Sheng, Y., Zhu, A.C., Robinson, S., Jiang, X., Dong, L., Chen, H., Su, R., Yin, Z., Li, W., et al. (2020). RNA Demethylase ALKBH5 Selectively Promotes Tumorigenesis and Cancer Stem Cell Self-Renewal in Acute Myeloid Leukemia. Cell Stem Cell *27*, 64-80 e69. 10.1016/j.stem.2020.04.009.

Shuai, S., Suzuki, H., Diaz-Navarro, A., Nadeu, F., Kumar, S.A., Gutierrez-Fernandez, A., Delgado, J., Pinyol, M., Lopez-Otin, C., Puente, X.S., et al. (2019). The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature *574*, 712-716. 10.1038/s41586-019-1651-z.

Signer, R.A., Magee, J.A., Salic, A., and Morrison, S.J. (2014). Haematopoietic stem cells require a highly regulated protein synthesis rate. Nature *509*, 49-54. 10.1038/nature13035.

Signer, R.A., Qi, L., Zhao, Z., Thompson, D., Sigova, A.A., Fan, Z.P., DeMartino, G.N., Young, R.A., Sonenberg, N., and Morrison, S.J. (2016). The rate of protein synthesis in hematopoietic stem cells is limited partly by 4E-BPs. Genes & development *30*, 1698-1703. 10.1101/gad.282756.116.

Simon, J.M., Hacker, K.E., Singh, D., Brannon, A.R., Parker, J.S., Weiser, M., Ho, T.H., Kuan, P.F., Jonasch, E., Furey, T.S., et al. (2014). Variation in chromatin accessibility in human kidney cancer links H3K36 methyltransferase loss with widespread RNA processing defects. Genome Res *24*, 241-250. 10.1101/gr.158253.113.

Siprashvili, Z., Webster, D.E., Johnston, D., Shenoy, R.M., Ungewickell, A.J., Bhaduri, A., Flockhart, R., Zarnegar, B.J., Che, Y., Meschi, F., et al. (2016). The noncoding RNAs SNORD50A and SNORD50B bind K-Ras and are recurrently deleted in human cancer. Nat Genet *48*, 53-58. 10.1038/ng.3452.

Sloan, K.E., Leisegang, M.S., Doebele, C., Ramirez, A.S., Simm, S., Safferthal, C., Kretschmer, J., Schorge, T., Markoutsa, S., Haag, S., et al. (2015). The association of late-acting snoRNPs with human pre-ribosomal complexes requires the RNA helicase DDX21. Nucleic Acids Res *43*, 553-564. 10.1093/nar/gku1291.

Sloan, K.E., Warda, A.S., Sharma, S., Entian, K.D., Lafontaine, D.L.J., and Bohnsack, M.T. (2017). Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. RNA Biol *14*, 1138-1152. 10.1080/15476286.2016.1259781.

Sonenberg, N., and Hinnebusch, A.G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell *136*, 731-745. 10.1016/j.cell.2009.01.042.

Su, H., Xu, T., Ganapathy, S., Shadfan, M., Long, M., Huang, T.H., Thompson, I., and Yuan, Z.M. (2014). Elevated snoRNA biogenesis is essential in breast cancer. Oncogene *33*, 1348-1358. 10.1038/onc.2013.89.

Sveen, A., Kilpinen, S., Ruusulehto, A., Lothe, R.A., and Skotheim, R.I. (2016). Aberrant RNA splicing in cancer; expression changes and driver mutations of splicing factor genes. Oncogene *35*, 2413-2427. 10.1038/onc.2015.318.

Tahmasebi, S., Khoutorsky, A., Mathews, M.B., and Sonenberg, N. (2018). Translation deregulation in human disease. Nat Rev Mol Cell Biol *19*, 791-807. 10.1038/s41580-018-0034-x.

Taoka, M., Nobe, Y., Yamaki, Y., Yamauchi, Y., Ishikawa, H., Takahashi, N., Nakayama, H., and Isobe, T. (2016). The complete chemical structure of Saccharomyces cerevisiae rRNA: partial pseudouridylation of U2345 in 25S rRNA by snoRNA snR9. Nucleic Acids Res *44*, 8951-8961. 10.1093/nar/gkw564.

Teittinen, K.J., Laiho, A., Uusimaki, A., Pursiheimo, J.P., Gyenesei, A., and Lohi, O. (2013). Expression of small nucleolar RNAs in leukemic cells. Cellular oncology *36*, 55-63. 10.1007/s13402-012-0113-5.

Tollervey, D., and Kiss, T. (1997). Function and synthesis of small nucleolar RNAs. Curr Opin Cell Biol *9*, 337-342. 10.1016/s0955-0674(97)80005-1.

Turowski, T.W., and Tollervey, D. (2015). Cotranscriptional events in eukaryotic ribosome synthesis. Wiley interdisciplinary reviews. RNA *6*, 129-139. 10.1002/wrna.1263.

Turunen, J.J., Niemela, E.H., Verma, B., and Frilander, M.J. (2013). The significant other: splicing by the minor spliceosome. Wiley interdisciplinary reviews. RNA *4*, 61-76. 10.1002/wrna.1141.

Vaklavas, C., Blume, S.W., and Grizzle, W.E. (2017). Translational Dysregulation in Cancer: Molecular Insights and Potential Clinical Applications in Biomarker Development. Front Oncol *7*, 158. 10.3389/fonc.2017.00158.

Valadkhan, S., Mohammadi, A., Jaladat, Y., and Geisler, S. (2009). Protein-free small nuclear RNAs catalyze a two-step splicing reaction. Proceedings of the National Academy of Sciences of the United States of America *106*, 11901-11906. 10.1073/pnas.0902020106.

Valadkhan, S., Mohammadi, A., Wachtel, C., and Manley, J.L. (2007). Protein-free spliceosomal snRNAs catalyze a reaction that resembles the first step of splicing. RNA *13*, 2300-2311. 10.1261/rna.626207.

Valleron, W., Laprevotte, E., Gautier, E.F., Quelen, C., Demur, C., Delabesse, E., Agirre, X., Prosper, F., Kiss, T., and Brousset, P. (2012). Specific small nucleolar RNA expression profiles in acute leukemia. Leukemia *26*, 2052-2060. 10.1038/leu.2012.111.

Vu, L.P., Cheng, Y., and Kharas, M.G. (2019). The Biology of m(6)A RNA Methylation in Normal and Malignant Hematopoiesis. Cancer Discov *9*, 25-33. 10.1158/2159-8290.CD-18-0959.

Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., and Burge, C.B. (2008). Alternative isoform regulation in human tissue transcriptomes. Nature *456*, 470-476. 10.1038/nature07509.

Wang, X., Li, Z.T., Yan, Y., Lin, P., Tang, W., Hasler, D., Meduri, R., Li, Y., Hua, M.M., Qi, H.T., et al. (2020). LARP7-Mediated U6 snRNA Modification Ensures Splicing Fidelity and Spermatogenesis in Mice. Mol Cell *77*, 999-1013 e1016. 10.1016/j.molcel.2020.01.002.

Watkins, N.J., and Bohnsack, M.T. (2012). The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. Wiley interdisciplinary reviews. RNA *3*, 397-414. 10.1002/wrna.117.

Weatheritt, R.J., Sterne-Weiler, T., and Blencowe, B.J. (2016). The ribosome-engaged landscape of alternative splicing. Nat Struct Mol Biol *23*, 1117-1123. 10.1038/nsmb.3317.

Will, C.L., and Luhrmann, R. (2011). Spliceosome structure and function. Cold Spring Harbor perspectives in biology *3*. 10.1101/cshperspect.a003707.

Wilson, A., Murphy, M.J., Oskarsson, T., Kaloulis, K., Bettess, M.D., Oser, G.M., Pasche, A.C., Knabenhans, C., Macdonald, H.R., and Trumpp, A. (2004). c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes & development *18*, 2747-2763. 10.1101/gad.313104.

Wilson, J.E., Powell, M.J., Hoover, S.E., and Sarnow, P. (2000). Naturally occurring dicistronic cricket paralysis virus RNA is regulated by two internal ribosome entry sites. Mol Cell Biol *20*, 4990-4999. 10.1128/MCB.20.14.4990-4999.2000.

Wu, G., Radwan, M.K., Xiao, M., Adachi, H., Fan, J., and Yu, Y.T. (2016). The TOR signaling pathway regulates starvation-induced pseudouridylation of yeast U2 snRNA. RNA *22*, 1146-1152. 10.1261/rna.056796.116.

Wu, G., Xiao, M., Yang, C., and Yu, Y.T. (2011). U2 snRNA is inducibly pseudouridylated at novel sites by Pus7p and snR81 RNP. Embo J *30*, 79-89. 10.1038/emboj.2010.316.

Xing, M., Liu, Q., Mao, C., Zeng, H., Zhang, X., Zhao, S., Chen, L., Liu, M., Shen, B., Guo, X., et al. (2020). The 18S rRNA m(6) A methyltransferase METTL5 promotes mouse embryonic stem cell differentiation. EMBO Rep *21*, e49863. 10.15252/embr.201949863.

Yankova, E., Blackaby, W., Albertella, M., Rak, J., De Braekeleer, E., Tsagkogeorga, G., Pilka, E.S., Aspris, D., Leggate, D., Hendrick, A.G., et al. (2021). Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature *593*, 597-601. 10.1038/s41586-021-03536-w.

Yokoyama, T., Machida, K., Iwasaki, W., Shigeta, T., Nishimoto, M., Takahashi, M., Sakamoto, A., Yonemochi, M., Harada, Y., Shigematsu, H., et al. (2019). HCV IRES Captures an Actively Translating 80S Ribosome. Mol Cell *74*, 1205-1214 e1208. 10.1016/j.molcel.2019.04.022.

Yoon, A., Peng, G., Brandenburger, Y., Zollo, O., Xu, W., Rego, E., and Ruggero, D. (2006). Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. Science *312*, 902-906. 312/5775/902 [pii]

10.1126/science.1123835.

Yu, Y.T., Shu, M.D., and Steitz, J.A. (1997). A new method for detecting sites of 2'-O-methylation in RNA molecules. RNA *3*, 324-331.

Yu, Y.T., Shu, M.D., and Steitz, J.A. (1998). Modifications of U2 snRNA are required for snRNP assembly and pre-mRNA splicing. Embo J *17*, 5783-5795. 10.1093/emboj/17.19.5783.

Zaher, H.S., and Green, R. (2009). Fidelity at the molecular level: lessons from protein synthesis. Cell *136*, 746-762. 10.1016/j.cell.2009.01.036.

Zaringhalam, M., and Papavasiliou, F.N. (2016). Pseudouridylation meets next-generation sequencing. Methods *107*, 63-72. 10.1016/j.ymeth.2016.03.001.

Zaro, B.W., Noh, J.J., Mascetti, V.L., Demeter, J., George, B., Zukowska, M., Gulati, G.S., Sinha, R., Flynn, R.A., Banuelos, A., et al. (2020). Proteomic analysis of young and old mouse hematopoietic stem cells and their progenitors reveals post-transcriptional regulation in stem cells. Elife *9*. 10.7554/eLife.62210.

Zhang, Y., Qian, J., Gu, C., and Yang, Y. (2021). Alternative splicing and cancer: a systematic review. Signal Transduct Target Ther *6*, 78. 10.1038/s41392-021-00486-7.

Zhao, X., Li, Z.H., Terns, R.M., Terns, M.P., and Yu, Y.T. (2002). An H/ACA guide RNA directs U2 pseudouridylation at two different sites in the branchpoint recognition region in Xenopus oocytes. RNA *8*, 1515-1525.

Zhao, X., and Yu, Y.T. (2004). Detection and quantitation of RNA base modifications. RNA *10*, 996-1002. 10.1261/rna.7110804.

Zhou, F., Liu, Y., Rohde, C., Pauli, C., Gerloff, D., Kohn, M., Misiak, D., Baumer, N., Cui, C., Gollner, S., et al. (2017). AML1-ETO requires enhanced C/D box snoRNA/RNP formation to induce self-renewal and leukaemia. Nat Cell Biol *19*, 844-855. 10.1038/ncb3563.