UPPSALA
UNIVERSITET

*Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1854

# PARN – The Tail End

## *Function and mechanisms of specificity and processivity*

MIKAEL NISSBECK

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2019

ISSN 1651-6214
ISBN 978-91-513-0752-7
urn:nbn:se:uu:diva-392998

Dissertation presented at Uppsala University to be publicly examined in B:21, Biomedicinskt centrum (BMC), Husargatan 3, Uppsala, Thursday, 31 October 2019 at 13:15 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: professor Renée Schroeder (University of Vienna).

**Abstract**
Nissbeck, M. 2019. PARN – The Tail End. Function and mechanisms of specificity and processivity. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1854. 46 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0752-7.

Poly(A)-specific ribonuclease (PARN) is an exoribonuclease that is processive, poly(A) specific and cap-binding. PARN deadenylates the poly(A) tails present on a subset of mRNAs and non-coding RNAs, including among others certain snoRNAs, miRNAs and precursor rRNAs.

Here, we have investigated molecular mechanisms behind PARN's specificity for adenine and ribose, essential properties for PARN's ability to degrade poly(A) on RNAs. We have applied enzyme kinetics and used divalent metal ions as mechanistic probes to show that PARN's poly(A) specificity is tightly linked to a translocation event during the hydrolytic cycle of PARN action.

To further understand the mechanism of PARN's processive mode of action we are developing a kinetic model that allow us to measure the probability of processive action for each round of the hydrolytic cycle. Our kinetic model will be general and applicable to the processive action of any processive enzymatic activity. In conclusion, our study has so far established a mechanistic link between PARN's processive mode of action, hydrolytic activity and preference for degrading poly(A).

Human patients with genetic lesions in *PARN* suffer from a spectrum of syndromes called telomere biology disorders (TBD), which are associated with short telomeres. PARN is involved in the maturation of the snoRNA telomerase RNA component (TERC) that is used as template during the elongation of the telomer by the telomerase. Point mutations in the gene for *PARN* have been identified in patients. We show that point mutations in PARN that perturb its deadenylation activity correlated with TBDs and developmental disorders.

Our mechanistic studies of PARN action will provide a framework for our understanding of PARN's physiological role and in extension the molecular basis for human diseases caused by perturbed PARN action. Our studies will also be of general interest for our detailed and mechanistic understanding of basic and essential mechanisms of gene expression and RNA biology.

*Keywords:* Poly(A) specific ribonuclease, Adenosine, Specificity, Processivty, Telomere biology disorders

*Mikael Nissbeck, Department of Cell and Molecular Biology, Microbiology, Box 596, Uppsala University, SE-75124 Uppsala, Sweden.*



ISSN 1651-6214
ISBN 978-91-513-0752-7
urn:nbn:se:uu:diva-392998 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-392998)

*Mormor*

# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Nissbeck**. **M**., Henriksson, N., Nilsson. P., Berndtsson, J., Popova, G. and Virtanen, A. (2019) Specificity for adenine is established during the hydrolytic cycle of poly(A)-specific ribonuclease (PARN). (*Submitted Manuscript*)

II **Nissbeck**, **M**. and Virtanen, A., (2019) Mechanisms of PARN action: Specificity and requirement for the 2'OH groups of the substrate. (*Manuscript*)

III **Nissbeck**, **M**., Emmerich, A., Ehrenberg, M. and Virtanen, A. (2019) Mechanisms of PARN action: Kinetic analysis of processive mode of degradation. (*Manuscript*)

IV Dhanraj, A., Gunja, S.M.R., Deveau, A.P., **Nissbeck**, **M**., Boonyawat, B., Coombs, A.J., Renieri, A., Mucciolo, A., Marozza, A., Buoni, S., Turner, L., Li, H., Jarrar, A., Sabanayagam, M., Kirby, M., Shago, M., Pinto, D., Berman, J.N., Scherer, S.W., Virtanen, A. and Dror, Y. (2015) Bone marrow failure and developmental delay caused by mutations in poly(A)-specific ribonuclease (PARN). *Journal of Medical Genetics*, 52(11), pp. 738–748.

V Dodson, L.M., Baldan, A., **Nissbeck**, **M**., Gunja S.M.R., Boonen, P.E., Aubert, G., Birchansky, S., Virtanen, A. and Bertuch. A.A. (2019) From incomplete penetrance with normal telomere length to severe disease and telomere shortening in a family with monoallelic and biallelic PARN pathogenic variants. *Human Mutation*. John Wiley & Sons, Ltd, (July), p. humu.23898. doi: 10.1002/humu.23898

# Contents

# Abbreviations

| | |
|---|---|
| A | Adenosine |
| ATP | Adenosine triphosphate |
| C | Cytosine |
| Caf1 | CCR4-assosiated factor 1 |
| CCR4 | carbon catabolite repressor 4 |
| DC | Dyskeratosis congenita |
| DcpS | Scavenger-decapping enzyme |
| DCP | Decapping enzymes |
| DDR | DNA damage response |
| EEP | exo-endonuclease-phosphatase |
| eIF | eukaryotic initiation factor |
| G | Guanine |
| HHS | Hoyeraal-Hreidersson syndrome |
| IPF | idiopathic pulmonary fibrosis |
| mRNA | Messenger RNA |
| miRNA | microRNA |
| U | Uracil |
| PABP | Poly(A) binding proteins |
| Pan | poly(A) nuclease |
| PAP | Poly(A) polymerase |
| PARN | poly(A)-specific ribonuclease |
| pre-RNA | Precursor RNA |
| RNAP | RNA polymerase |
| RRM | RNA recognition motif |
| rRNA | Ribosomal RNA |
| scaRNA | small cajal body specific RNA |
| snRNA | spliceosomal RNA |
| snoRNA | small nucleolar RNA |
| tRNA | Transfer RNA |
| T | Thymine |
| TBD | Telomer biology disorders |
| TERC | Telomeric RNA component |
| TERT | Telomeric reverse transcriptase |
| Xrn1 | 5’-3’ exoribonuclease 1 |

# Introduction

The RNA poly(A) tail was discovered by Edmonds, Vaughan and Nakazato (Edmonds, Vaughan and Nakazato, 1971) a decade after the enzymatic function that polymerizes the poly(A) tail on the 3'end of RNA was found (Edmonds and Abrams, 1960). The poly(A) tail is a polymer of adenine nucleotides that is polymerized to the 3'end of the RNA by poly(A) polymerase (PAP). The addition of the poly(A) tail is a post-transcriptional modification on RNA. PAP builds the poly(A) tail from adenosine triphosphate (ATP) molecules without a template, unlike transcription or replication of the genome, which both require a template DNA molecule for their synthesis. The polymerization of the poly(A) tail is aided by poly(A) binding proteins (PABP) that also stabilizes the newly formed poly(A) sequences as well as creating a platform for PAP to continue the polymerization of the poly(A) tail (Bienroth, Keller and Wahle, 1993). The polymerization of the mRNA poly(A) tail continues until approximately 200-300 adenines have been incorporated (Sheets and Wickens, 1989). PABP was first purified and analyzed from polysomes by Blobel (Blobel, 1973). The purified protein associates with the 3'end located poly(A) tail. It has been found that the poly(A)/PABP complex makes the polyadenylated RNA resistant to 3' degradation by RNases. There is a perception of the poly(A) tail that it stabilizes the mRNA. However, the stabilization of the poly(A) tail arises from the binding of PABP to the poly(A) tail and thereby protects the polyadenylated 3'end from nucleases. As a matter of fact, a poly(A) tail in prokaryotes is a signal for degradation of the adenylated RNA, while a free poly(A) tail is readily degraded by deadenylases in the absence of PABP in eukaryotes with one known exception, *i.e.* of the PABP dependent deadenylase Pan2/Pan3.

## Synthesis and maturation of RNAs

The genetic information is primarily stored in the sequence of the genomic DNA. To gain access to this information the genomic DNA is transcribed to RNA. In the eukaryotic cell there are three different RNA polymerases that transcribe the major classes of RNA in the cell: RNA polymerase I (RNAPI) transcribes precursor ribosomal RNAs (pre-rRNA), RNAPII transcribes precursor messenger RNA (pre-mRNA), spliceosomal RNA (pre-snRNA) and microRNA (pre-miRNA), and RNAPIII transcribes transfer RNA (pre-tRNA)

and one of the precursor rRNA (pre-rRNA). All the transcribed RNAs have to be matured before the RNA becomes functional. The maturation pathways for RNA depend on the type of RNA that is matured. The maturation process is a highly regulated process. Furthermore, during the maturation process the RNA is quality checked and even chemically modified to ensure that the RNA receives the intended function.

## mRNA maturation

The maturation of the pre-mRNA starts shortly after transcription has been initiated by RNA polymerase II. The 5'end of the growing mRNA is capped when approximately twenty nucleotides have been transcribed (Shatkin, 1976; Proudfoot, Furger and Dye, 2002). Capping occurs at the tri-phosphate of the first transcribed nucleotide. The first transcribed nucleotide and the first step of capping is the hydrolysis of the terminal phosphate of the triphosphate ($pppGp(Np)_x$). This generates a diphosphate at the 5'end of the mRNA ($ppGp(Np)_x$). In the second step a guanosine triphosphate residue is hydrolyzed to guanosine monophosphate and transferred, by guanylyltransferase, to the diphosphate of the hydrolyzed 5'end of the pre-mRNA generating ($GpppGp(Np)_x$). In the last step of capping the amine at the seventh position of the guanine base becomes methylated by N7G-methyltransferrase to generate the matured 5'-end located cap of the mRNA ($m^7GpppGp(Np)_x$) (Shatkin and Manley, 2000). After the cap has been added to the 5'end the RNA polymeras II enters into the elongation phase (Ho and Shuman, 1999). The transcription process copies the entire sequence at the template DNA to generate a mix of coding (exons) and non-coding (introns), in the final matured mRNA the introns have been removed (Berget, Moore and Sharp, 1977; Chow *et al.*, 1977). The introns are removed by the spliceosome, a large ribonucleoprotein complex where the recognition of the exon-intron junction, cleavage and ligation of the mRNA are achieved by RNAs in the complex (Will and Lührmann, 2011). The final major modification to the pre-mRNA is the cleavage of the mRNA from the transcription machinery and polyadenylation of the 3'end (Chan, Choi and Shi, 2011). The mRNA contains the instructions for the ribosome to translate the mRNA into a protein. Both the 5'cap and the poly(A) tail are important elements during initiation of translation (Hinnebusch and Lorsch, 2012), PABP interacts with the scaffold protein eukaryotic initiation factor 4G (eIF4G) (Tarun and Sachs, 1996) and eIF4G also interacts with the cap binding protein eIF4E (Marcotrigiano *et al.*, 1997). The complexes cap-eIF4E-eIF4G-PABP-poly(A) bring both ends of the mRNA together and circularizes the mRNA (Wells *et al.*, 1998).

### Non-coding RNA maturation

All the precursor RNAs are transcribed from the genome, this generates a copy of the genetic sequence. For example the precursor for the ribosomal RNAs 18S, 5.8S and 28S are transcribed in the same pre-RNA molecule (Tomecki, Sikorski and Zakrzewska-Placzek, 2017). In the pre-rRNA molecule RNA sequences at the 5'end, 3'end and between the functional rRNAs will not be included in the functional rRNAs in the ribosome. The enzymatic activities associated with maturation of the pre-RNA to functional RNA are endo- and exonucleases. Endonucleases cut the phosphodiester bond in the RNA molecule and removes larger pieces from the pre-rRNA. In the maturation of the rRNA all the precursors to 18S, 5.8S and 28S rRNA are released from the pre-rRNA by endonucleases. After the individual precursors have been released they are further trimmed by exonucleases and other enzymatic activities. Endonucleases recognize a particular sequence or a structural element in the precursor RNA that guide the endonucleolytic cleavage of the RNA molecule. Splicing of the intron is an example of endonucleolytic activity, which requires two endonucleolytic cleavages of the RNA molecule to join the exons. The endonucleolytic cleavage of the RNA strand might still leave extra nucleotides on the RNA that has to be removed by exonucleases before the RNA becomes functional. Exonucleases remove nucleotides one by one from the ends of the RNA. Exonucleases are directional as they can only digest the RNA strand in either the 5'-3' direction or the 3'-5' direction. One interesting property of exonucleases is that they leave the end of the RNA molecule chemically the same as before the end nucleotide was removed. Thus, exonucleases create the substrate for the next exonucleolytic reaction. In contrast, endonucleases dissociate from the cleavage site after each round of catalysis as the substrate, *i.e.* the scissile phosphodiester bond, is consumed for each round of catalysis. Endo- and exonucleases mature the rRNA to the correct length. In addition, many pre-RNAs of different classes undergo internal modifications of specific bases. Internal modifications like pseudouridylation and 2'-O-methylation of specific nucleotides are frequently guided by small nucleolar RNA (snoRNA) respective small cajal body-specific RNA (scaRNA) (Darzacq *et al.*, 2002; Kiss, Fayet-Lebaron and Jády, 2010; Yu, Ge and Yu, 2011). All rRNAs undergo a number of post-transcriptional modifications before they are matured. The modifications of the pre-rRNA ensure their function as well as providing possible points of regulation of the rRNA synthesis.

## Deadenylation

Little more than half of a decade passed after the discovery of the poly(A) tail until the deadenylation activity of a poly(A) substrate was discovered (Lazarus and Sporin, 1967). Deadenylation is the enzymatic process where the poly(A)

tail of the mRNA is removed from the 3'end one adenine at the time (Lazarus and Sporin, 1967; Abraham and Jacob, 1978; MÜLLER *et al.*, 1978; Schröder *et al.*, 1980). Newton's third law states that "*for every action, there is an equal opposite reaction*". Deadenylation is the equal but opposite reaction to polyadenylation. The deadenylase reaction releases one hydrolyzed adenosine monophosphate (AMP) for each round of hydrolysis and after each hydrolysis a new 3' adenine nucleotide will be exposed at the end of the shortened poly(A) tail (Astrom, Astrom and Virtanen, 1992). The hydrolysis reactions continue until the poly(A) tail has been removed from the mRNA, leaving the mRNA body untouched (Aström, Aström and Virtanen, 1991).

## mRNA turnover

Given that deadenylation is the equal but opposite reaction to polyadenylation, it follows that turnover of RNA is the equal and opposite reaction to transcription. The steady state level of a particular mRNA depends on the rate of transcription and the rate of turnover. Together these processes regulate the mRNA level inside the cell (Ross, 1995). In mRNA turnover the removal of the poly(A) tail is the first and rate limiting step (Meyer, Temme and Wahle, 2004; Parker and Song, 2004). When the poly(A) tail has been removed from mRNA the remaining mRNA body will be degraded from either the 3'end or the 5'end. In the 3'end mRNA degradation pathway, the mRNA body will be degraded by the exosome, all the way to the 5'end, which contains the first transcribed base and cap structure. The cap structure and the first transcribed base are finally degraded by scavenger-decapping enzyme (DcpS).

In the 5' end degradation pathway, the cap on the mRNA is first removed by the decapping enzyme complex DCP1/DCP2, before the mRNA body is degraded. After the cap has been removed from the mRNA the body will be degraded by the 5' to 3' exoribonuclease 1 (Xrn1) (reviewed in (Parker and Song, 2004; Garneau, Wilusz and Wilusz, 2007; Goldstrohm and Wickens, 2008). Deadenylation of the mRNA is primarily dependent on two different deadenylases, the CCR4-associated factor 1 (Caf1) and poly(A) nuclease 2 (Pan2) deadenylases. Caf1 and the deadenylase carbon catabolite repressor protein 4 (CCR4) are part of the major cytoplasmic CCR4/Caf1/NOT deadenylation complex (Liu *et al.*, 1998; Tucker *et al.*, 2002). Pan2 is in complex with two Pan3 proteins (Jonas *et al.*, 2014; Schäfer *et al.*, 2014; Wolf *et al.*, 2014). The deadenylation activity of the Pan2/Pan3 complex is stimulated when the poly(A) tail is in complex with PABP (Lowell and Sachs, 1992; Boeck *et al.*, 1996). Caf1 has a stricter adenosine specificity than Pan2. For example, Pan2 has the capacity to degrade into the mRNA body whereas Caf1 stops when the poly(A) tail has been removed (Tang *et al.*, 2019). It has been suggested that both Caf1 and Pan2 are required to deadenylate the poly(A) tail of one mRNA. Pan2 removes the majority of poly(A) associated with PABP and leaves a poly(A) stub on the mRNA that Caf1 deadenylates.

Caf1, Pan2 and poly(A)-specific ribonuclease (PARN) belong to the DnaQ-like family of nucleases and they coordinate the catalytically essential divalent metal ions in the DEDD active site. CCR4 is a member of the exonuclease-endonuclease-phosphatase (EEP) family of nucleases and the active site consist of the highly conserved amino acids motif NEDDH (Yan, 2014) that coordinates the catalytically essential divalent metal ions (Harnisch *et al.*, 2012). The active sites in both DEDD and EEP coordinate at least two divalent $Mg^{2+}$ metal ions that in turn interact with the scissile phosphodiester bond. These four deadenylases (Caf1, Pan2, PARN and CCR4) appears to be equal. However, the substrate recognition and in particular the recognition of the adenine differs between the four deadenylases.

This thesis focuses on three aspects of PARN action: *i*) specificity for adenine and ribose; *ii*) processivity mode of action and finally *iii*) the biological function of PARN activity.

# PARN – The enzyme

Poly(A)-specific ribonuclease (PARN) is a deadenylase that removes poly(A) tails from 3' polyadenylated RNAs. PARN removes the 3'end exposed adenine nucleotide of the poly(A) tail by hydrolysis. The hydrolysis reaction starts with a hydroxide ion making a nucleophilic attack on the scissile phosphodiester bond through a transition state where a new bond is formed between the attacking hydroxide ion and the targeted phosphodiester bond. One AMP residue will be released and the poly(A) tail will be shortened by one nucleotide for each round of hydrolysis.

## Structure and domains of PARN

PARN has three structural domains, the nuclease domain and two RNA binding domains, *i.e.* the R3H and the RNA recognition motif (RRM) (Figure 1). The R3H domain is located in the middle of the nuclease domain and very little is known of its function.

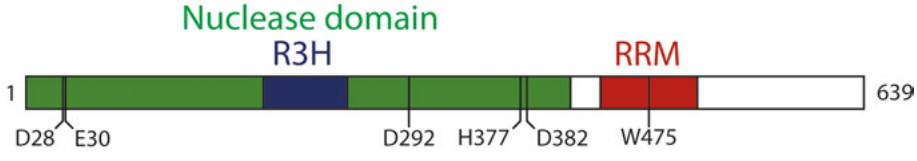


*Figure 1*. Structural domain organization of the polypeptide of PARN

Currently three different crystal structures have been solved for PARN; an apo-structure of human PARN (PDB: 2A1S, figure 2A), a poly(A) associated structure, *i.e.* human PARN co-crystalized with an $A_{10}$ substrate (PDB: 2A1R, figure 2B) and a structure of mouse PARN co-crystalized with the cap moiety $m^7$GpppG (PDB: 3D45, figure 2C) (Wu *et al.*, 2005, 2009). The apo- and the substrate structures of PARN were obtained with a truncated variant of PARN that lacked both the RRM and the C-terminal domain of PARN. All of the structures of PARN show that PARN dimerizes through the nuclease domain. The dimerization surface is close to the active site and dimerization is important for the stability of the active site (Wu *et al.*, 2005). The apo-structure of PARN shows the structure of the nuclease domain and the R3H domain.

The poly(A) substrate structure of PARN shows how the last three adenosine nucleotides of a poly(A) substrate are bound to the active site region. Unfortunately, the poly(A)/PARN structure does not resolve the R3H domain (Wu *et al.*, 2005). The cap-structure of PARN resolves the nuclease domain and the RRM but it does not reveal the R3H domain (Wu *et al.*, 2009). The cap-structure also shows how the cap moiety interacts with the RRM and the nuclease domain of PARN. The dimeric structure of the cap-structure complex shows that the RRM of each monomeric unit has different positions in relation to the nuclease domain. The two structures are referred to as open and closed conformations. In the open conformation the RRM is located further away from the active site whereas the RRM has moved close to the active site in the closed conformation (Wu *et al.*, 2009).

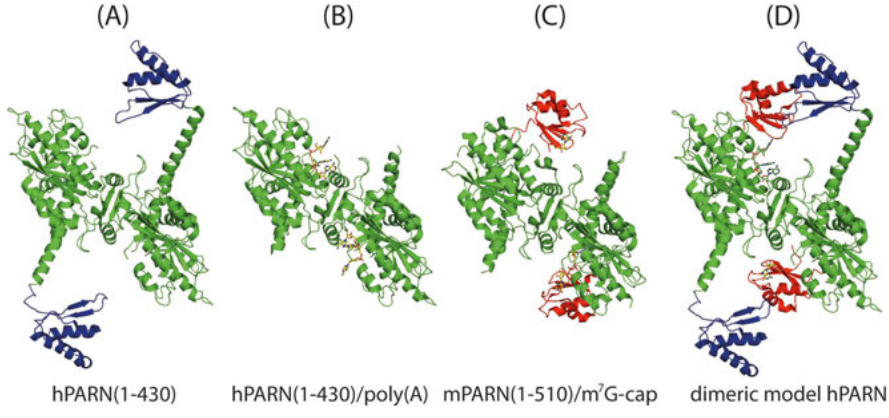


*Figure 2.* A) Apo-structure of PARN (PDB:2A1S) B). Structure of PARN co-crystalized with poly(A) (PDB:2A1R). C) Structure of PARN co-crystalized with the $m^7$GpppG cap moiety (PDB:3D45). D) Dimeric model of PARN from the different structures of PARN.

## Nuclease domain

The active site of PARN belongs to the DnaQ-like nuclease superfamily and consist of three aspartic acid and one glutamic acid that form an acidic core, referred to as the DEDD-core (Yang, 2011). The DEDD motif coordinates at least two divalent metal ions (Ren, Kirsebom and Virtanen, 2004). At least one of the divalent metal ions coordinates in turn to the phosphate pro-Rp oxygen of the scissile bond (Ren, Kirsebom and Virtanen, 2004). In the active site there is also a catalytically important histidine residue that most likely positions the hydroxide ion for the nucleophilic attack on the scissile phosphodiester bond (Wu *et al.*, 2005; Virtanen *et al.*, 2013). PARN is classified as a DEDDh nuclease.

## RNA recognition motif (RRM)

The RRM of PARN is highly conserved in different species encoding PARN. The RRM has a similar fold to the RRMs found in for instance, PABP and cap binding protein (CBP). The RRM of PARN binds efficiently to poly(A) tails of six adenine residues length or longer (Nilsson *et al.*, 2007). Likewise, full length PARN also binds poly(A) tails with similar efficiency and length dependence (Henriksson *et al.*, 2010). The RRM interacts slightly better to poly(A) than to random RNA (Nilsson *et al.*, 2007; Henriksson *et al.*, 2010). The interaction between the RRM and the poly(A) (or RNA) indicates that the RRM is involved in the degradation of the poly(A) substrate. When PARN is degrading a 20-adenines residue long substrate ($A_{20}$) an intermediate product of four adenines accumulates in the reactions (**Paper I** and (Henriksson *et al.*, 2010)). Interestingly, the minimum distance from the 3' end located adenine residue in the active site to the RRM corresponds to the length of a poly(A) tail consisting of four adenine residues. In **Paper III** we used an RRM truncated variant of PARN and this mutant did not reveal a prominent accumulation of the $A_4$ reaction intermediate. This observation is in keeping with the proposal that the poly(A) tail is associated with the RRM before it enters into the active site. Furthermore, our studies have shown that the RRM stimulates the rate of PARN mediated deadenylation.

The $m^7$GpppG cap structure interacts with the RRM at a site that is distinct from the poly(A) interaction surface of the RRM (Nilsson *et al.*, 2007). The crystal structure of PARN with the cap revealed the molecular basis for the cap-stimulated PARN-mediated poly(A) degradation. The deadenylation activity increases on 5'end $m^7$GpppG capped substrates compared to uncapped (Dehlin *et al.*, 2000; Gao *et al.*, 2000; Martínez *et al.*, 2000; Martînez *et al.*, 2001). The crystal structure showed that the methylated guanine residue of the cap structure stacks with the tryptophan residue 475 of the RRM of PARN while the first transcribed guanine of the dinucleotide cap moiety reaches and interacts with the active site region of PARN, which is located in the nuclease domain (Wu *et al.*, 2009). Interestingly, when the dinucleotide cap structure is titrated into the deadenylase reaction it stimulates the degradation reaction at low concentrations but inhibits the reaction at high concentrations of the cap (Martînez *et al.*, 2001). The inhibition of PARN activity by the $m^7$GpppG cap analogue supports the structural observations that the cap interacts with the active site. This defines the 5' end cap structure as an allosteric regulator of PARN activity. The allosteric regulation of the cap on PARN activity and its binding to PARN leads to an intriguing property of dimeric PARN. Dimeric PARN has the ability to interact with both ends of the mRNA, *i.e.* to simultaneously interact with both the 3'end located poly(A) tail and the 5'end located cap structure. In essence, one of the active sites binds the cap whereas the other active site binds the poly(A) tail and this is where hydrolysis also will take place.

A dimeric model of PARN (Figure 2D)(Wu *et al.*, 2009) binding both ends of the targeted mRNA has been the basis for our working model of PARN action (Figure 3, (Virtanen *et al.*, 2013)). In this model the poly(A) tail is threaded into the active site by the RRM in one of the monomers of the dimer and the active site of the other monomer interacts with the 5'end cap structure of the mRNA (Figure 3). As mentioned previously, the RRM in the cap structure has two different conformations in regards to the nuclease domain, an open and a closed conformation. The moving distance between the two conformations is approximately one adenine nucleotide in length. We predict that the substrate will be fed into the active sit by the RRM when the RRM rotates from the open conformation to the closed. At the same time in the other dimer the first transcribed base will be pulled out from the active site region. When the active site of PARN has been loaded with a new adenine nucleotide the next phosphodiester bond is ready to be cleaved. We postulate that hydrolysis occurs when the RRM opens up to the open conformation. In the opposite RRM, where the cap moiety is bound, the RRM is moving from the open conformation to closed conformation for each round of hydrolysis. The working model of dimeric PARN action indicates that the allosteric regulation by the cap structure of PARN activity could be mediated from the allosteric cap binding site to the hydrolytic active site on the other monomeric subunit through the nuclease domain dimerization faces.

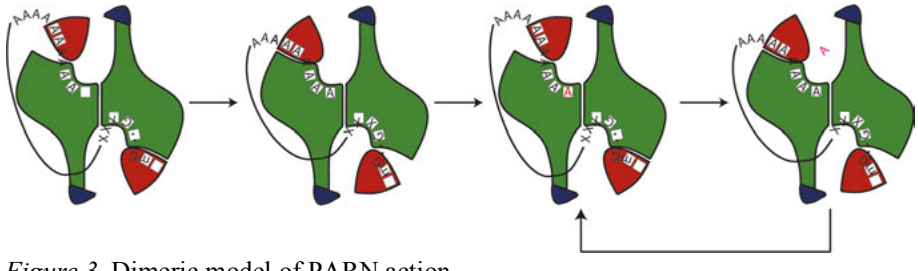

*Figure 3.* Dimeric model of PARN action.

The distance between the allosteric site and the catalytically active site is rather long for an allosteric effect. A revised tetrameric model could provide a reasonable explanation for the allosteric stimulation by the cap moiety (Figure 4). A tetrameric model for PARN is based on three observations: first the "molecular size argument" which is based on observations from both bovine and recombinant PARN, where deadenylase activity by PARN is found in fraction larger than a dimer when size fractionated (Martínez *et al.*, 2000; He and Yan, 2014). Secondly, "experimental x-linking data" crosslinking of PARN reviles a cross-linked complex being larger than a dimer of PARN when fractionated on the SDS-PAGE (Martínez *et al.*, 2000). The third published observation is the "RRM dimerization", which is based on the observation that a purified RRM of PARN, when fractionated by size exclusion chromatography, reveals

a dimeric and a monomeric peak of the RRM (Niedzwiecka *et al.*, 2016). Together these three published observations indicate that both PARN and the RRM by itself can form oligomers. Therefore, we propose that PARN could act as a dimer of dimers. The first dimerization surface is located in the nuclease domain and the second dimerization surface of PARN is between two RRMs. An interesting possibility when comparing a tetrameric and a dimeric model of PARN is that cap moiety could be located in the RRM of one PARN dimer whereas the hydrolytic site could be located in the other dimer of PARN. The RRM of PARN also resembles the RRM found in PABP, where two RRM interacts to forms a continues surface for poly(A) binding (Deo *et al.*, 1999). The dimerization of the RRM in a tetrameric model could therefore also play a role in increasing the poly(A)/RNA binding surface besides the poly(A) binding RRM in direct contact with the allosteric cap binding RRM.

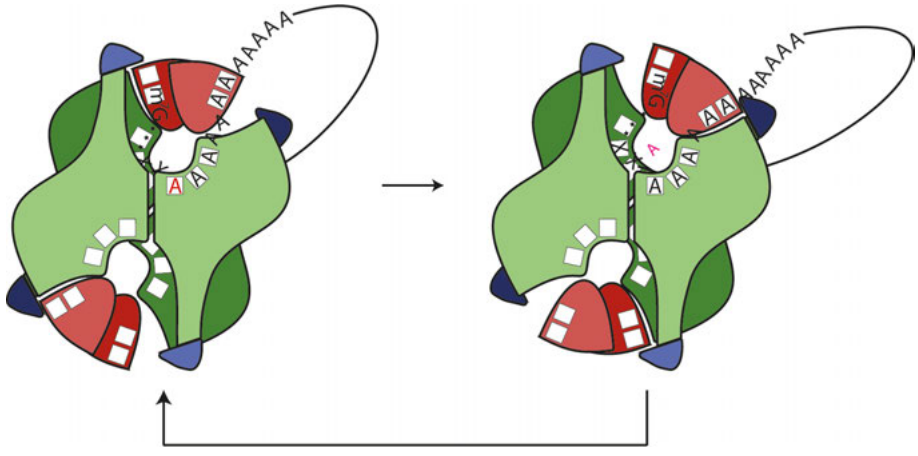

*Figure 4.* Proposed tetrameric model of PARN action.

# Specificity

PARN hydrolyses the scissile phosphodiester bond that joins the 3'end and penultimate located adenine nucleotides in the poly(A) tail. The substrate for the catalytic reaction of PARN is the scissile phosphodiester bond between these two nucleotides. To enable a site specific and adenine dependent hydrolytic reaction at this bond, both the adenine base and the ribose of the nucleotide need to be recognized by PARN. The molecular mechanism that distinguishes an adenine from the other bases has not been revealed for PARN. A problem with the adenine recognition in PARN is the lack of visible interactions between the polypeptide of PARN and the adenine bases in the structure of the poly(A)/PARN complex. Similarly, a low number of interactions between the adenine bases of a poly(A) tail has been observed in Pan2. In Pan2 it has been proposed that adenine specificity is associated with the helical A-form structure of the poly(A) tail (Tang *et al.*, 2019). In the deadenylase CNOT6L multiple interactions between the adenine base and the enzyme provide adenine specificity (Wang *et al.*, 2010). In the active site of PARN the ribose backbone of the poly(A) substrate is well-coordinated to the PARN polypeptide (se figure 5 **paper II**).

The difference between RNA and DNA is one hydroxyl group, located at the 2' carbon of the sugar ring of RNA. In DNA this hydroxyl is replaced by a hydrogen. The nucleotide sequence of RNA is determined by the order of the bases adenine (A), guanine (G), cytosine (C) and uracil (U). In DNA U is replaced by a thymine (T) base. A and G are purine bases that have a double ring structure. The bases U, T and C are pyrimidines consisting a single ring structure. A and G share the same chemical structure but adenine has one amine group bound to the carbon in the sixth position of the purine ring whereas guanine has a double bonded oxygen at this position. Furthermore, guanine has an additional amine group at the second carbon of the purine structure. The differences between the pyrimidine bases C and U are that C has an amine group linked to the fourth carbon of the pyrimidine whereas U has an oxygen double bonded to the same position.

In PARN there have to be two mechanisms of recognition that work in tandem. One needs to recognize the 2´hydroxyl to distinguish RNA from DNA. The other mechanism needs to discriminate an adenine base from the other three bases, G, U and C. In **paper I** we investigated the mechanism behind adenine specificity and in **paper II** we investigated the mechanism behind ribose dependency.

The approach in **papers I** and **II** was based on a kinetic model for the degradation of an $A_3$ trinucleotide substrate (**paper I)**. The kinetic model included kinetic steps involved in the complete degradation of an $A_3$ substrate. To develop the kinetic model we defined three binding pockets, called binding pockets "-1", "-2" and "-3". Each pocket binds one each of the three adenines in the trinucleotide $A_3$. The presence of three binding sites was based on the positions of the three 3'end terminal adenine bases, which were visible in the crystal substrate structure of the poly(A)/PARN complex (Wu *et al.*, 2005). The DEDDh hydrolytic core of PARN is located between binding pockets "-1" and "-2". This is where hydrolysis of the scissile phosphodiester bond occurs. The first step in the kinetic model represents the association of the $A_3$ substrate with the active site region of PARN when the 3'end located adenine residue enters binding pocket "-3". After the initial binding of the $A_3$ trinucleotide substrate to binding pocket "-3" the 3'end located adenine residue translocates into binding pocket "-2". The next adenine in the $A_3$ trinucleotide will at the same time enter into binding pocket "-3". This step is then followed by a second translocation event that fills all three binding pockets with adenine nucleotides, se figure 5A **paper I**. When binding pockets "-1" and "-2" are filled with adenine nucleotides the phosphodiester bond linking the two bases in pockets "-1" and "-2" will be placed in the DEDDh hydrolytic core of PARN and hydrolysis can occur. After hydrolysis the $A_2$ intermediate reaction product sits in binding pockets "-3" and "-2" and the released AMP sits in binding pocket "-1". Before the dinucleotide intermediate can translocate into the hydrolytic core the released AMP nucleotide has to diffuse away and leave binding pocket "-1" empty. To completely degrade the $A_3$ substrate the $A_2$ intermediate product needs to be translocated into the hydrolytic core. A second hydrolytic reaction takes place to generate two AMP mononucleotides. Taken together, the complete degradation of an $A_3$ substrate requires two hydrolytic cycles. The first hydrolytic cycle encompasses the association of the $A_3$ substrate with PARN, two subsequent translocation events and one hydrolytic reaction. The second hydrolytic cycle encompasses the translocation of the $A_2$ intermediate into the hydrolytic core and the subsequent hydrolysis. In the kinetic model the first hydrolytic cycle will be represented by the observed kinetic rate constant $k_{obs1}$, while the second hydrolytic cycle is described by the observed kinetic rate constant $k_{obs2}$.

In **paper I** we found that the replacement of the divalent metal ion $Mg^{2+}$ with $Mn^{2+}$ reduced PARN's specificity for adenine. In the presence of $Mg^{2+}$ PARN was most efficiently degrading a 20 adenine residues long ($A_{20}$) substrate followed by uracil ($U_{20}$) and cytosine ($C_{20}$) substrates. In the presence of $Mn^{2+}$ the uracil and cytosine homopolymeric substrates were as equally well degraded as the 20 nucleotides long adenine substrate. We also noted that an $A_4$ intermediate product appeared in the presence of $Mg^{2+}$. The appearance of this intermediate was significantly reduced in the presence of $Mn^{2+}$. Interestingly, the accumulation of an $A_4$ intermediate corresponds to the length

between the hydrolytic core of PARN and the RRM in its closed conformation. The loss of adenine specificity in the presence of $Mn^{2+}$ suggested to us that we could use $Mn^{2+}$ as a probe to investigate the mechanism of adenine specificity in the presence of $Mg^{2+}$.

Specificity for adenine was also observed when we used short homo-trinucleotides as the substrates in the presence of $Mg^{2+}$. $A_3$ trinucleotides were preferred over guanine, uracil and cytosine containing trinucleotide substrates. As observed with longer substrates this preference was reduced in the presence of $Mn^{2+}$. One key observation from our PARN titration experiments with the trinucleotide substrates was that neither of the pyrimidine containing trinucleotide substrate ($U_3$ or $C_3$) were completely degraded to their respective final mononucleotide end products in $Mg^{2+}$, while they were completely degraded in the presence of $Mn^{2+}$. Time course experiments with the $A_3$ substrate in enzyme excess showed that the second hydrolytic cycle was rate limiting in the presence of $Mg^{2+}$ while this cycle was not rate limiting in the presence of $Mn^{2+}$. These sets of observations suggested to us that we could use the disappearance of the dinucleotide intermediate, which is represented by the $k_{obs2}$ parameter, to investigate the mechanism of adenine specificity in PARN.

In **paper I** we concluded that the adenine specificity in PARN is achieved by a dynamic process when the adenine base in binding pocket "-3" translocates into binding pocket "-2". When a pyrimidine base is placed in binding pocket "-3" (*e.g.* when using UAA and CAA as the substrates) the rate of accumulation of the dinucleotide intermediate (UA or CA) was similar to the rate of accumulation of the dinucleotide intermediate from the $A_3$ substrate. However, the disappearance of the 5'end pyrimidine was very slow in the presence of $Mg^{2+}$ compared to the corresponding reaction when using the $A_3$ substrate. This was similar to what we found for the pyrimidine homo-trinucleotide substrates where the pyrimidine dinucleotide intermediate was not degraded by PARN in the presence of $Mg^{2+}$.

In **paper II** we found that in the presence of $Mg^{2+}$ the tri-deoxynucleotide substrate ($dA_3$) was resistant to PARN activity. However, under the same conditions in the presence of $Mn^{2+}$ the tri-deoxynucleotide substrate was degraded, albeit slowly. This indicates that the 2'OH is important for the completion of PARN's hydrolytic cycle and that $Mn^{2+}$ to some extent can bypass this requirement.

To further investigate the 2'OH dependency of PARN we introduced a deoxyribose in either the 3'end, penultimate or the 5'end position of a trinucleotide substrate. In the presence of $Mg^{2+}$ and during the first hydrolytic cycle the substrate with the deoxyribose at the 3' end of the substrate was the most affected, followed by the substrate with the deoxyribose in the penultimate position. The smallest effect was seen when the deoxy substitution was placed at the 5'end located nucleotide of the trinucleotide substrate. After the 3' end located deoxyribose adenine nucleotide had been released the hydrolysis of the $A_2$ intermediate was as efficient as for the $A_2$ intermediate of the $A_3$

substrate in the presence of $Mg^{2+}$. In our kinetic model of PARN degrading the trinucleotide substrate the 3'end located nucleotide has to translocate through all binding pockets in the active site region of PARN to position the phosphodiester bond in the hydrolytic site. Our measurements suggest that the 2'OH in the 3'end of the substrate is important for the loading of the phosphodiester bond in the hydrolytic site. If the deoxyribose is placed at the penultimate or the 5'end position of the substrate even the first hydrolytic cycle is affected by the absence of a 2'OH in both positions. In the structure of the poly(A)/PARN complex the 2'OH in the penultimate position is hydrogen bonded through a water molecule to D292 of the active site (se Figure 5A **paper II**). The penultimate 2'OH is most likely involved in the stabilization of the phosphodiester bond during hydrolysis. In the same structure of PARN the 5'end located ribose is unfortunately not resolved. It is intriguing that a 5'end located ribose affects the hydrolytic reaction of the 3'end located adenine. Similar observations, *i.e.* communication between the bases located in binding pocket "-3" and "-1", were also made in **paper I** where both a 5'end located guanine and cytosine affected the first hydrolytic cycle. This implies that short range communications occur in the active site of PARN. In **paper I** we theorized that the communication between the adenine bases in binding pocket "-1" and "-3" was mediated by a third and transient divalent metal ion, similar to the 3$^{rd}$ metal ion that has been visualized in DNA polymerase η (Nakamura *et al.*, 2012; Gao and Yang, 2016). Previous metal ion rescue experiments where the oxygen at the pro-Rp position of the phosphodiester bond was replaced by sulfur could be rescued by replacing $Mg^{2+}$ by $Mn^{2+}$ (Ren, Kirsebom and Virtanen, 2004).

Taken together we imagine that the molecular mechanism for adenine specificity is linked to the event when the poly(A) tail enters from the RRM and is translocated into the hydrolytic site of PARN and that occurs for each round of the hydrolytic cycle when an adenine with its scissile phosphodiester bond is pushed into the hydrolytic site by the rotation of the RRM. During the loading of the active site there is a reorientation of the adenine base when it moves form binding pocket "-3" to "-2". Our results indicate that the adenine base in binding pocket "-3" is recognized before the base moves into position "-2" and the phosphodiester bond enters the hydrolytic site of PARN. The feeding of the substrate into the active site by the RRM continues until the poly(A) tail is removed. At this point the body of the RNA substrate will enter into the active site. We postulate that PARN will stall its hydrolytic activity when the first nucleotide of the RNA body that enters tries to move from binding pocket "-3" to "-2". Due to the staling the RNA/PARN complex dissociates and the deadenylated RNA is released from PARN.

# Processivity

Nossal and Singer observed that RNase II did not release the homo polymeric adenine substrate until several rounds of hydrolysis had occurred. This ability of the enzyme to hold on to the polymeric substrate while performing several rounds of hydrolysis before releasing the fully degrade end product was termed *processivity* (Nossal and Singer, 1968). It was later revealed from the crystal structure of RNase II that three nucleic acid binding domains binds to the RNA upstream of the active site that channels the RNA into the active site (Zuo *et al.*, 2006).

Most if not all processive enzymes/enzyme complexes bind the polymeric substrate within a groove. The polymeric substrate that is within the groove of the enzyme contains more than one monomeric subunit of the polymeric substrate. For instance, PAP is a processive enzyme that synthesises the polymeric poly(A) tail on mRNA from the monomeric ATP substrates. PAP interacts with the four last incorporated adenosine residues of the nascent poly(A) tail (Balbo and Bohm, 2007). This implies that the grooving poly(A) tail has to translocate one step, corresponding in length to the distance between the phosphodiester bonds in a poly(A) polymer. Thus, the newly added adenosine residue moves out of the active site, which then will be ready for the next ATP hydrolysis reaction. Similar translocation mechanism as in PAP can be found in the more complex DNA replication (Kornberg, 1988) and transcription (Cheung and Cramer, 2012) machineries. These machineries incorporate repeatably one nucleotide residue for each round of catalysis. The identity of the base is determined by the DNA template. After each step of polymerization one step of translocation follows. During this step the template/product double helices moves away the distance of one nucleotide and the active site is ready for the next reaction. Both DNA replication and RNA transcription machineries utilize so called processivity factors (also called elongation factors in RNA transcription) that clamp the template and in many cases increases the rate of elongation of the polymeric product (Zhuang and Ai, 2010; Hartzog and Kaplan, 2011). PAP, DNA replication and RNA transcription are examples of processive reactions where the product is created by a processive mechanism. In all of these processes the substrates for the reaction are the incoming nucleotides and the 3'end of the polymer being extended.

RNase II, Rrp44, Xrn1 and PARN are examples of processive enzymes, where mechanisms of processivity have been investigated. In the cases of Rrp44, which is a 3' to 5' exoribonuclease of the exosome, and Xrn1, which is the 5' to 3' exonuclease that participate in the degradation of the mRNA body, the RNA substrate interacts in grooves present in the nucleases. Three or more nucleotides of the RNA interact with the groove of the enzyme before the RNA substrate enters into the active site (Jinek, Coyle and Doudna, 2011). The interaction with nucleotides in the substrate upstream of the scissile phosphodiester bond indicates that the substrate needs to interact with the enzyme before it is translocated into the active site. In the case of PAP, DNA replication and RNA transcription, the polymeric product is translocated out from the active site after the incorporation of the added nucleotide. In contrast, in processive exoribonucleases, the polymeric substrate is translocated into the active site before each round of hydrolysis.

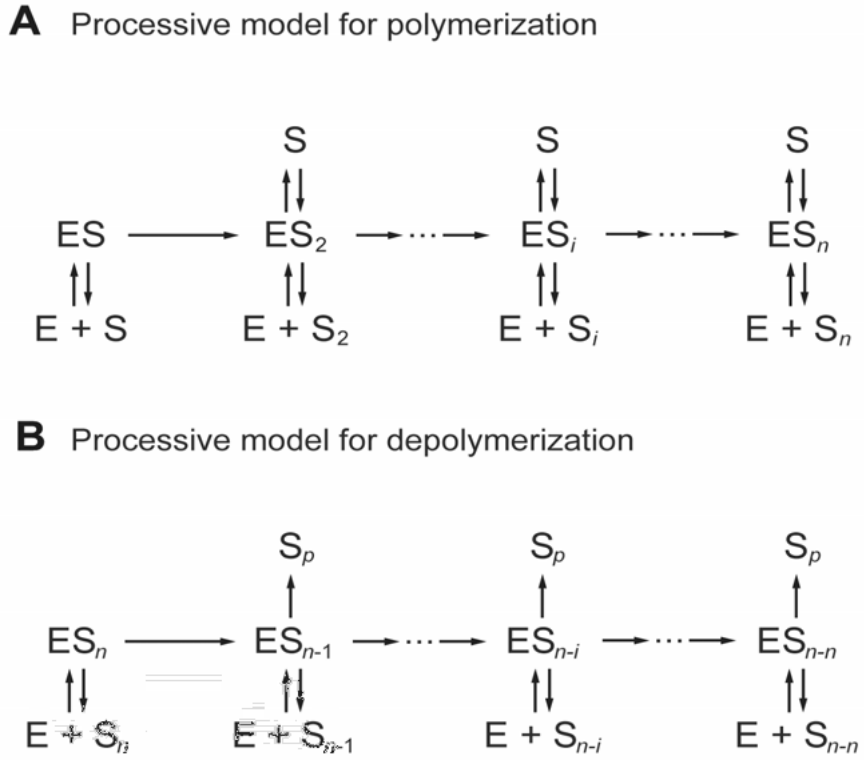


*Figure 5.* Reaction schemes for A) polymerising and B) depolymerising processive reactions

A distributive enzyme binds the substrate once and the catalyses the formation of the product from the substrate. The catalytic reaction is followed by the dissociation of the enzyme/product complex. Processive enzymes can be dived in two classes, polymerizing and depolymerizing (Figure 5). In the

polymerization pathway the first step encompasses the association of the enzyme and first monomeric substrate. In the second step the hydrolytic reaction occurs, *i.e.* the first nucleotide becomes incorporated. Instead of releasing the polymeric product the product is translocated out from the active site creating an open active site for the next incoming nucleotide substrate to be incorporated into the growing polymer (Figure 5A). For a depolymerizing processive enzyme the polymeric substrate stays associated with the enzyme. For each round of hydrolysis one monomer of the polymeric substrate is released from the enzyme and the hydrolysed substrate translocates into the active site before the next round of hydrolysis (Figure 5B). The key event in all processive enzymes is the translocation of the product/substrate in/out of the active site. During the translocation event the enzyme partially dissociates the substrate/product and re-associates with it again at the distance of one monomeric subunit. In processive enzymes the forward reaction (*i.e.* translocation and the catalytic reaction) is in competition with the dissociation of the entire enzyme substrate/product complex. This competition event is reflected by the processivity parameter.

**Table 1**. Calculated processivity parameter for different processive enzymes.

| **Enzyme** | **Extent of processivity** | **Processivity parameter** |
|---|---|---|
| Yeast RNAP II | 1'000'000 | 0.999997 |
| Taq RNAP | 10'000 | 0.9997 |
| RNA-dependent RNAP | 2'500 | 0.999 |
| Reverse transcriptase | 50 | 0.94 |
| Heicase PcrA | 20 | 0.86 |
| TRAMP | 2 | 0.22 |

Table 1 is adopted from Breyer and Matthews 2001 (Breyer and Matthews, 2001) and extent of processivity for TRAMP complex was adopted from Jia *et al.* 2011 (Jia *et al.*, 2011). The processivity parameter was calculated with the formula $p^n$=0.05, assuming that the minimum of final end product is 5% of the total substrate. n represents the "extent of processivity".

A processivity parameter describes the probability for the forward reaction to occur in competition with the dissociation event of the enzyme/product complex. In Table 1 an estimated processivity parameter has been calculated from tabulated extent of determined processivity of different enzymes. For example, Taq RNAP acts processively and can incorporate 10'000 of nucleotides before the enzyme complex is dissociated. This gives a processivity parameter of 0.9997. This translates to that for every incorporation of a nucleotide by Taq RNAP the probability of dissociating the enzyme/product complex is 0.03%

The processivity property of PARN was experimentally verified by applying a kinetic model of processivity (Martînez *et al.*, 2001). In order to experimentally show that a reaction pathway is processive the reaction has

to be performed with substrate concentration that are much high than the $K_M$ concentration. This makes sure that the enzyme always works at its maximum rate of capacity, $V_{max}$. The second requirement of the processivity experimental assay is to compare a time course experiments with a high ($3x K_M$) and higher ($15x K_M$) initial substrate concentration. If the reaction is distributive the appearance of the first fully degraded polymer will depend on the initial substrate concentration. Increasing the initial substrate concentration will prolong the first appearance of the fully deadenylated end product. In a processive reaction the appearance of the fully deadenylated end product will not depend on the initial substrate concentration, due to the fact that a single association event between the substrate and enzyme is followed by multiple rounds of hydrolysis.

In **paper I** we used this approach to determine if PARN degrades trinucleotide substrates processively. We found that the accumulation of the 5'end located fully deadenylated end product ($A_1$) appeared at the same time independent on the initial substrate concentrations ($3x K_M$ and $15x K_M$). The appearance of the end product shows that $A_2$ can be degraded in a processive mode of action, thus the $A_2$ intermediate product can be translocated into the active site without dissociating from the active site after it has been generated by the hydrolysis of the $A_3$ trinucleotide substrate.

The amount of $A_2$ that is degraded in a processive manner directly after A3 has been hydrolysed is low. In **paper III** we have refined a kinetic processive model to be able to calcuate a processivity parameter from a similar scheme as in figure 5B. In **paper III** we used adenosine substrates of different lengths to investigate if the processivity parameter changed due to the length of the substrate. We used substrates with the lengths of $A_{10}$, $A_5$, $A_4$ and $A_3$. The substrates were provided at an initial concentrations of $3x K_M$, $10x K_M$, $20x K_M$ and $30x K_M$ (as determined for the $A_3$ trinucleotide substrate) and we followed the reactions during the time course. We used full length PARN and a RRM and C-terminal truncated PARN (PARN(1-436)) as the enzymatic source. The turnover rates were calculated based on model 1 from the experiment (Figure 4, **paper III**). We found that the short $A_4$ and $A_3$ substrates were degraded with similar rates when using either full length PARN or RRM truncated PARN. In contrast, for the longer $A_{10}$ substrate the degradation rate was reduced for the RRM truncated version of PARN compared to full length PARN. This indicates that for long substrates the RRM is involved in the degradation of the substrate, possibly acting as a processivity parameter. Interestingly, the A4 intermediate accumulated when $A_{10}$ was degraded by the full length enzyme while is appearance was reduced when the RRM truncated version of PARN was used. Furthermore, when the $A_4$ intermediate was degraded the turnover rate of the $A_3$ product is increased. Actually, the $A_3$ intermediate product was consumed faster than both $A_4$ and $A_2$. The initial approximations indicate that the reaction of degrading $A_8$ degraded from the substrate A10 is 40-60% processive. The equivalent reaction for the RRM truncated PARN is 30-40% processive. This shows that the RRM is a structure that influences the

processivity parameter of PARN. The results of **Paper III** are in agreement with the finding in **paper I** that the active site harbours an intrinsic processivity property and that the substrate is actively translocated into the active site even if the substrate will not be able to bind any parts of PARN that are located outside the active site region. The reason being that PARN in the absence of the RRM clearly degrades longer substrates processively.

# PARN mutations associated with telomere biology disorders

The biochemical work to understand enzyme action is straight forward after the enzyme has been purified, *i.e.* to investigate the biochemical properties of the purified enzyme with different substrates and to perturb the enzyme's activity by mutagenesis. The biochemical work gives a picture of how the enzyme works mechanistically and also predicts the function it might have in cells. The initial studies made with PARN always assumed that PARN was involved in mRNA metabolism due to its cap binding property and deadenylation activity. In the last years, more information has emerged that tells us a different story of the function of PARN. A story that could not have been theorized from the biochemical properties of PARN. **Paper IV** and **Paper V** describe human patients with mono- and bi-allelic mutations in the gene for PARN. We (**Paper IV**) and others (Moon *et al.*, 2015; Stuart *et al.*, 2015; Tummala *et al.*, 2015) showed that a deficiency in PARN could lead to developmental disorders/delay as well as telomere biology disorders (TBDs). The final part of this thesis will discuss TBDs and how low activity mutants of PARN relates to telomer biology.

## Telomeres

Telomeres are located at the ends of linear chromosomes and protect the chromosomes from degradation. Unlike the prokaryotic chromosome that is circular the eukaryotic chromosomes are linear with true ends. However, a free double stranded DNA end is a signal for the DNA damage response (DDR) mechanisms that signals that a double stranded break of the DNA molecule has occurred. The telomere region of a human linear chromosome needs to avoid activation of DDR signal. For example, the telomeres need to be protected from devastating recombination events of the chromosome by avoiding ligation to other linear chromosomes. The protection from nuclease activity on the mRNA poly(A) tail is gained from the interaction with PABP. A similar protection strategy is found for the telomeres where the shelterin complex binds to the telomere sequence (Lange, 2010). The telomeres also protect the chromosomes from exo- and nonnucleolytic degradation of linear DNA (Armanios and Blackburn, 2012; Blackburn, Epel and Lin, 2015).

In humans the telomeres are built up by a tandem repeat of six-nucleotides with the sequence $(TTAGGG)_n$ (Moyzis *et al.*, 1988). The tandem hexa-nucleotide repeat was first described in *Tetrahymena* (Blackburn and Gall, 1978). The presence of a repeat at the end of the chromosomes is a general property of linear eukaryotic chromosomes. At birth the telomere is about 10 kb long in humans. The telomere length shortens for every somatic cell replication event and when the telomeres become too short (≤3 kb) the cell enters into senescence (Hayflick and Moorhead, 1961). The cellular machinery that adds the hexa-nucleotide sequence of the telomere at the end of the chromosome is called telomerase and was first described in *Tetrahymena* (Greider and Blackburn, 1985) and later in human HeLa cells (Morin, 1989). The highest telomerase activity can be found in germ cells (ovaries and testis) and stem cells. The telomerase is a ribonucleoprotein complex that contains the telomeric reverse transcriptase (TERT) that transcribes the six-nucleotide repeat sequence using the telomerase RNA component (TERC) as the template (Greider and Blackburn, 1985; Morin, 1989). The telomerase RNA component is a 451 nt long RNA in humans (Feng J *et al.*, 1995). The template for the hexa-nucleotide repeat is located within the so called pseudoknot domain of TERC (Chen, Blasco and Greider, 2000). In the 3'end of TERC a so called snoRNA H/ACA box RNA structure is located. The H/ACA box RNA has a stem loop structure and interacts with the highly conserved protein dyskerin (Kiss, Fayet-Lebaron and Jády, 2010). Mutation in the gene the encodes dyskerin affects the steady state levels of TERC, the H/ACA box small nucleolar RNAs (snoRNA) and the small cajal body RNAs (scaRNA) (Angrisani *et al.*, 2014). Mutations in the gene for dyskerin were found in patients with a rare human disorder called x-linked dyskeratosis congenita (DC) (Heiss *et al.*, 1998). Patients with DC have mutations in genes encoding dyskerin, TERT or TERC. Mutations in these genes impair the function of the telomerase and result in short telomeres at the ends of the chromosomes (Mitchell, Wood and Collins, 1999; Vulliamy *et al.*, 2006; Alter *et al.*, 2007). DC manifests in childhood and patients show frequently three characteristics phenotypes, *i.e.* skin hyperpigmentation, oral leukoplakia and nail dystrophy. Patients with DC die early and usually from the development of lethal disorders like bone marrow failure, pulmonary fibrosis and/or cancer (Vulliamy *et al.*, 2006; Alter *et al.*, 2007). Shortening of the telomere is a natural process that occurs when somatic and stem cells divide. The shorting is due to an incomplete replication of the lagging strand of the replicated DNA duplex at the end of the chromosome (de Lange *et al.*, 1990; Harley, Futcher and Greider, 1990; Hastie *et al.*, 1990; Lindsey *et al.*, 1991). Somatic cells show very low or no telomerase activity. In contrast, stem cells express telomerase activity constituently and the telomeres are actively and constituently elongated in such cells (Blackburn, Epel and Lin, 2015).

## Telomere biology disorders

The onset of TBDs depends on the length of the telomere. The severity of the disorder is also related to the length of the telomere (Armanios and Blackburn, 2012). The most severe disorder and also the earliest onset TBD is Hoyeraal-Hreidersson syndrome (HHS), which is a particularly severe form of dyskeratosis congenita (DC). The least severe TBD, which also is the latest in life to be manifested is idiopathic pulmonary fibrosis (IPF) (Bertuch, 2016). Patients with IPF suffers from lung tissue that deteriorates irreversible. The manifestation of IPF occurs at ages above 50 years old. The cause of IPF is likely related to the lack of alveolar epithelial stem cells. The molecular basis of DC was revealed when a mutation in the gene encoding dyskerin was linked to the disorder. The onset of HHS occurs during infancy and patients have global developmental delay, immunodeficiency, cerebellar hypoplasia and bone marrow failure. Patients with HHS have very short telomeres and the symptoms are most likely due to a global lack of stem cells. The onset and severity of TBDs correlate to the length of the telomeres at birth (Armanios and Blackburn, 2012).

## PARN deficiency in patients

In **paper IV** and **paper V** we studied patients with bi-allelic mutations in the PARN gene. The two most affected patients were called "patient 1" in **paper IV** and "proband" in **paper V**. The same terminology will be used throughout this thesis when discussing the two patients. In both patient 1 and proband one of the alleles encoding *PARN* was deleted while the other allele in each patient contained a point mutation that affected the amino acid sequence of the point mutated allele. Both mutated *PARN* alleles in the patients were inherited from their parents. All four parents have one fully functional *PARN* gene and one affected allele. Their telomeres are slightly shorter that the average for their age-group. The deleted PARN alleles in both patients generate truncated and inactive polypeptides of PARN. Both patients suffer from global developmental delay, immunodeficiency, cerebellar hypoplasia and bone marrow failure. They were both diagnosed with the severe form of DC referred to as HHS.

Ribosome profiling of cells from patient 1 showed a reduction in the abundance of the mature small ribosome subunit 30S that effected the assembly of the 80S ribosome. We hypothesized that the ribosome deficiency might be caused by an increase in the oligadenylated state of sno- and scaRNAs. Berndt and colleagues had recently shown that the oligoadenylated state of snoRNAs and scaRNAs of the H/ACA class were affected in cells where PARN activity had been transiently knocked down (Berndt *et al.*, 2012). In both patients we found that the oligoadenylated state of sno- and scaRNA population of the H/ACA was increased. SnoRNA are part of the maturation machinery of the

ribosomal RNAs where they participate in the pseudouridylation process. Pseudouridylation improves base paring to adenosine (Ganot, Bortolin and Kiss, 1997; Kiss, Fayet-Lebaron and Jády, 2010). Besides affecting snoRNAs that are involved in pseudouridylation process PARN *per se* is also involved in the actual maturation process of the 18S rRNA (Tafforeau *et al.*, 2013; Ishikawa *et al.*, 2017; Montellese *et al.*, 2017). We found that the Proband had an increased amount of untrimmed 18S rRNA. This correlated with a reduced the level of the mature small subunit of the ribosome. We concluded that the ribosome deficiency in both patients very likely was linked to disrupted maturation of the 18S rRNA of the small ribosomal subunit and to perturbed maturation of sno- and scaRNAs.

Both patients have telomeres much shorter than the 1$^{st}$ percentile. The short telomere implies a problem with the telomerase activity. When the oligoadenylated state of TERC was analyzed we found that the level oligoadenylated TERC was increased and that the total amount of TERC was reduced. Our observation of the increased level of oligoadenylated TERC has also been documented in other DC and HHS patients with short telomeres (Moon *et al.*, 2015). It has been proposed that PARN during maturation of the snoRNA of the H/ACA class removes an oligo(A) tail stub that has been added transiently by the noncanonical poly(A) polymerase PAPD5 (Berndt *et al.*, 2012). PARN deficiency would then affect the final deadenylation of the PAPD5 of adenylated immature snoRNAs. It is conceivable that this deficiency in the maturation process will reduce or inactivate the function of the matured snoRNA of the H/ACA type.

## Biochemical characterization of the PARN mutation

To investigate if the point mutated alleles in patient 1 and proband encoded deficient versions of the PARN polypeptide we generated recombinant versions of each point mutated PARN polypeptide. The point mutated PARN polypeptides were expressed, purified and tested for their deadenylation activity *in vitro* (**paper IV** and **paper V)**.

The point mutation found in patient 1 (**paper IV**) causes an amino acid change from arginine to a tryptophan at position 349 (R349W) of the full-length polypeptide. In our *in vitro* assays the R349W mutated PARN polypeptide revealed a 50-fold drop in activity. We also introduced an alanine substitution mutation at the same position (R349A). Interestingly, the R349A PARN polypeptide was not affected in the *in vitro* deadenylation activity assay if compared to the activity of the wildtype PARN polypeptide. This indicates that the presence of a tryptophan residue at this particular position was devastating for PARN activity. At present we don't understand why a tryptophan would be particularly devastating. The amino acid R349 is located in the

nuclease domain of PARN and near the interface where the RRM will be placed in the RRM closed conformation of PARN (Figure 6). Based on this we speculate that a tryptophan at this position could interfere with the motion of the RRM relative the nuclease domain during each round of hydrolysis (Figure 7).

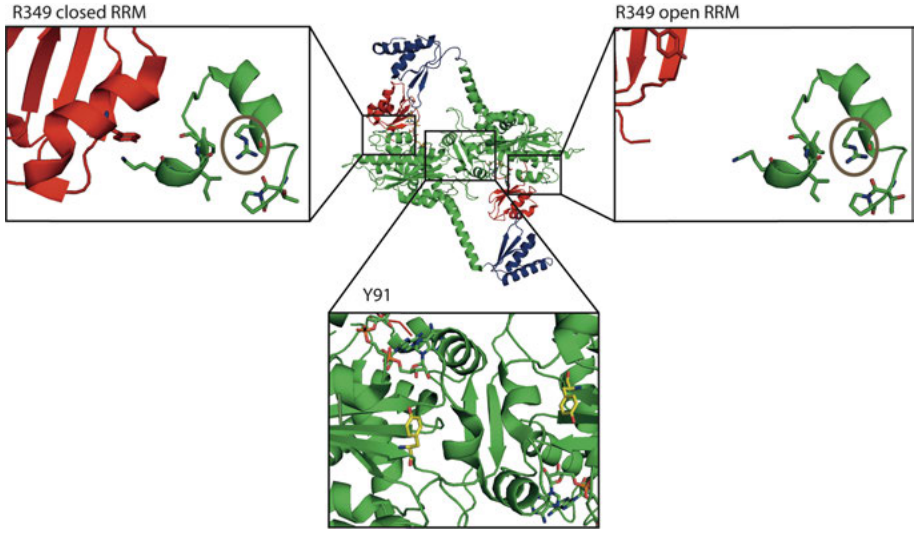


*Figure 6.* Locations of the mutation in PARN, patient 1 (two R349 panels) and proband (Y91 panel).

The point mutation in proband (**paper V**) is a cysteine substitution of a tyrosine at position 91 in the polypeptide of PARN. We generated a recombinant version of the Y91C PARN polypeptide and investigated its *in vitro* deadenylation activity. Also, in this case we found that PARN's deadenylating activity was affected in the mutant polypeptide. We investigated the mutant's activity on both long substrates ($A_{20}$) and short trinucleotide substrates ($A_3$). In both cases PARN's deadenylating activity was reduced. To quantify the defect, we determined the rate constants $k_{obs1}$ and $k_{obs2}$ for the degradation of the trinucleotide $A_3$. Both rate constants were reduced 30-fold compared to wildtype. The Y91C mutation is located close to the nuclease dimer interface of PARN (figure 6). We postulated that the mutation could interfere with the dimerization of PARN through its nuclease domains, which in turn could affect the active site of PARN (Figure 7).

It was surprising that mutations in the PARN gene could cause TBD. To ensure that none of the known genes associated with TBDs was the cause for the short telomeres in the patients, we investigated if any of those previously identified genes were mutated in patient 1 (**paper IV**). No other mutations were found in patient 1in any of the known genes linked to TBDs. In summary, both patient 1 and proband had telomeres that were much shorter than the 1$^{st}$

percentile. Both suffered from HHS, a particularly severe form of TBD. Both patient 1 and proband were bi-allelic in the PARN gene and in both cases one allele was deleted while the other allele carried a for PARN activity devastating amino acid substitution. Taken together, our biochemical characterization of the mutated PARN polypeptides strongly supported the conclusion that mutations in PARN could lead to the manifestation of TBD.

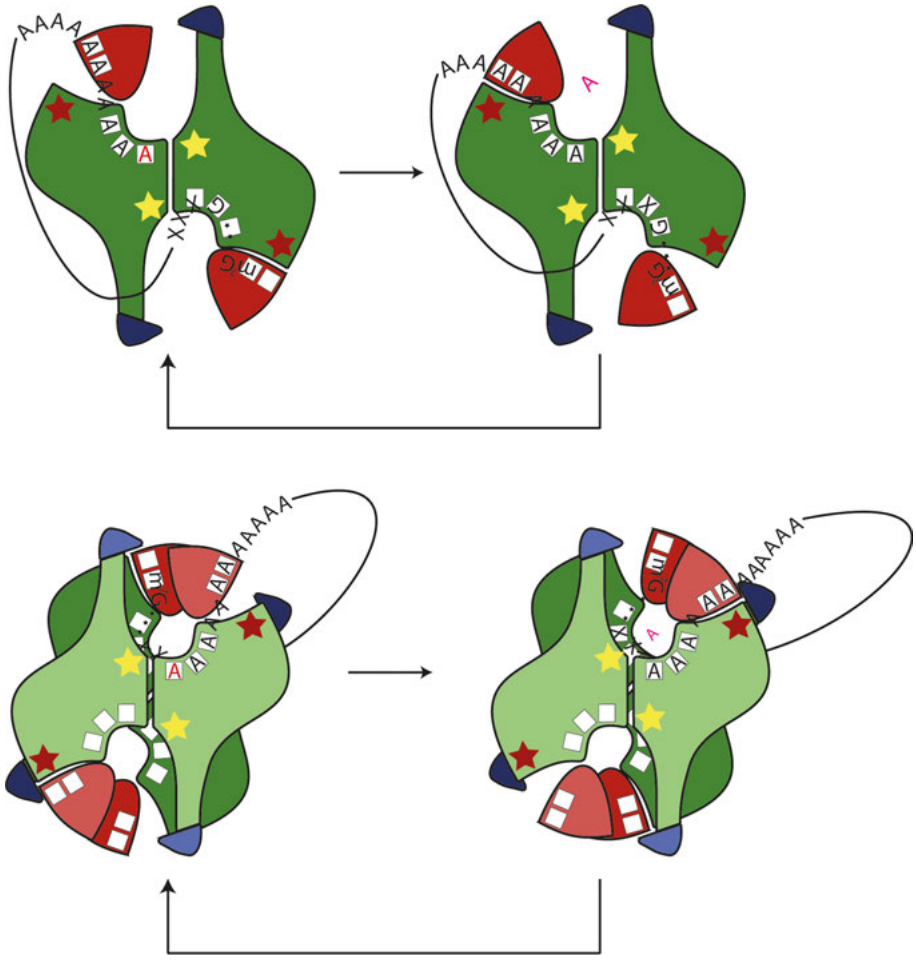

*Figure 7.* Dimeric (top) and tetrameric (bottom) models of PARN action depicting the location of the mutation from patient 1 (red stars) and proband (yellow stars) in PARN.

# Concluding remarks

PARN has been dubbed as "*the best studied deadenylase*" by Elliott and Ladomery (Elliot and Ladomery, 2016) in their 2nd edition of "Molecular Biology of RNA". This statement was done even without mentioning the association of PARN and telomere biology. The work presented in this thesis has certainly added more knowledge to "the best studied deadenylase", both functionally and mechanistically.

During my time as a PhD student the view of PARN has changed from a deadenylase that might degrade the poly(A) tail of an mRNA to a deadenylase that is part of the maturation of H/ACA RNAs and many other RNAs (se introduction in **paper I**). After the initial papers on PARN in relation to the telomeres were published in 2015 an explosion of new intriguing and interesting information has emerged about PARN. One conclusion of all the new information is that PARN is an enzyme "that regulates the regulators". PARN can now be defined as a deadenylase that deadenylates short poly(A) tails of RNAs playing crucial roles in several other essential processes.

The interaction between the cap moiety and PARN is a unique function for PARN. If the cap is important for substrate selection in the cell remains to be investigated. An intriguing part of TERC biology is that it has a trimethylated cap at the 5'end. The simulation of the cap moiety on the deadenylation activity in PARN associated with structural arrangements of PARN. Intriguingly, cap stimulation is more plausible in a tetrameric structural arrangement of PARN than in its dimeric form. Both the dimer and the possible tetrameric structural arrangements of PARN indicate two functions of PARN, one with and one without cap stimulation. In the cell both might be used the proposed tetramer could well be the PARN used for capped RNAs whereas the dimeric form of PARN could be predominant for regulating uncapped RNAs.

The enzymatic functions of PARN is well established and we now think that specificity for adenine arises from the dynamic processive associated with the loading of the substrate into the active site. We believe that the rotation of the adenine base when it translocates from binding pocket "-3" to "-2" of the active site is when adenine specificity is established.

# Sammanfattning på Svenska

Deadenylering är den enzymatiska processen som degraderar en poly(A) svans från en budbärare RNA molekyl (mRNA). mRNA är en RNA molekyl som syntetiseras/transkriberas i kärnan av våra celler med utgångspunkt från sekvensen i arvsmassans DNA. mRNA molekylerna innehåller ritningar för hur proteiner ska sättas samman i ribosomen, d.v.s. cellens proteinsyntesmaskineri. Tidigt under transkriberingsprocessen modifieras 5' änden av mRNA molekylen med en s.k. kapp-struktur, var på transkriptionen av mRNA fortsätter. När mRNA molekylen är färdigtranskriberad från DNA strängen så klyvs den av från transkriptionsmaskineriet. På det avkluvna 3' änden av mRNA molekylen syntetiseras en poly(A) svans från ATP molekyler med hjälp av enzymet poly(A) polymeras. I våra celler sker en konstant nysyntes av alla maskinerier t.ex av ribosomer och andra biomolekyler för att garantera funktionen hos dessa. När ett mRNA molekyler blir för gamla eller inte ska användas måste de degraderas. Processen för att degradera en mRNA molekyl sker i flera steg. Först tas poly(A) svansen bort därefter degraderas resterande delar av mRNAt molekylen.

Poly(A) specifikt exoribonukleas (PARN) är ett deadenylas som degraderar RNA molekylens poly(A) svans. En adeninenhet tas bort åt gången och PARN fortsätter till dess alla adeninenheter tagits bort. Exonukleas har sedan datorspelet Pacman uppfanns alltid blivit representerat av den gula bollen som äter mindre vita bollar. I Pacman analogin är Pacman exonukleaset som äter upp en nuklinsyramolekyl. PARN är därmed ett Pacman exonukleas som äter endast adeniner i poly(A) svansen. Andra nukleinsyrabaser som guanin, cytosin och urasil äts inte av PARN. En del av avhandlingen handlar om hur PARN kan känna igen adenin och hur andra baser inte degraderas av PARN. Adenin känns igen sker när PARN translokerar, d.v.s. matar in poly(A) svansen in i aktiva sätet. När adeninerna i poly(A) svansen ska in i de aktiva sätet passerar de en tröskel som de måste rotera över för att komma in. Om det är en annan bas så har den svårt att komma över denna tröskel och kommer därmed inte in i det aktiva sätet. För att visa detta använde vi oss av två olika divalenta metaljoner, $Mg^{2+}$ och $Mn^{2+}$. När $Mn^{2+}$ användes som metalljon i PARN reaktionerna så försvann tröskeln och baserna kunde passera in det aktiva sätet. Vi säger att PARN blev minder basspecifik. I Pacman analogin så kan PARN skilja på smaken på de olika baserna när $Mg^{2+}$ används som metalljon. Men i närvaro metaljonen Mn2+ har PARN svårt att skilja på bassmakerna, alla baserna smakar lika bra. Man kan säga att PARN Pacmannen

smakar på basen innan den äter basen och i närvaro av $Mg^{2+}$ spottas andra baser är adenin ut.

En fråga som är viktig att förstå är om PARN tar av en adenin från poly(A) svansen och därefter släpper poly(A) svansen innan nästa adenin tas av. Man säger att PARN då arbetar distributivt. En annan variant är att PARN äter upp hela poly(A) svansen utan att släppa taget. Man säger då att PARN arbetar processivt. Vi fann att PARN med största sannolikhet degraderar stora delar poly(A) svans utan att släppa RNA molekylen.

Ett viktigt genombrott i PARN-forskningen var när vi fann att PARN är inblandat i mognadsprocessen för en RNA molekyl som påverkar våra egna kromosomers ändar. Ändarna kallas telomerer. Telomerer finns i varje ände av våra kromosomer och skyddar kromosomerna från nedbrytning. Telomererna skydda även kromosomerna från rekombineringar/omstruktureringar som kan ske när kromosomer går sönder. När vi födds är telomeränderna på våra kromosomer som längst och för varje celldelning och när vi blir äldre blir telomerena kortare och kortare. Förkortningen av telomerena är en naturlig process som orsakas av att DNA replikationprocessen inte kan kopiera hela kromosomens ändar. Telomeren är en cellulär klocka som när den blir för kort hindrar cellen från att fortsätta att dela sig. I celler som stamceller och könsceller så finns de ett enzymatiskt komplex som förlänger telomererna på kromosomerna. Enzymet kallas för telomeras. Telomeraset består av ett protein och RNA molekyl. I RNA molekylen finns informationen för sekvensen som telomeren är uppbyggd av och ett omvänt transcriptas (TRET) som förlänger DNA strängen från RNA mallen. RNA mallen kallas för telomeras RNA komponent (TERC).

Upptäckterna om PARN cellulära funktion kom från att studera patienter med mutationer i båda generna för PARN. Patienterna hade ärvt de defekta PARN generna från sina föräldrar som båda två hade förutom den defekta även en fullt funktionell PARN gen. En av de muterade PARN generna hos patienterna saknar en stor del och genererar ett inaktivt och förkortat PARN protein. I den andra genen hos patienterna är en aminosyra muterat. En viktig fråga var därför om det punktmuterade PARN enzymet fortfarande var aktivt. När deadenylering testades på de muterade varianterna av PARN fann vi att aktiveten var reducerad och att patienterna därmed hade en mycket låg PARN funktion kvar i cellerna. När vi tittade på hur olika RNA molekyler hade reagerat på den låga PARN funktionen fann vi att telomer RNA (TERC) hade en poly(A) svans kvar i 3'ändan av RNA molekylen. För att TERC ska vara funktionellt måste poly(A) svansen tas bort för att telomeraset i sin tur ska vara funktionellt. När telomeraset inte är funktionellt förlängs inte telomererna effektive och patienterna får mycket korta telomerer.

Förkorta telomerer leder till att stamceller kommer att sluta dela sig. Olika sjukdomar kan uppkomma p.g.a. att man har för korta telomerer. Den allvarligaste är Hoyeraal-Hreidersson syndromet. Patienter med Hoyeraal-Hreidersson lider av allvarlig utvecklingsstörningar som, cerebellar hypoplasi,

benmärgsförlust och nedsatt immunförsvar. Patienterna med låg PARN aktivitet som vi studerade led av Hoyeraal-Hreidersson. De blev diagnostiserade vid ca 6 månades ålder. Andra sjukdomar som orsakas av felaktigt PARN är dyskeratos congenita. Patienter med dyskeratos congenita utvecklar slemhinneförändringar i munnen, onormal hudpigmentering och nedsatt benmärgs funktion vid tidig ålder. Den telomer sjukdom som uppkommer senast är lungfibros, som dyker upp vid åldern 40-50 år. Allvarligheten i telomersjukdomarna beror på telomerlängden. Ju kortare telomer ju tidigare uppkommer sjukdomssymptomen och ju allvarligare symptom får patienterna.

# Acknowledgements

In the end I would like to mention and thank:

My supervisor Anders for letting me into and play around in the lab and for many valuable discussion (some scientific and other not so scientific) and guidance.

My co-supervisors Sam and Måns.

Niklas, Harri, Magnus, Lei and Mattias for the warm welcoming to the poly(A) lab.

Sethu and the project students Gergana, Jens, Sangeetha, Andrew and Filip for your aid in all the PARN projects.

Ram and Nina.

Our collaborators: In Toronto Yigal, Santhosh and Hongbing. In Texas Alison, Lois and Penelope, and to those involved with the PARN patient projects that I have not have the pleasure to meet.

Göransson-Sandviken Stipendiefond.

Everyone at department that have aided me through my PhD.

And finally, friends and especially my family.

Tack så mycket för all hjälp!

# References


Abraham, A. K. and Jacob, S. T. (1978) 'Hydrolysis of poly (A) to adenine nucleotides by purified poly (A) polymerase.', *Proceedings of the National Academy of Sciences of the United States of America*, 75(5), pp. 2085–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27783%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC392495.

Alter, B. P. *et al.* (2007) 'Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita', *Blood*, 110(5), pp. 1439–1447. doi: 10.1182/blood-2007-02-075598.

Angrisani, A. *et al.* (2014) 'Human dyskerin: Beyond telomeres', *Biological Chemistry*, 395(6), pp. 593–610. doi: 10.1515/hsz-2013-0287.

Armanios, M. and Blackburn, E. H. (2012) 'The telomere syndromes.', *Nature reviews. Genetics*, 13(10), pp. 693–704. doi: 10.1038/nrg3246.

Astrom, J., Astrom, A. and Virtanen, A. (1992) 'Properties of a HeLa cell 3' exonuclease specific for degrading poly(A) tails of mammalian mRNA.', *The Journal of biological chemistry*. United States, 267(25), pp. 18154–18159.

Aström, J., Aström, A. and Virtanen, A. (1991) 'In vitro deadenylation of mammalian mRNA by a HeLa cell 3′ exonuclease.', *The EMBO Journal*, 10(10), pp. 3067–3071. doi: 10.1002/j.1460-2075.1991.tb07858.x.

Balbo, P. B. and Bohm, A. (2007) 'Mechanism of Poly(A) Polymerase: Structure of the Enzyme-MgATP-RNA Ternary Complex and Kinetic Analysis', *Structure*, 15(9), pp. 1117–1131. doi: 10.1016/j.str.2007.07.010.

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*, 74(8), pp. 3171–3175. doi: 10.1073/pnas.74.8.3171.

Berndt, H. *et al.* (2012) 'Maturation of mammalian H/ACA box snoRNAs: PAPD5-dependent adenylation and PARN-dependent trimming', *Rna*, 18(5), pp. 958–972. doi: 10.1261/rna.032292.112.

Bertuch, A. A. (2016) 'The molecular genetics of the telomere biology disorders', *RNA Biology*, 13(8), pp. 696–706. doi: 10.1080/15476286.2015.1094596.

Bienroth, S., Keller, W. and Wahle, E. (1993) 'Assembly of a processive messenger RNA polyadenylation complex.', *The EMBO Journal*, 12(2), pp. 585–594. doi: 10.1002/j.1460-2075.1993.tb05690.x.

Blackburn, E. H., Epel, E. S. and Lin, J. (2015) 'Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection', *Science*, 350(6265), pp. 1193–1198. doi: 10.1126/science.aab3389.

Blackburn, E. H. and Gall, J. G. (1978) 'A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena', *Journal of Molecular Biology*, 120(1), pp. 33–53. doi: 10.1016/0022-2836(78)90294-2.

Blobel, G. (1973) '78,000', 70(3), pp. 924–928.

Boeck, R. *et al.* (1996) 'The yeast Pan2 protein is required for poly(A)-binding protein-stimulated poly(A)-nuclease activity', *Journal of Biological Chemistry*, 271(1), pp. 432–438. doi: 10.1074/jbc.271.1.432.

Breyer, W. A. and Matthews, B. W. (2001) 'A structural basis for processivity', *Protein Science*, 10(9), pp. 1699–1711. doi: 10.1110/ps.10301.
Chan, S., Choi, E. A. and Shi, Y. (2011) 'Pre-mRNA 3′-end processing complex assembly and function', *Wiley Interdisciplinary Reviews: RNA*, 2(3), pp. 321–335. doi: 10.1002/wrna.54.
Chen, J., Blasco, M. A. and Greider, C. W. (2000) 'Secondary Structure of Vertebrate Telomerase RNA telomerase RNA genes, and then full-length genomic', *Cell*, 100(Figure 1), pp. 503–514.
Cheung, A. C. M. and Cramer, P. (2012) 'A movie of RNA polymerase II transcription', *Cell*. Elsevier Inc., 149(7), pp. 1431–1437. doi: 10.1016/j.cell.2012.06.006.
Chow, L. T. *et al.* (1977) 'An amazing sequence arrangement at the 5′ ends of adenovirus 2 messenger RNA', *Cell*, 12(1), pp. 1–8. doi: 10.1016/0092-8674(77)90180-5.
Darzacq, X. *et al.* (2002) 'Cajal body-specific small nuclear RNAs: a novel class of 2'-O-methylation and pseudouridylation guide RNAs.', *The EMBO journal*, 21(11), pp. 2746–56. doi: 10.1093/emboj/21.11.2746.
Dehlin, E. *et al.* (2000) 'Cap-dependent deadenylation of mRNA', *EMBO Journal*, 19(5), pp. 1079–1086. doi: 10.1093/emboj/19.5.1079.
Deo, R. C. *et al.* (1999) 'Recognition of polyadenylate RNA by the poly(A)-binding protein', *Cell*, 98(6), pp. 835–845. doi: 10.1016/S0092-8674(00)81517-2.
Edmonds, M. and Abrams, R. (1960) 'Polynucleotide Biosynthesis: Formation of a Sequence of Adenylate Units from Adenosine Triphosphate by an Enzyme from Thymus Nuclei', *Journal of Biological Chemistry* , 235(4), pp. 1142–1149. Available at: http://www.jbc.org/content/235/4/1142.short.
Edmonds, M., Vaughan, M. H. and Nakazato, H. (1971) 'Polyadenylic Acid Sequences in the Heterogeneous Nuclear RNA and Rapidly-Labeled Polyribosomal RNA of HeLa Cells : Possible Evidence for a Precursor Relationship', 68(6), pp. 1336–1340.
Elliot, D. and Ladomery, M. (2016) *Molecular Biology of RNA*. Second Edi.
Feng J *et al.* (1995) 'The RNA component of human telomerase', *Science*, 269(5228), pp. 1236–1241.
Ganot, P., Bortolin, M. L. and Kiss, T. (1997) 'Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs', *Cell*, 89(5), pp. 799–809. doi: 10.1016/S0092-8674(00)80263-9.
Gao, M. *et al.* (2000) 'Interaction between a Poly(A)-Specific Ribonuclease and the 5 Cap Influences mRNA Deadenylation Rates In Vitro The 3 poly(A) tail, in association with specific poly(A) binding protein(s) (PABP), also plays a broad role in', *Molecular Cell*, 5, pp. 479–488.
Gao, Y. and Yang, W. (2016) 'Capture of a third Mg2+ is essential for catalyzing DNA synthesis', *Science*, 352(6291), pp. 1334–1337. doi: 10.1126/science.aad9633.
Garneau, N. L., Wilusz, J. and Wilusz, C. J. (2007) 'The highways and byways of mRNA decay', *Nature Reviews Molecular Cell Biology*, 8(2), pp. 113–126. doi: 10.1038/nrm2104.
Goldstrohm, A. C. and Wickens, M. (2008) 'Multifunctional deadenylase complexes diversify mRNA control', *Nature Reviews Molecular Cell Biology*, 9(4), pp. 337–344. doi: 10.1038/nrm2370.
Greider, C. W. and Blackburn, E. H. (1985) 'Identification of a specific telomere terminal transferase activity in tetrahymena extracts', *Cell*, 43(2 PART 1), pp. 405–413. doi: 10.1016/0092-8674(85)90170-9.

Harley, C. B., Futcher, A. B. and Greider, C. W. (1990) 'Telomeres shorten during ageing of human fibroblasts', *Nature*, 345(6274), pp. 458–460. doi: 10.1038/345458a0.

Harnisch, C. *et al.* (2012) *Activity and Function of Deadenylases*. 1st edn, *Enzymes*. 1st edn. Elsevier Inc. doi: 10.1016/B978-0-12-404740-2.00009-4.

Hartzog, G. A. and Kaplan, C. D. (2011) 'Competing for the Clamp: Promoting RNA Polymerase Processivity and Managing the Transition from Initiation to Elongation', *Molecular Cell*. Elsevier Inc., 43(2), pp. 161–163. doi: 10.1016/j.molcel.2011.07.002.

Hastie, N. D. *et al.* (1990) 'Telomere reduction in human colorectal carcinoma and with ageing', *Nature*, 346(6287), pp. 866–868. doi: 10.1038/346866a0.

Hayflick, L. and Moorhead, P. S. (1961) 'The serial cultivation of human diploid cell strains', *Experimental Cell Research*, 25(3), pp. 585–621. doi: 10.1016/0014-4827(61)90192-6.

He, G. J. and Yan, Y. Bin (2014) 'Self-association of poly(A)-specific ribonuclease (PARN) triggered by the R3H domain', *Biochimica et Biophysica Acta - Proteins and Proteomics*. Elsevier B.V., 1844(12), pp. 2077–2085. doi: 10.1016/j.bbapap.2014.09.010.

Heiss, N. S. *et al.* (1998) 'X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions', *Nature Genetics*, 19(1), pp. 32–38. doi: 10.1038/ng0598-32.

Henriksson, N. *et al.* (2010) 'Recognition of Adenosine Residues by the Active Site of', *Journal of Biological Chemistry*, 285(1), pp. 163–170. doi: 10.1074/jbc.M109.043893.

Hinnebusch, A. G. and Lorsch, J. R. (2012) 'The mechanism of eukaryotic translation initiation: New insights and challenges', *Cold Spring Harbor Perspectives in Biology*, 4(10), pp. 1–27. doi: 10.1101/cshperspect.a011544.

Ho, C. K. and Shuman, S. (1999) 'Distinct roles for CTD Ser-2 and Ser-5 phosphorylation in the recruitment and allosteric activation of mammalian mRNA capping enzyme', *Molecular Cell*, 3(3), pp. 405–411. doi: 10.1016/S1097-2765(00)80468-2.

Ishikawa, H. *et al.* (2017) 'Poly(A)-specific ribonuclease regulates the processing of small-subunit rRNAs in human cells', *Nucleic Acids Research*, 45(6), pp. 3437–3447. doi: 10.1093/nar/gkw1047.

Jia, H. *et al.* (2011) 'The RNA Helicase Mtr4p Modulates Polyadenylation in the TRAMP Complex', *Cell*. Elsevier Inc., 145(6), pp. 890–901. doi: 10.1016/j.cell.2011.05.010.

Jinek, M., Coyle, S. M. and Doudna, J. A. (2011) 'Coupled 5' Nucleotide Recognition and Processivity in Xrn1-Mediated mRNA Decay', *Molecular Cell*. Elsevier Inc., 41(5), pp. 600–608. doi: 10.1016/j.molcel.2011.02.004.

Jonas, S. *et al.* (2014) 'An asymmetric PAN3 dimer recruits a single PAN2 exonuclease to mediate mRNA deadenylation and decay', *Nature Structural and Molecular Biology*, 21(7), pp. 599–608. doi: 10.1038/nsmb.2837.

Kiss, T., Fayet-Lebaron, E. and Jády, B. E. (2010) 'Box H/ACA Small Ribonucleoproteins', *Molecular Cell*, 37(5), pp. 597–606. doi: 10.1016/j.molcel.2010.01.032.

Kornberg, A. (1988) 'DNA replication.', *Biochimica et biophysica acta*, 951(2–3), pp. 235–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2850007.

de Lange, T. *et al.* (1990) 'Structure and variability of human chromosome ends.', *Molecular and Cellular Biology*, 10(2), pp. 518–527. doi: 10.1128/mcb.10.2.518.

Lange, T. de (2010) 'How Shelterin Solves the Telomere End-Protection Problem', *Cold Spring Harbor Symposia on Quantitative Biology*, 75, pp. 167–177. doi: 10.1101/sqb.2010.75.017.

Lazarus, H. M. and Sporin, M. B. (1967) 'Purification and properties of a nuclear exoribonuclease from Ehrlich ascites tumor cells.', *Proceedings of the National Academy of Sciences*, 57(5), pp. 1386–1393. doi: 10.1073/pnas.57.5.1386.

Lindsey, J. *et al.* (1991) 'In vivo loss of telomeric repeats with age in humans', *Mutation Research DNAging*, 256(1), pp. 45–48. doi: 10.1016/0921-8734(91)90032-7.

Liu, H. Y. *et al.* (1998) 'The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively', *EMBO Journal*, 17(4), pp. 1096–1106. doi: 10.1093/emboj/17.4.1096.

Lowell, J. E. and Sachs, A. B. (1992) 'by the yeast poly ( A ) nuclease', *Genes & Development*, pp. 2088–2099. doi: 10.1101/gad.6.11.2088.

Marcotrigiano, J. *et al.* (1997) 'Cocrystal structure of the messenger RNA 5' cap-binding protein (elF4E) bound to 7-methyl-GDP', *Cell*, 89(6), pp. 951–961. doi: 10.1016/S0092-8674(00)80280-9.

Martínez, J. *et al.* (2000) 'A 54-kDa fragment of the poly(A)-specific ribonuclease is an oligomeric, processive, and cap-interacting poly(A)-specific 3' exonuclease', *Journal of Biological Chemistry*, 275(31), pp. 24222–24230. doi: 10.1074/jbc.M001705200.

Martînez, J. *et al.* (2001) 'The mRNA Cap Structure Stimulates Rate of Poly(A) Removal and Amplifies Processivity of Degradation', *Journal of Biological Chemistry*, 276(30), pp. 27923–27929. doi: 10.1074/jbc.M102270200.

Meyer, S., Temme, C. and Wahle, E. (2004) 'Messenger RNA turnover in eukaryotes: Pathways and enzymes', *Critical Reviews in Biochemistry and Molecular Biology*, 39(4), pp. 197–216. doi: 10.1080/10409230490513991.

Mitchell, J. R., Wood, E. and Collins, K. (1999) 'A telomerase component is defective in the human disease dyskeratosis congenita', *Nature*, 402(6761), pp. 551–555. doi: 10.1038/990141.

Montellese, C. *et al.* (2017) 'Poly(A)-specific ribonuclease is a nuclear ribosome biogenesis factor involved in human 18S rRNA maturation', *Nucleic Acids Research*, 45(11), pp. 6822–6836. doi: 10.1093/nar/gkx253.

Moon, D. H. *et al.* (2015) 'Poly(A)-specific ribonuclease (PARN) mediates 3′-end maturation of the telomerase RNA component', *Nature Genetics*. Nature Publishing Group, 47(12), pp. 1482–1488. doi: 10.1038/ng.3423.

Morin, G. B. (1989) 'The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats', *Cell*, 59(3), pp. 521–529. doi: 10.1016/0092-8674(89)90035-4.

Moyzis, R. K. *et al.* (1988) 'A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes.', *Proceedings of the National Academy of Sciences*, 85(18), pp. 6622–6626. doi: 10.1073/pnas.85.18.6622.

MÜLLER, W. E. G. *et al.* (1978) 'Control of Enzymic Hydrolysis of Polyadenylate Segment of Messenger RNA: Role of Polyadenylate-Associated Proteins', *European Journal of Biochemistry*, 86(1), pp. 283–290. doi: 10.1111/j.1432-1033.1978.tb12309.x.

Nakamura, T. *et al.* (2012) 'Watching DNA polymerase η make a phosphodiester bond', *Nature*. Nature Publishing Group, 487(7406), pp. 196–201. doi: 10.1038/nature11181.

Niedzwiecka, A. *et al.* (2016) 'Molecular recognition of mRNA 5′ cap by 3′ poly(A)-specific ribonuclease (PARN) differs from interactions known for other cap-binding proteins', *Biochimica et Biophysica Acta - Proteins and Proteomics*. Elsevier B.V., 1864(4), pp. 331–345. doi: 10.1016/j.bbapap.2016.01.002.

Nilsson, P. *et al.* (2007) 'A multifunctional RNA recognition motif in poly(A)-specific ribonuclease with cap and poly(A) binding properties', *Journal of Biological Chemistry*, 282(45), pp. 32902–32911. doi: 10.1074/jbc.M702375200.

Nossal, N. G. and Singer, M. F. (1968) 'The Processive Degradation of Individual Polyribonucleotide Chains', *Journal of Biological Chemistry*, 243(5), pp. 913–922. Available at: http://www.jbc.org/content/243/5/913.abstract.

Parker, R. and Song, H. (2004) 'The enzymes and control of eukaryotic mRNA turnover', *Nature Structural and Molecular Biology*, 11(2), pp. 121–127. doi: 10.1038/nsmb724.

Proudfoot, N. J., Furger, A. and Dye, M. J. (2002) 'Integrating mRNA processing with transcription', *Cell*, 108(4), pp. 501–512. doi: 10.1016/S0092-8674(02)00617-7.

Ren, Y. G., Kirsebom, L. A. and Virtanen, A. (2004) 'Coordination of divalent metal ions in the active site of poly(A)-specific ribonuclease', *Journal of Biological Chemistry*, 279(47), pp. 48702–48706. doi: 10.1074/jbc.M403858200.

Ross, J. (1995) 'mRNA stability in mammalian cells.', *Microbiological reviews*, 59(3), pp. 423–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7565413.

Schäfer, I. B. *et al.* (2014) 'The structure of the Pan2-Pan3 core complex reveals cross-talk between deadenylase and pseudokinase', *Nature Structural and Molecular Biology*. Nature Publishing Group, 21(7), pp. 591–598. doi: 10.1038/nsmb.2834.

Schröder, H. C. *et al.* (1980) 'Purification and characterization of a poly(A)-specific exoribonuclease from calf thymus.', *Journal of Biological Chemistry*, 255(10), pp. 4535–4538.

Shatkin, A. J. (1976) 'Capping of eucaryotic mRNAs', *Cell*, 9(4 PART 2), pp. 645–653. doi: 10.1016/0092-8674(76)90128-8.

Shatkin, A. J. and Manley, J. L. (2000) 'The ends of the affair: Capping and polyadenylation', *Nature Structural Biology*, 7(10), pp. 838–842. doi: 10.1038/79583.

Sheets, M. D. and Wickens, M. (1989) 'Two phases in the addition of a poly(A) tail.', *Genes & development*, 3(9), pp. 1401–1412. doi: 10.1101/gad.3.9.1401.

Stuart, B. D. *et al.* (2015) 'Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening', *Nature Genetics*. Nature Publishing Group, 47(5), pp. 512–517. doi: 10.1038/ng.3278.

Tafforeau, L. *et al.* (2013) 'The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of pre-rRNA processing factors', *Molecular Cell*. Elsevier Inc., 51(4), pp. 539–551. doi: 10.1016/j.molcel.2013.08.011.

Tang, T. T. L. *et al.* (2019) 'The intrinsic structure of poly(A) RNA determines the specificity of Pan2 and Caf1 deadenylases', *Nature Structural and Molecular Biology*. Springer US, 26(6), pp. 433–442. doi: 10.1038/s41594-019-0227-9.

Tarun, S. Z. and Sachs, A. B. (1996) 'Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G.', *The EMBO Journal*, 15(24), pp. 7168–7177. doi: 10.1002/j.1460-2075.1996.tb01108.x.

Tomecki, R., Sikorski, P. J. and Zakrzewska-Placzek, M. (2017) 'Comparison of preribosomal RNA processing pathways in yeast, plant and human cells – focus on coordinated action of endo- and exoribonucleases', *FEBS Letters*, 591(13), pp. 1801–1850. doi: 10.1002/1873-3468.12682.

Tucker, M. *et al.* (2002) 'Ccr4p is the catalytic subunit of a Ccr4p/Pop2p/Notp mRNA deadenylase complex in Saccharomyces cerevisiae', *EMBO Journal*, 21(6), pp. 1427–1436. doi: 10.1093/emboj/21.6.1427.

Tummala, H. *et al.* (2015) 'Poly(A)-specific ribonuclease deficiency impacts telomere biology and causes dyskeratosis congenita', *Journal of Clinical Investigation*, 125(5), pp. 2151–2160. doi: 10.1172/JCI78963.

Virtanen, A. *et al.* (2013) 'Poly(A)-specific ribonuclease (PARN): An allosterically regulated, processive and mRNA cap-interacting deadenylase', *Critical Reviews in Biochemistry and Molecular Biology*, 48(2), pp. 192–209. doi: 10.3109/10409238.2013.771132.

Vulliamy, T. J. *et al.* (2006) 'Mutations in dyskeratosis congenita: Their impact on telomere length and the diversity of clinical presentation', *Blood*, 107(7), pp. 2680–2685. doi: 10.1182/blood-2005-07-2622.

Wang, H. *et al.* (2010) 'Crystal structure of the human CNOT6L nuclease domain reveals strict poly(A) substrate specificity', *EMBO Journal*. Nature Publishing Group, 29(15), pp. 2566–2576. doi: 10.1038/emboj.2010.152.

Wells, S. E. *et al.* (1998) 'Circularization of mRNA by Eukaryotic Translation Initiation Factors', 2, pp. 135–140. doi: https://doi.org/10.1016/S1097-2765(00)80122-7.

Will, C. L. and Lührmann, R. (2011) 'Spliceosome structure and function', *Cold Spring Harbor Perspectives in Biology*, 3(7), pp. 1–2. doi: 10.1101/cshperspect.a003707.

Wolf, J. *et al.* (2014) ' Structural basis for P an3 binding to P an2 and its function in mRNA recruitment and deadenylation ', *The EMBO Journal*, 33(14), pp. 1514–1526. doi: 10.15252/embj.201488373.

Wu, M. *et al.* (2005) 'Structural insight into poly(A) binding and catalytic mechanism of human PARN', *EMBO Journal*, 24(23), pp. 4082–4093. doi: 10.1038/sj.emboj.7600869.

Wu, M. *et al.* (2009) 'Structural Basis of m7GpppG Binding to Poly(A)-Specific Ribonuclease', *Structure*, 17(2), pp. 276–286. doi: 10.1016/j.str.2008.11.012.

Yan, Y. Bin (2014) 'Deadenylation: Enzymes, regulation, and functional implications', *Wiley Interdisciplinary Reviews: RNA*, 5(3), pp. 421–443. doi: 10.1002/wrna.1221.

Yang, W. (2011) *Nucleases: Diversity of structure, function and mechanism*, *Quarterly Reviews of Biophysics*. doi: 10.1017/S0033583510000181.

Yu, A. T., Ge, J. and Yu, Y. T. (2011) 'Pseudouridines in spliceosomal snRNAs', *Protein and Cell*, 2(9), pp. 712–725. doi: 10.1007/s13238-011-1087-1.

Zhuang, Z. and Ai, Y. (2010) 'Processivity factor of DNA polymerase and its expanding role in normal and translesion DNA synthesis', *Biochimica et Biophysica Acta - Proteins and Proteomics*. Elsevier B.V., 1804(5), pp. 1081–1093. doi: 10.1016/j.bbapap.2009.06.018.

Zuo, Y. *et al.* (2006) 'Structural Basis for Processivity and Single-Strand Specificity of RNase II', *Molecular Cell*, 24(1), pp. 149–156. doi: 10.1016/j.molcel.2006.09.004.

Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1854

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-392998

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2019