Cryo-EM Aided Design of Multivalent Aptamer Nanostructures for Enhanced Targeting of hCD40L for Immune Modulation

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Abstract:
Aptamers targeting therapeutically relevant biomolecules bare great potential in disease treatment and the regulation of cellular processes outperforming widely used antibodies in size, ease of production and immunogenicity. We propose a workflow to design tailored, multivalent RNA-aptamer structures with enhanced targeting and therapeutic potential based on a precise understanding of the aptamers structure and mechanism of action.
Here, I focus on aptamers binding to human trimeric protein CD40 ligand (hCD40L), allowing inhibition of hCD40L-CD40 interaction, a key costimulatory immune checkpoint in the T-cell mediated B-cell activation with great potential in immunotherapy. After characterization of the protein-aptamer interactions using cryo-EM structural analysis, we determined important binding interactions and used the structural information to guide the design of tailored multimeric architectures. To that end, we employ self-assembled RNA nanostructures, which allow precise three-dimensional positioning of monomeric aptamer subunits for enhanced affinity towards the protein target.
Our data shows three aptamers simultaneously binding to trimeric hCD40L, enabling the generation of trimeric aptameric RNA nanostructures, for both 2'-fluoro and locked nucleic acid modified aptamers. These newly designed, multivalent, self-assembled RNA nanostructures show enhanced binding properties to hCD40L. Structural validation by cryo-EM and further functional characterization using cell-based assays confirmed the successful assembly and the positive effect of multimerization on inhibiting hCD40L-CD40 interaction and B-cell proliferation in co-culture cell studies. The implementation of this cryo-EM–guided structural design approach, has an enormous innovative potential that will help improving our understanding of RNA folding and function while enabling the development of high affinity RNA binders for biomedical and sensing applications.

Chemical modification of mRNA for enhanced stability and protein expression

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Messenger RNA (mRNA) therapeutics have gained heightened interest over the past decade, driven by advances in mRNA synthesis and intracellular delivery. However, the potential of mRNA in protein replacement therapy remains largely unmet, primarily due to the inherent immunogenicity and limited stability of RNA, which impair the sustained protein translation required for therapeutic efficacy. Chemical modification of nucleotides represents a promising strategy to overcome these limitations, as demonstrated by their successful implementation in small interfering RNA (siRNA) and antisense oligonucleotide (ASO) therapeutics. However, this strategy has remained largely unexplored within the field of mRNA, as conventional polymerases used in in vitro transcription (IVT) show poor incorporation of modified nucleotides.

To address this challenge, we employed a panel of mutated polymerases to systematically investigate the transcriptional incorporation efficiency of five different chemically modified nucleotides and their downstream effects on mRNA translation and stability. Transcriptional efficacy varied as a function of the chemical nature of the nucleotide modification, the degree of canonical nucleotide substitution, and the specific mutant polymerase employed. Moreover, modified pyrimidines, particularly uracil analogues, demonstrated superior transcriptional incorporation efficiency. The transcriptional studies also revealed that sterically bulky modified guanine nucleotides yielded improved output when transcripts were co-transcriptionally capped using CleanCap technology, thereby bypassing incorporation challenges at the 5’ terminus.

The modified transcripts were subsequently evaluated for translational potential using two different mammalian expression systems: a cell-free rabbit reticulocyte lysate (RRL) and a HEK293 cell-based system. Across both systems, chemical modifications generally reduced translational output and, in some cases, suppressed it. Unexpectedly, identical modified transcripts revealed contradictory translational results between the two systems, highlighting the context-dependent impacts of chemical modifications on translation. Whether the detected translational suppression, observed with some modified nucleotides, is a result of ribosomal impairment or a limitation in assay sensitivity is actively investigated using higher-sensitivity assays.

Collectively, these findings demonstrate feasible incorporation of chemically modified nucleotides into transcripts and their translational impacts. Current efforts focus on further investigating translation efficiency of these modified mRNAs, as well as evaluating their intracellular stability half-life, which are key factors influencing therapeutic efficacy that open new avenues for protein replacement therapies.

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Title: Identification and functional studies of novel microproteins derived from lncRNAs in HCC

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Hepatocellular carcinoma (HCC) poses a significant challenge in oncology due to its aggressive nature, molecular complexity, and limited treatment options. HCC is largely immune-evasive, highlighting the urgent need for novel therapeutic strategies. Searching for novel targets, we are exploring the potential of long non-coding RNA (lncRNAs)-derived microproteins.

Here, we employed an integrated multi-omics strategy combining ribosome profiling (Ribo-Seq), mass spectrometry (MS), and HLA immunopeptidomics to systematically identify and validate microproteins expressed in HCC. Candidate expression was further validated by transcript stabilization following nonsense-mediated decay (NMD) inhibition, and we evaluated modulation by integrated stress response (ISR) activation, and localization after overexpression of myc-tagged versions. Functional relevance was evaluated using targeted knockdown and rescue experiments, coupled with quantitative cell proliferation assays.

Our bioinformatic analyses revealed a robust subset of microproteins derived from lncRNAs or upstream open reading frames (uORFs), detectable in over 10% of HCC patient samples. By combining our data with further public data we selected ~150 genes with potential to express non-canonical proteins. Bioinformatic studies indicated that many of them are shared between different tumors, correlate with overall survival and associate with functions relevant for tumor growth. We selected 40 candidates for further validation. NMD inhibition led to increased transcript levels of most candidates, supporting their potential translation. Cellular stress assays revealed differential expression patterns of the candidate lncRNAs, suggesting their involvement in stress-related pathways. Functional screening of 19 candidates identified six microproteins with oncogenic properties and two with tumor-suppressive activity. MP overexpression was confirmed by Western blotting and immunofluorescence, revealing heterogeneous expression levels and distinct subcellular localization patterns, with predominant cytoplasmic foci localization.

One oncogenic candidate, termed **NISR**, was selected for in-depth characterization. Silencing of NISR significantly reduced cell proliferation and induced a stress response phenotype. Transcriptomic profiling demonstrated that NISR depletion disrupts ribosomal RNA maturation, alters nucleolar integrity, and impairs translation initiation, likely through enhanced activation of the integrated stress response. Consistently, NISR knockdown increased stress-reporter signaling both under basal conditions and following stress induction. Furthermore, NISR expression was dynamically regulated in response to sorafenib and other tyrosine kinase inhibitors (TKIs), suggesting a potential role in therapy response and adaptive resistance mechanisms. Interestingly, NISR overexpression showed the reverse phenotype, confirming that the MP was functional and not the lncRNA.

Our findings suggest that MPs encoded by lncRNAs represent a novel class of regulators in HCC, with potential implications for both cancer progression and therapy response. Functional characterization of these molecules, such as NISR, may open new avenues for targeted treatment strategies and resistance mitigation in advanced HCC.

DNA Carrier–Enhanced Solid-State Nanopore Sensing for Detection and Quantification of RNA Modifications

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With the evident biological significance of native RNA modifications, as well as the growing interest in utilising chemically introduced RNA modifications to enhance RNA therapeutics, there is an ongoing need for methods to detect and quantify the broad variety of such modifications. Solid-state nanopore sensing holds potential as a novel approach to detect and quantify both native and chemically introduced RNA modifications. These nanopores, typically fabricated from quartz with nanometre-scale diameters, enable voltage-driven translocation of biomolecules such as nucleic acids and proteins, which can be detected as drops in ionic current. While this method does not allow direct sequencing or detection of small RNA modifications, specificity can be introduced using a DNA carrier with selective binding regions, and modification signals can be amplified via biotinylation and subsequent streptavidin binding.

Based on these principles, we have demonstrated the detection and quantification of multiple RNA modifications, including amino-, formyl-, and sialic acid–modified residues. RNA strands were annealed to DNA carriers, selectively biotinylated, and incubated with monovalent streptavidin prior to nanopore translocation. By analysing the number of bound streptavidin molecules per molecule, we developed a model enabling quantitative assessment of modification levels from nanopore readouts. Furthermore, we established orthogonal biotinylation strategies, showing that selective labelling of distinct modification types can be achieved depending on the biotinylation chemistry, enabling modification-specific detection. As a proof-of-principle for biologically relevant targets, we are currently investigating the detection of formylcytosine modifications in isolated $tRNA^{Met}$ from HEK cells. Preliminary results indicate feasibility, highlighting the potential applicability of the approach beyond synthetic systems and towards native RNA samples.

Ongoing and future work focuses on expanding multiplexed detection through combined orthogonal labelling strategies, investigating the influence of modification spacing, and exploring additional modification types and chemistries. The approach is envisioned as a rapid and scalable method for detecting and quantifying native as well as chemically introduced RNA modifications.

Tumor-specific microproteins derived from lncRNAs as antigens for anticancer vaccination

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Triple-negative breast cancer (TNBC) and hepatocellular carcinoma (HCC) are two of the most deadly tumors due to their poor responses to conventional treatments including immunotherapy. Searching for novel therapies, we are exploring the potential of long non-coding RNA (lncRNAs)-derived microproteins. Ribosome profiling (RiboSeq) and RNASeq analyses performed in tumor samples from 10 patients with HCC and 10 with TNBC, allowed the identification of thousands of novel non-canonical ORFs (ncORFs). Bioinformatic studies show that these ncORFs can initiate at AUG codons or all possible single point mutations from AUG, suggesting an aberrant pairing with a Met-tRNA. This aberrant initiation is not allowed by an enriched Kozak sequence, but by sequences rich in GC. In fact, ncORFs are high in GCs and this impacts amino acid composition. RiboSeq data was combined with mass spectrometry (MS), immunopeptidomics data from the same liver tumors and related cell lines and with public data to obtain a map of lncRNA-derived microproteins (MPs). Inhibition of several of the candidates resulted in altered cell growth, suggesting that some coding lncRNAs may play relevant roles in tumor proliferation. To determine whether some lncRNA-derived MPs may be promising antigens for anticancer vaccination, we selected those identified by RiboSeq and/or MS (public data or our own ligandome or microproteome data), that are upregulated in TNBC and/or HCC and are not expressed from healthy tissues except testis (according to GTEX database). Following this pipeline, we have identified lncRNA-derived MPs containing epitopes with high HLA class I/II binding affinities after studies *in silico*, *in vitro* and in transgenic mice models. To evaluate which mechanisms best aid in microprotein processing and presentation *in vivo*, we have developed different expression vectors varying in domain composition using control epitopes as immunogens. All vectors are based on autoreplicative RNAs. Their immunogenicity and antitumor therapeutic ability were evaluated in murine tumor models, with one construct yielding high rates of progression-free survival and complete responses. This system will be used to express the selected tumor-specific MPs, hopefully resulting in a vaccine with potential to enhance the antitumor efficacy of immune checkpoint inhibitors.

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Understanding the role of RNA in the regulation of ORC1 in the DNA replication

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Abstract (poster presentation)

Accurate DNA replication is essential for genome stability and is initiated at specific genomic sites by the **Origin Recognition Complex (ORC)** during the M/G1 transition. Its key subunit, **ORC1**, binds chromatin to mark replication origins and coordinates the sequential recruitment of initiation factors, ensuring proper origin licensing and firing while preventing re-replication through its degradation in S phase [1, 2]. ORC1 does not recognize a defined DNA consensus sequence in human cells, and only a subset of licensed origins is activated each cycle, leaving the mechanisms of origin selection unresolved. Emerging evidence indicates that ORC1 interacts with RNAs transcribed near replication origins, with RNA binding positively correlating with origin activity. This function is mediated by the **intrinsically disordered region (IDR)** of ORC1, which enables dynamic interactions, including RNA binding, phosphorylation-dependent regulation, and **liquid–liquid phase separation (LLPS)**, leading to the formation of **biomolecular condensates** [3, 4].

We propose that RNA sequence features (e.g., GC content) and spatiotemporal dynamics regulate ORC1 function and replication origin selection by modulating its phase separation and promoting origin licensing. To test this hypothesis, we performed *in silico* predictions [5, 6, 7] of RNA-binding affinity and LLPS propensity for ORC1 variants annotated in the ClinVar database [8]. Based on these analyses, we selected and generated five **Meier–Gorlin syndrome (MGS)**-associated mutations (C186R, A372V, R396W, G399D, R477Q) located within the IDR of ORC1 [9, 10]. For functional studies, these variants were engineered for recombinant IDR expression in bacteria and full-length ORC1 expression in mammalian cells, fused to HaloTag for imaging and functional analyses.

We show that some Meier–Gorlin syndrome (MGS)-associated ORC1 variants exhibit reduced RNA-binding affinity *in vitro* and altered behavior *in vivo*, including decreased number and size of nuclear foci (IG399D, R477Q), reduced liquid-like dynamics as measured by FRAP (G399D), diminished replication origin activation (R396W, G399D, R477Q) and altered chromatin binding (G399D, R477Q). Together, these findings provide new insight into how RNA regulates ORC1 activity and replication origin selection and suggest novel avenues for targeting replication stress and genome instability in disease.

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Genes and lncRNAs Involved in the progression from Early Transcriptomic Response to Methotrexate Resistance in HT29 Colon Cancer Cells

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Methotrexate (MTX) resistance hampers therapeutic efficacy in cancer. To gain insight into the molecular mechanisms underlying MTX resistance in colorectal cancer (CRC), we investigated and compared the transcriptional profile associated with early drug response in parental HT29 cells with the previously reported data found in established MTX-resistant cells. In parallel, given the growing relevance of lncRNAs in CRC, we also explored their differential expression in response to the drug and integrated these data into lncRNA–mRNA regulatory networks linked to MTX resistance. Finally, the functional relevance and validation of selected molecular targets was evaluated using Polypurine Reverse Hoogsteen (PPRH) therapeutic oligonucleotides as gene-silencing tools.

Differentially expressed genes (DEGs) found in 48h MTX-treated parental cells revealed transcriptional changes in this early exposure stage related with stress response, proliferation, migration, and metabolic rewiring. Among these, Caveolin-1 (CAV1) emerged as the highest overexpressed protein-coding hub in common with long-term resistant cells.

Analysis of the differentially expressed lncRNAs in both parental and established resistant cells revealed their prominent role in the early stages of MTX exposure. Notably, MALAT1 and NEAT1 were overexpressed in both MTX-treated and resistant cells, whereas H19 displayed a context-dependent expression pattern, since it was overexpressed during short-term MTX exposure in parental cells but downregulated in resistant cells. Integration of lncRNA and protein-coding gene expression data identified MALAT1 and NEAT1 as key regulatory hubs directly associated with CAV1. In addition, these two overexpressed lncRNAs can be sponging miR-224 and miR-552, that are found downregulated in MTX-resistant HT29 cells (GSE28547), which in turn interact with CAV1 mRNA releasing miRNA-mediated repression of CAV1 and promoting its overexpression.

To functionally validate the role of these hubs in MTX resistance, PPRHs were designed against the selected lncRNAs and CAV1. Gene silencing mediated by PPRH transfection enhanced MTX-induced cytotoxicity, supporting the contribution of MALAT1, NEAT1, H19, and CAV1 to the development of drug resistance.

These results propose a new mechanistic lncRNA–miRNA–gene axis linking lncRNA dysregulation to CAV1 upregulation in MTX-resistant colorectal cancer cells which is further supported by the functional validation data showing that NEAT1 and MALAT1 silencing reduces CAV1 expression.

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Adenoviral Delivery of RNA-PPRHs against cMYC in Cervical, Hepatocellular Carcinoma and Breast Cells

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Polypurine reverse Hoogsteen hairpins (PPRHs) are non-modified nucleic acid structures which form triplex DNA complexes that selectively interfere with gene transcription. We previously reported that RNA-based PPRHs maintained their gene-silencing capacity when expressed intracellularly from either plasmid or viral vectors. In this study, we designed a recombinant adenovirus type 5 encoding four PPRHs targeting two sequences against the promoter region, one against intron 1 and another against intron 2 of the oncogene cMYC, together with a GFP reporter (Ad5-MYC). Viral internalization, PPRH expression, and biological activity were assessed in HeLa, HepG2, and T47D cancer cell lines.

Efficient viral transduction was confirmed by fluorescence microscopy and flow cytometry. Quantitative PCR (RT-qPCR) demonstrated strong intracellular expression of the RNA-PPRHs three days after infection, and a significant downregulation of cMYC and its downstream effector CCND1 at the mRNA level in all cell lines. These effects were followed by a reduction in cMYC and cyclin D1 protein levels and decreased cell viability, particularly at higher multiplicities of infection.

Our results establish adenoviral vectors as an effective delivery and expression system for RNA-PPRHs and demonstrate their functional activity against a therapeutic relevant oncogene such as MYC. This work highlights the potential of combining the PPRH technology with adenoviral vectors to expand the applicability of PPRH-mediated gene silencing strategies in cancer gene therapy.

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Toxic RNA versus Toxic Protein: The Role of CGG-Expanded RNA and Polyglycine Protein in *FMR1* Repeat Expansion Syndromes

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Fragile X chromosome-associated tremor and ataxia syndrome (FXTAS) is a neurodegenerative genetic disorder caused by an expansion of CGG trinucleotide repeats (CGGexp) involving 55-200 CGG repeats in the 5'UTR region of the *FMR1* gene encoding the FMRP protein, located on the X chromosome. Symptoms develop gradually in the presence of intranuclear protein aggregates in neurons and astrocytes, accompanied by an increase in FMR1 mRNA levels. Currently, little is known about the exact molecular basis of FXTAS, although several processes have been proposed.

So far, suggested mechanisms of FXTAS pathogenesis include: [1] sequestration by toxic *FMR1* mRNAs from CGGexp of RNA-binding proteins (RBPs) crucial for miRNA maturation and alternative splicing, and [2] biosynthesis of aggregation-prone polyglycine (FMRpolyG) due to non-canonical repeat-associated non-AUG codon-initiated translation (RAN translation).

The project aims to investigate the role of CGG-Expanded RNA and mutant FMRpolyG in FXTAS pathogenesis by deriving stable *in vitro* and *in vivo* models (*Danio rerio*). It seeks to distinguish the effects of toxic protein and RNA from the mutated *FMR1* gene and to clarify how FMRpolyG aggregates contribute to the disease. Understanding these mechanisms is crucial for developing future therapies. The resulting models may serve as tools to test treatments targeting toxic *FMR1* products or reducing protein aggregation.

The host tRNA epitranscriptome: a selective pressure driving viral convergent evolution

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Most human viral genomes are enriched in suboptimal A/U-ending codons, typically associated with reduced translation efficiency due to limited cognate tRNA availability. How these viruses nevertheless achieve efficient protein synthesis remains unclear. In both chikungunya virus (CHIKV) and coronaviruses, we showed that infection induces oxidative stress responses that ultimately remodel the host tRNA epitranscriptome, reprogram codon optimality, and enhance translation of suboptimal codons enriched in both viral genomes and host stress-response genes. Whether this strategy represents a general principle across viruses remains unknown. To address this, we performed a comprehensive analysis of codon frequency and relative synonymous codon usage (RSCU) across all known human-infecting viruses. Most viral genera, including both DNA and RNA viruses, are enriched in the same four A-ending codons whose decoding in favored by the tRNA modification mcm^5/mcm^5s^2, although notable exceptions exist. We hypothesize that this enrichment reflects convergent evolution to align viral codon usage with the stress-induced tRNA modification environment of infected cells. To test this experimentally, we compared two DNA viruses with contrasting codon profiles: Vaccinia virus (VACV), enriched in mcm^5/mcm^5s^2-dependent codons, and Herpes simplex virus type 1 (HSV-1), which lacks this enrichment. Consistently, VACV infection increased mcm^5/mcm^5s^2 levels and upregulated the corresponding writer enzymes, whereas HSV-1 downregulated this pathway. These opposing effects extended to the oxidative stress response: VACV induced the antioxidant enzyme GPx1—whose translation depends on mcm^5/mcm^5s^2-modified tRNAs—while HSV-1 reduced GPx1 expression and failed to control oxidative burst. Together, these results identify a shared viral adaptation to the stress-altered tRNA epitranscriptome during infection that enhances viral protein production, and highlight tRNA-modifying enzymes as potential targets for broad-spectrum antiviral strategies whose efficacy may be predicted by viral codon usage, including in newly emerging viruses.

tRNA-derived fragments generated in Huntington's disease trigger differential immune activation

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The small RNA (sRNA) landscape is altered in Huntington's disease (HD), with multiple studies reporting the dysregulation of specific sRNA classes, including microRNAs (miRNAs) and tRNA fragments (tRFs), in patients' brains. tRFs are generated by the cleavage of mature cytoplasmic tRNAs, and are known to accumulate in cells under pathological conditions, where they can not only engage canonical stress-response pathways, but also activate RNA-sensing machinery, thereby triggering downstream inflammatory signaling pathways. Through deep sRNA-sequencing, our group identified tRFs as the most abundant sRNA species dysregulated in human HD post-mortem putamen samples, and subsequent qRT-PCR analysis of selected candidate sequences validated their upregulation in HD vs control individuals, in independent, age- and sex-matched samples. Interestingly, this increase was not observed in the homologous brain region of the R6/1 murine model of HD.

To further investigate this, we evaluated the potential immunogenicity of a subset of upregulated tRFs and miRNAs in human THP-1 cells differentiated into macrophages, a commonly used model with well-characterized RNA-sensing machinery. Exposure to the selected sRNA species elicited distinct inflammatory profiles that were both dose-dependent and sequence-specific. Immunogenicity was assessed by measuring the release of pro-inflammatory cytokines to the culture supernatant, as well as by evaluating the ability of the sequences to induce the expression of RNA-sensing molecules. Finally, we confirmed the immunogenic effect of tRFs in vivo by intrastriatal injection of specific candidates followed by qPCR quantification of cytokines.

Together, the stress-induced increase in tRNA fragmentation and the immunogenic properties of these sRNA species may contribute to the neuroinflammatory process associated with HD.

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Impact of dsRNA on cellular viability and reduction of dsRNA in *in vitro* transcribed mRNA via enzymatic optimization and polishing

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Presenting: Nicolo Sacchetti, Ph.D.

mRNA has become a widely-used therapeutic tool in recent history due to the ability of researchers to modify templates for specific genes of interest and to produce mRNA quickly. mRNA's overall scalability is an attractive feature, as is its rapid and strong biological response. After mRNA synthesis through the process of in vitro transcription (IVT) is completed, several unwanted contaminants require removal before the mRNA material may be used as a therapeutic. Most of these contaminants, which include enzymes, DNA, unused nucleotides, DTT, spermidine, and salts, are necessary additions for a successful IVT reaction and are well-characterized and easy to remove. Similarly, removal of the phosphate generated during the IVT is trivial. However, there is one contaminant generated during the IVT process that is difficult to separate from the desired mRNA: double-stranded RNA (dsRNA). There are several ways dsRNA can be generated, including via a turnaround transcript, the annealing of small and incomplete mRNA fragments, or the reading of the RNA or DNA template in the reverse direction. Regardless how dsRNA is generated, the product has a dramatic impact on biological activity as cells recognize dsRNA as viral RNA and mount an immune response. Here, we show the drastic impact dsRNA can have on cellular health and on expression of a protein encoded by the mRNA. We also propose two solutions to reduce dsRNA in mRNA drug product. Our first proposed method for reducing dsRNA is utilizing T7 RNA polymerases that have been specifically engineered to prevent the production of dsRNA in the IVT reaction and our second proposed method is a cellulose-based purification method that can significantly reduce dsRNA in final mRNA drug product. We have seen dramatic reduction of dsRNA using both methods in our research, which has resulted in improved cellular health and protein expression.

Long non-coding RNA *OIL1* regulates centrosome replication through G-quadruplex secondary structures.

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Genomic instability is a hallmark of cancer and frequently arises from defects in DNA replication and centrosome homeostasis. Although the Origin Recognition Complex (ORC) plays a central role in replication initiation, the noncanonical regulatory mechanisms governing its activity remain poorly understood. Here, we found in RIP-seq and iCLIP assays identify the long non-coding RNA (lncRNA) *OIL1* as a critical regulator of ORC function and centrosome stability.

We show that *OIL1* directly interacts with ORC1 and associates with key centrosomal proteins, including γ-TUBULIN. Using sucrose gradient fractionation, we demonstrate their co-migration, thereby supporting a functional link between replication control and structural inheritance. Moreover, knockdown of either *OIL1* or ORC leads to centrosome amplification. Functional analyses further reveal that this regulatory mechanism depends on a defined region of *OIL1* with a high propensity to form G-quadruplex (G4) structures, which are required for ORC1 binding.

Moreover, both *OIL1* depletion or G4 deletion confers resistance to microtubule-stabilizing chemotherapeutics, such as paclitaxel. This disruption of genome integrity is accompanied by elevated reactive oxygen species (ROS) levels and increased migratory and invasive potential.

Together, our findings uncover an RNA-mediated mechanism that links ORC1 activity to centrosome homeostasis and identify a G4-forming lncRNA as key centrosomal regulator and therapeutic response.

Editing the m6A mark in mHTT RNA: Exploring a novel therapeutic strategy in HD

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Huntington's disease (HD) is caused by a CAG repeat expansion in exon 1 of the *HTT* gene, leading to the production of toxic huntingtin protein and selective striatal neurodegeneration. Recent evidence highlights HTT1a, a short transcript generated by incomplete splicing and cryptic intronic polyadenylation, as a key pathogenic RNA species that encodes the aggregation-prone HTT exon 1 protein and forms nuclear RNA assemblies.

Our recent work demonstrates that N6-methyladenosine (m6A) RNA methylation is altered during HD progression, with pronounced enrichment in *HTT* intron 1 and HTT1a transcripts in knock-in mouse models. This intronic m6A signature is conserved in human HD samples and depends on CAG repeat expansion. Functional modulation of m6A, either through inhibition of the methyltransferase METTL3 or by site-specific RNA demethylation, reduces HTT1a levels, supporting a direct role for m6A in regulating its biogenesis.

Here, we investigate whether targeting m6A within *HTT* intron 1 using an AAV9-P31-delivered mini-dCas13X.1–ALKBH5 epitranscriptomic editing system can modulate HTT1a expression in vivo. We assess editing efficiency, changes in HTT1a and full-length *HTT* transcript levels, subcellular RNA localization, and downstream effects on motor function and huntingtin aggregation in HdhQ7/Q111 mice. Our findings aim to establish proof of principle for a novel RNA-based therapeutic strategy in HD.

The RNA editing damage response as a driving force of tumour adaptation

Topic: RNA Modifications & Epitranscriptomics

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Keywords: Genomic Instability, Cancer, DNA Repair, ADAR, RNA Editing

Abstract

Heterogeneity and cell plasticity have a crucial role in cellular reprogramming and adaptation to adverse conditions. Regulation of DNA damage response and RNA editing have been described as a strategy against genomic instability, a threaten to cell viability. However, when cellular checkpoints are altered in pluricellular organisms, these adaptational mechanisms may compromise homeostasis by promoting malignant tumour development. MCF10-derived cell lines have been used as a cancer progression model due to high heterogeneity of breast cancer. Our main purpose was to describe the effects of RNA editing damage response in breast cancer progression as a driving force of tumour adaptation. RNA-sequencing analysis was performed to characterize MCF10-derived cell lines and its RNA editing activity after DNA damage. Differential expression analysis, RNA modification, functional enrichment and alternative splicing analysis were computed to determine transcriptome alterations and downstream effects in regulatory pathways involved in cell viability and tumour adaptation. Lowest variation in gene expression profile was found between malignant cell lines, even though they were developed by different tumour progression protocols. By studying changes after DNA damage, a limited number of differential expressed genes were described as a short-term response to irradiation, involved in DNA damage response pathways. Downstream analysis of alterations in RNA editing events as a response to DNA damage identified a vast list of edited genes involved in oncogenic interest pathways: p53 regulation and DNA resection, as a critical first step in DNA repair by homologous recombination. However, alternative splicing isoforms could not be identified as a product of RNA editing in these conditions.

Expanding RNA Aptamer Functionality via Transcriptional Incorporation of Bio-Inspired Nucleotide Modifications

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With the growing interest in RNA-based therapeutics and diagnostics, expanding the chemical diversity of RNA has become essential for generating aptamers with enhanced stability and novel binding capabilities. Here, we present an enzymatic transcription strategy using a mutant T7 RNA polymerase to incorporate a broad range of amino acid– and glycosyl-inspired nucleotide modifications, alongside stabilizing ribose chemistries such as 2'-fluoro and 2'-deoxy groups. We systematically evaluated the compatibility of these modified nucleotides with transcription and downstream amplification, confirming efficient incorporation and generally low misincorporation rates. Crucially, the modified nucleotides were well tolerated throughout the sequencing process, demonstrating their robustness in aptamer evolution workflows. As a proof of concept, we selected a Histidine-U–modified aptamer that binds Influenza hemagglutinin with low-nanomolar affinity, demonstrating that introducing bio-inspired chemical functionalities during transcription can enhance aptamer performance and expand their molecular recognition capabilities. This work establishes a versatile platform for selecting next-generation chemically modified aptamers and highlights the potential of transcriptionally introduced modifications to broaden the scope of targets and functions accessible to RNA aptamers.

Title: Novel regulatory elements at the 5' end of the MBNL1 locus with potential impact on MBNL1 expression

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MBNL family proteins are key regulators of alternative splicing (AS). They play an essential role in organism development by promoting an AS pattern characteristic of fully differentiated cells. The MBNL family consists of three paralogs: MBNL1, MBNL2, and MBNL3, whose expression is highly tissue- and development-stage-specific. MBNL1, the focus of this proposed research, primarily functions in skeletal muscles.

Myotonic dystrophy type 1 (DM1) is a multisystemic disorder caused by an excessive number of CTG repeat expansions (more than 50) in the 3' untranslated region (3'UTR) of the DMPK (DM1 protein kinase) gene. Expression of this mutant gene leads to the production of mRNA containing expanded toxic CUG repeats. This mutant RNA forms stable hairpin-like structures that sequester MBNL proteins into nuclear ribonucleoprotein foci, depleting the cell of functional MBNL activity.

The resulting functional deficiency of MBNL proteins causes profound molecular changes in the cell, including a reversion to the fetal splicing pattern of MBNL-dependent alternative exons and impaired cell proliferation and differentiation.

Half of human mRNAs have one or more uORF (short, open reading frames placed upstream from main coding sequence) in their 5'UTR. Functional uORFs constitute a mechanism regulating the translation of the main open reading frames (ORFs) either in a negative or (less often) positive manner and depending on changing internal conditions (e.g., tissue specificity, developmental stage) or environmental factors (such as abiotic and biotic stress). The 5'UTR of the MBNL1 transcript, approximately 2 kb in length, contains multiple upstream open reading frames (uORFs). Global ribosome profiling (Ribo-seq), which maps translation initiation sites and ribosome occupancy across transcripts in mammalian cells, has revealed several potentially functional uORFs in this region.

The research aim is to investigate whether the Ribo-seq-revealed uORFs within **MBNL1 5'UTR** constitute the functional regulatory elements fine-tuning the MBNL1 expression on the protein level. We investigated this using a variety of plasmid reporter constructs containing different promoters and different luciferase assay systems.

Towards a Molecular Understanding of Human rDNA Silencing by the Nucleolar Remodelling Complex and the Promoter-associated RNA

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Introduction. The Nucleolar Remodelling Complex (NoRC) is a two-subunit, 330 kDa complex that repositions nucleosomes at rDNA promoter repeats, placing them over the transcription start site and thereby silencing ribosomal RNA (rRNA) genes.

The promoter-associated RNA (pRNA) is a 188-nucleotide ncRNA (~60 kDa) that recruits NoRC to rRNA gene repeats — and hence to the nucleolus — and has been shown to inhibit NoRC's remodelling activity *in vitro*.

Objective. This work aims to determine cryo-EM structures of **(1)** NoRC–nucleosome complexes, to unveil how NoRC recognises and remodels its substrate, and **(2)** NoRC–pRNA complexes, to elucidate how pRNA inhibits NoRC's remodelling activity.

Results. We present our ongoing efforts to express and purify NoRC, and to optimise a chromatin remodelling assay that will enable us to identify shorter pRNA variants capable of inhibiting NoRC. Defining a minimal inhibitory pRNA will reduce conformational flexibility within the complex and improve the prospects of obtaining high-resolution structures.

Perspectives. These structures will deepen our understanding of human gene silencing, in particular of ncRNA-mediated rDNA silencing.

Differential regulation of histone H1 subtypes by N6-methyladenosine RNA methylation.

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Histone H1 is involved in the regulation of chromatin structure and gene expression. Up to seven H1 subtypes or variants are expressed in human somatic cells. The H1 complement, defined as the subtype composition and proportions in a given cell, is highly variable depending on the cell type, cell cycle stage, developmental context, and several diseases such as cancer. This variability results from the combined action of multiple regulatory processes.Epitranscriptomic modifications have emerged as a new regulatory layer capable of controlling all aspects of mRNA metabolism.

In this work, we examined the role of the most prevalent mRNA modification, N6-methyladenosine (m6A), in the regulation of H1 subtypes. MeRIP-seq showed that H1.0 and H1.4 are enriched in m6A, whereas H1.2 displays intermediate levels of this mark. We found that m6A inhibition alters transcript and protein levels, de novo transcription, and ribosome occupancy at the translation start site of specific H1 subtypes.Pull-down experiments using biotinylated subtype-specific probes followed by mass spectrometry, as well as RNA immunoprecipitation coupled with RT-qPCR, allowed the identification of IGF2BP1, hnRNPD, and YTHDF2 as m6A readers of H1 subtypes. Integration of the functional studies involving m6A inhibition and partial depletion of these m6A readers led us to propose the first model of the differential regulation of H1 subtypes by m6A.In this model, m6A promotes the degradation of H1.0 mRNA mediated by YTHDF2, while it enhances both the stability and the translation of H1.2 mRNA through the binding of the readers IGF2BP1 and hnRNPD, respectively. In addition, in the case of H1.4, m6A promotes its transcription and stimulates its translation via hnRNPD binding.

These findings suggest that m6A participates in the subtype-specific regulation of H1 subtypes, adding another layer to their complex regulation and contributing to the variability of the H1 complement in cancer.

Low-input mass spectrometry method to monitor ~65 ribonucleoside modifications in a single run

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Post-transcriptional RNA modification technologies have advanced significantly in recent years, being liquid chromatography-tandem mass spectrometry (LC–MS/MS) the gold standard method for the identification and quantification of ribonucleoside modifications due to its sensitivity and accuracy. However, the analysis of ribonucleosides in real biological samples remains challenging because of limited sample amounts and the presence of positional isomers and structurally similar chemical species.

Here, we present a mass spectrometry approach for low-input samples that enables the quantification of more than 65 ribonucleosides in a single analytical run. The method is optimized for samples down to 15 ng and it enables unequivocal identify different positional isomers.

Initially, mixtures of commercially available modified and unmodified ribonucleosides were analyzed using an Orbitrap-based mass spectrometer coupled to nanoLC, employing an optimized data-independent acquisition strategy. Reference retention times and fragmentation patterns were determined for each ribonucleoside to enable unambiguous identification and discrimination of positional isomers. Longitudinal injections were subsequently performed to assess ribonucleoside stability, while serial dilutions were used to determine detection limits and quantitative ranges for each compound.

Finally, the method was used to characterize tRNA modifications purified from real biological samples. Data was analyzed with a newly developed Skyline workflow that provides a user-friendly framework for data processing and visualization, including quality control metrics and statistical analysis across biological conditions.

Title: RetroZearch – Large scale search and characterization of retrozymes in plant genomes.

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Abstract:

Retrozymes are small non-autonomous retroelements containing Hammerhead ribozymes, which are responsible for the self-cleavage and circularization of their transcripts. These mobile genetic elements were recently discovered in plants and animals, though their origin, regulation, and mechanisms of retrotransposition remain poorly understood. The main objective of this work was to perform a large-scale search for retrozymes in the National Center for Biotechnology Information (NCBI) genomic databases, focusing mainly on angiosperms, to establish a foundation for future studies.

To this end, the RetroZearch program was developed, a bioinformatics tool implemented in Python and based on Infernal, an algorithm for RNA sequence and structure search. This program was used to analyze more than 2000 plant species genomes, identifying more than 8000 retrozyme sequences distributed across 271 plant species, predominantly dicotyledons. In addition, comparative analyses based on sequence homology demonstrated the presence of homologous retrozymes in evolutionarily distant plants, supporting the hypothesis of horizontal transfer of these elements among species.

To validate *in silico* findings, we characterized retrozymes in *Helianthus annuus*, an economically important crop. Molecular studies in three cultivars of this species revealed abundant expression of its retrozymes as linear and circular RNAs in both floral tissues and seeds, but not in mature leaves, suggesting a tight transcriptional regulation of these elements during development.

Our study provides the most extensive catalog of plant retrozymes to date and uncovers tissue-specific expression patterns in sunflower, laying the groundwork for future functional studies of these enigmatic mobile elements.

Aptamer-Guided Strategy to Enhance ASO Therapeutics

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RNA interference (RNAi) therapies, including antisense oligonucleotides (ASOs), have made remarkable strides in the treatment of genetic liver diseases. This success is largely due to advances in oligonucleotide chemistry and the development of GalNAc conjugation to further enhance liver-specific delivery. Despite this progress, several critical challenges remain: restricted tissue-specific targeting, limited ASO internalization, inefficient endosomal escape, and restricted access to the nuclear compartment. ASOs designed to degrade nascent mRNAs depend on RNase H activity, which is predominantly nuclear. Likewise, ASOs aimed at modulating splicing must act on immature mRNAs, which are exclusively localized in the nucleus.

In this context, we have identified oligonucleotide aptamers (high-affinity single-stranded oligonucleotide ligands) capable of mediating cellular uptake and nuclear localization. This has been achieved after several rounds of Cell-SELEX. Starting rounds selected aptamers moving from the extracellular media to the interior of the cells. These were followed by rounds selecting aptamers from the media to the nucleus, and from the cytoplasm to the nucleus. Final rounds were done for aptamers capable of translocating nucleic acid cargos to nuclear fractions. Several aptamers were selected with capacity to internalize all target cells in just 6 hours, some of them with specific tropism. One of the candidates, called NuTrans1, outperformed the others in nuclear translocation. Interestingly, when NuTrans1 was conjugated to an ASO targeting a nuclear long non-coding RNA (lncRNA) and added to the cell media, we observed reduced lncRNA levels. Using similar conditions, the ASO alone had no effect. NuTrans1-ASO was also capable of reducing the levels of an artificial circular RNAs.

In summary, we describe how aptamers can be selected to enhance intracellular trafficking, particularly nuclear delivery, and improve cell-type specificity of ASO-based therapies.

Investigating the landscape of non-B DNA and RNA structures in Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the exon 1 of the Huntingtin (HTT) gene, paralleled by the abnormal expansion of a glutamine stretch in the N-terminal region of the HTT protein. The toxic accumulation of both the aberrant mRNA and protein in HD patients causes dysfunction and loss of cortical and striatal neurons, leading to progressive and fatal motor, psychiatric and cognitive symptoms. Over the years, several molecular mechanisms have been proposed that specify the onset of the pathogenesis of HD revealing the involvement of multiple proteins, in particular RNA binding proteins, in the development of this and others neurodegenerative diseases. In this line, we and other labs have reported the mis-regulation of key proteins underpinning altered RNA processing such as mRNA splicing and polyadenylation in the brains of HD patients and mouse models of HD. Preliminary data highlighted the aberrant accumulation in HD context of specific DNA and RNA helicases that tightly regulate the assembly and disassembly of non-B DNA and RNA structures, namely R-loops and G-quadruplexes. Accumulating evidences suggest that these non-canonical nucleic acids structures, enriched in functional genomic regions, are fundamental actors in maintaining genome integrity and RNA homeostasis in different tissue and cellular environments. Notably, since their deregulation could specify the onset of cancer and neurodegeneration diseases they are becoming promising candidates for the development of targeted therapies. Here we present our data on the occurrence and abundance of R-loops and G-quadruplexes and their related protein regulators in the brains of HD patients and mouse models of HD, pointing to a more global characterization of these non-canonical nucleic acids structures in the physiological and pathological mammalian brain.

The RNA structural logic of cellular iron regulation: SIREs 3.0 for iron-responsive elements (IREs) discovery

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Cellular iron homeostasis is regulated by an **RNA-protein mechanism**: **iron-responsive elements (IREs)** are RNA hairpins in 5' or 3' UTRs that recruit **iron-regulatory proteins (IRP1/IRP2)** to couple cellular iron availability to translational control or mRNA stability, with binding favored when cellular iron is low. Despite decades of study, functional IRE motifs extend beyond the canonical consensus; subtle stem-loop differences can shift affinity and likely contribute to context- and factor-specific recognition. Because transcriptome-wide IRP interaction datasets are limited and show incomplete concordance, **computational, structure-aware prioritization is still needed**.

We present **SIREs,** a web server for predicting IRE candidates from user-provided sequences. SIREs scans the submitted sequence for IRE-like candidates, evaluates RNA secondary-structure and motif constraints, and returns candidate stem-loops with an interpretable confidence score and graphical summaries to support shortlist selection.

Here **we introduce SIREs 3.0,** rebuilt from Perl to Python and redeployed as a modern web application to improve maintainability and interoperability with current RNA toolkits. In SIREs 3.0, we add gene- and transcript-centric queries: the server retrieves annotated sequences and enables rapid screening from a single identifier. We also map each predicted IRE to **transcript coordinates** and report distances to key landmarks (5' end, start codon, stop codon). Algorithmically, SIREs 3.0 **expands non-canonical IRE motif handling** and **refines confidence scoring** by integrating motif quality, secondary-structure support, thermodynamic stability, and mutation-informed evidence from the literature.

We **benchmark** SIREs 3.0 against experimentally validated IREs and complementary predictors: on curated sets, SIREs 3.0 recovers >80% of validated IREs and improves performance relative to alternative tools. As a complementary analysis layer, we explore structure-focused follow-ups, including 3D template comparisons to known IRP-IRE architectures and *in silico* mutational scans to assess fold robustness and highlight substitutions likely to alter IRP recognition.

Together, SIREs 3.0 provides an interpretable, structure-aware pipeline for IRE discovery and prioritization: from single sequences to transcriptome-scale case studies, supporting testable IRP-IRE hypotheses for the iron/RNA community.

SIREs 3.0 is accessible at https://www.sires-webserver.eu/.

Integrated regulation of RNA splicing and A-to-I editing in pancreatic cancer cells

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal human cancers, characterized by late diagnosis, limited therapeutic options, and rapid development of chemoresistance. Despite extensive genomic characterization implicating *KRAS* mutations and widespread loss of key tumor suppressors, translation of these findings into effective therapies has been limited. Increasing evidence points to RNA-processing alterations, including alternative splicing and RNA editing, as critical contributors to PDAC initiation, progression, and therapeutic failure. Spliceosome dysfunction, aberrant isoform expression, and deregulation of splicing factors are observed not only in tumor tissues but also in histologically normal regions adjacent to PDAC lesions, suggesting that RNA-processing alterations may represent early molecular events in tumor evolution. In parallel, dysregulated adenosine-to-inosine (A-to-I) RNA editing by ADAR enzymes has emerged as an additional layer of post-transcriptional control with broad implications in tumor behavior, immune modulation, and cell fate determination.

Within this regulatory landscape, the splicing-associated protein PRPF40A has recently gained attention as a potential oncogenic driver. PRPF40A participates in early spliceosome assembly through interactions with SF1, SF3A1, and U1 snRNP components, and its modular WW and FF domains also engage with regulatory proteins beyond the spliceosome. PRPF40A is frequently overexpressed in cancer, correlates with hypoxia-related signatures, and is enriched in PDAC transcriptomic datasets, supporting a functional role in tumor progression and stress adaptation.

Here, we uncover a dual role for PRPF40A in coordinating alternative splicing and ADAR-mediated RNA editing programs that collectively sustain PDAC malignancy. Collectively, our findings indicate that PRPF40A contributes to the coordination of RNA-processing pathways and supports PDAC cell survival, consistent with its potential relevance as a biomarker and therapeutic target.

Understanding lncRNA NIHCOLE effect on DNA damage repair

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Tumor development requires extensive genome reprogramming that equips cancer cells with mechanisms to tolerate replicative stress and DNA damage. In hepatocellular carcinoma (HCC), resistance to radio- and chemotherapy is largely driven by enhanced repair of DNA double-strand breaks (DSBs) via the non-homologous end joining (NHEJ) pathway. We previously identified the long non-coding RNA (lncRNA) NIHCOLE as a cancer-specific RNA that promotes NHEJ and confers a survival advantage to HCC cells. NIHCOLE expression correlates with poor prognosis and is required for HCC cell survival, and efficient DNA repair following genotoxic stress, suggesting NIHCOLE´s involvement in resistance to therapy. Looking for functionality, we have previously shown that NIHCOLE directly interacts with core NHEJ components, including the Ku70-Ku80 heterodimer, DNA-PKcs and APLF. Single-molecule DNA forceps and NHEJ reconstitution assays confirmed that NIHCOLE enhances DNA-end bridging and ligation efficiency. In HCC cells, depletion of NIHCOLE impairs DSB repair, as evidenced by γH2AX accumulation and comet assays.

Structural predictions identified a small motif within NIHCOLE, structural motif 3 (SM3), with an enhanced affinity to bind the Ku heterodimer versus other stem loop-structures of similar size (around 50 nucleotides) within NIHCOLE. We have recently found that SM3 can dimerize, and this is required to bind to two Ku heterodimers. This may be related to function, as broken DNA should also bind to two Ku complexes. SHAPE studies have determined the structure of both monomer and dimer RNAs and the kissing loop where dimerization starts. Interestingly, these structures share similar features with other well-known Ku binding RNAs, raising the possibility that these RNAs also have dimerization potential. Guided by the SM3 structure, we have designed different SM3 mutants predicted to enhance or decrease dimerization or interfere with dimerization *in trans*. We will show how these mutants affect RNA dimerization, binding to Ku and additional NHEJ factors and ligation of two DNA ends in the presence of XRCC4/Ligase4. We believe that these SM3 mimics could improve the therapeutic efficacy of chemo- and radiotherapy in patients with HCC.

This submission is intended for **poster presentation.**

Is translational regulation a viable strategy to enhance protein production in the yeast *Komagataella phaffii*?

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Translation is the cellular process in which the genetic information carried by mRNA is interpreted to synthesize proteins. mRNA levels alone often fail to predict protein abundance, revealing the importance of regulatory mechanisms that act at the level of translation. Such control becomes particularly relevant under cellular stress, when protein synthesis must be rapidly reprogrammed. In the context of improving heterologous protein production in the yeast *Komagataella phaffii* (formerly *Pichia pastoris*), most recent efforts have centered on enhancing secretion pathways and developing methanol-free expression systems. Our laboratory has engineered alkaline pH-responsive promoters that become activated when the culture medium is alkalinized. At a laboratory level, these promoters are a promising tool as they perform similarly to conventional methanol-inducible promoters. Despite these advances, tuning translation remains a relatively unexplored avenue for increasing protein output. To address this gap, we apply an integrative omics framework combining ribosome footprinting sequencing, RNA-seq, and proteomics to characterize the translatome of *K. phaffii* under alkaline conditions. Our goals are i) to study translational control under alkaline pH and ii) to assess whether there are any codon-usage patterns associated with genes that undergo differential translation in response to elevated pH. These insights may guide the rational design of codon-optimized heterologous genes, eventually improving protein output.

Extracellular Small RNA signatures in Cerebrospinal Fluid reveal early molecular alterations in Huntington's Disease

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the HTT gene, leading to widespread transcriptional alterations. Small non-coding RNAs (sRNAs), including microRNAs (miRNAs) and tRNA-derived fragments (tRFs), are emerging biomarkers detectable in biofluids that may reflect disease-related molecular changes. However, their potential as cerebrospinal fluid (CSF) biomarkers across early HD stages remains poorly defined.

Here, we investigated a panel of extracellular sRNAs in HD gene expansion carriers (HDGEC) from brain tissue to CSF. Candidate miRNAs and tRFs were selected through an extensive small RNA sequencing (sRNA-seq) analysis pipeline based on data from human brain samples, combined with prior literature. The dysregulation pattern of candidate sRNAs was validated by qRT-PCR in putamen and cortex samples from HDGEC and control individuals (n=8 per group). The selected sRNA panel was subsequently analyzed in CSF from HDGEC and age-matched healthy controls (CTL), for which detailed clinical characterization, including motor, cognitive, and behavioral assessments, as well as brain MRI data, was available. CSF neurofilament light chain (NfL) was also measured to evaluate associations with sRNA levels.

Analysis of CSF from 68 individuals (48 HDGEC and 20 CTL) revealed that several sRNAs, including tRF-Gly-GCC, tRF-Glu-CTC, miR-451a, and let-7a-5p, were already deregulated at early premanifest stages. These sRNAs were associated with clinical and neurodegeneration-related measures. Importantly, combinations of sRNAs showed stronger associations with clinical and disease progression variables than individual markers and improved discrimination between very early disease stages when combined with NfL.

Overall, these findings indicate that CSF sRNAs are deregulated at early stages of HD and may capture disease-related molecular alterations. Combined sRNA signatures may provide clinically relevant information complementary to established fluid biomarkers and support their potential as early biomarkers in Huntington's disease.

Interferon signaling inhibits DNA end resection through ISGylation. Aicardi–Goutières syndrome as disease model.

Topic: RNA in disease

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Keywords: Genomic Instability, Cancer, DNA Repair, Interferon, Aicardi–Goutières Syndrome

Abstract

Aicardi–Goutières syndrome (AGS) is an early onset encephalopathy characterized by an abnormal neurological development and elevated levels of inflammation makers in the cerebrospinal fluid. So far, there have been nine genes whose mutations lead either to a malfunction of DNA/RNA metabolism, or the hyperfunction of the pattern recognition system, promoting the activation of the interferon cascade in AGS. Here, we report how DNA end resection, fundamental for DNA double strand break (DSB) repair, is impaired in AGS models. We observed how in AGS backgrounds there is an accumulation of DNA:RNA hybrids, a nucleic acid species known to trigger the innate immune response. Indeed, when we enhanced DNA:RNA hybrid resolution, we could suppress the inflammatory and DSB repair phenotypes observed in AGS. Furthermore, at the mechanistic level, we studied the ubiquitin-like protein interferon-stimulated gene 15 (ISG15) as a post-translational modification mediating this process. In summary, we propose that upon DNA:RNA hybrid accumulation, interferon signaling drives the expression of ISG15, and that ISGylation (ISG15 conjugation) tethers the anti-resection factor CCAR2 to the DSB, inhibiting DNA end resection and leading to chromosomal instability.

Post-transcriptional control restrains the IL17–GCSF axis and limits neutrophil-driven breast-cancer metastasis

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Inflammatory cues remodel the tumor secretome, yet how stress-coupled translational control in the tumor niche programs this intercellular communication remains unclear. Although translational control has been studied mainly as a cell-autonomous driver of tumor growth, chronic inflammation, hypoxia and nutrient limitation impose endoplasmic-reticulum (ER) stress that can rewire translation of secreted factors and thereby tumor–immune crosstalk. Here we show that Cytoplasmic polyadenylation element-binding protein 4 (CPEB4) integrates pro-inflammatory environmental stress cues into mRNA stabilization in tumor cells establishing a negative-feedback branch of IL-17 signaling. Ribosome profiling and RIP-seq define two IL-17-regulated mRNA classes: a feedback-limiting module that is bound by and requires CPEB4 for cytoplasmic polyadenylation, stability and translation, and a CPEB4-independent branch exemplified by Csf3. Loss of CPEB4 collapses the former while leaving the latter hyperactive, uncoupling post-transcriptional restraint from transcriptionally linked outputs. This shift elevates tumor-derived G-CSF, drives systemic neutrophilia and accelerates pre-metastatic-niche maturation and lung metastasis in a breast-cancer model. Our data establish CPEB4 as a post-transcriptional rheostat that shapes tumor–immune crosstalk and suggest a novel strategy for targeting the IL-17–G-CSF axis for metastasis-prone breast cancer.

Liquid-liquid phase separation of the m6A RNA demethylase ALKBH9B: An IDR-dependent mechanism driving viral infection in *Arabidopsis*

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Abstract:

N^6-methyladenosine (m^6A) is a critical epitranscriptomic mark regulating plant-virus interactions. In *Arabidopsis thaliana*, the m^6A demethylase ALKBH9B is a key proviral factor during Alfalfa Mosaic Virus (AMV) infection. While ALKBH9B cytoplasmic localization and viral interplay are mediated by specific Nuclear Export Signals (NES) and Coat Protein (CP) interaction domains, its functional assembly into biomolecular condensates remains the most intriguing aspect of its regulation.

In this study, we demonstrate that ALKBH9B undergoes liquid-liquid phase separation (LLPS) driven by its intrinsically disordered regions (IDRs). Computational mapping identified three candidate IDRs, but only the C-terminal IDR3 proved essential for protein condensation. Specifically, we show that an Arginine-Glycine (RG) motif within IDR3 is the primary molecular determinant for this process. Both IDR3 deletion (ΔIDR3) and RG-to-Alanine substitutions completely abolished granule formation *in vitro* and reduced significantly granule formation *in vivo*.

Biophysical characterization revealed that ALKBH9B LLPS is promoted by the crowder PEG and disrupted by 1,6-hexanediol, suggesting that weak hydrophobic interactions drive the assembly. Interestingly, the process was insensitive to high NaCl concentrations, indicating a non-electrostatic nature. Northwestern assays confirmed that IDR3/RG motifs are essential for RNA binding, mediating a re-entrant phase behavior: while RNA scaffolds initial condensation, excessive RNA concentrations inhibit granule formation, highlighting the importance of protein-to-RNA stoichiometry. Finally, FRAP experiments in living cells demonstrated rapid signal recovery and high internal dynamism, confirming the fluid, liquid-like properties and constant molecular exchange of these ALKBH9B condensates with the cytoplasm.

The functional relevance of LLPS was validated through transient expression assays, where co-infiltration of AMV with phase-separation-deficient mutants (ΔIDR3 and RG-substitutions) resulted in significantly lower viral accumulation levels compared to the wild-type ALKBH9B.

To further elucidate this role, we performed a comprehensive RNA-Seq and m^6A-Seq analysis comparing Col-0 and *alkbh9b* knockout plants under both mock

and AMV-infected conditions. This four-way comparative approach identified numerous candidates related to m^6A machinery and RNA-binding proteins (RBPs).

In conclusion, this work demonstrates that ALKBH9B-mediated phase separation is a strict requirement for its proviral role. We propose that ALKBH9B does not function merely as a solitary enzyme, but as a dynamic scaffold that organizes RNA-associated factors into functional condensates.

Uncovering the role of m1A modification of RNA in Therapy-Induced Senescence

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Even though tumors initially respond to chemotherapy, patients often develop resistance, leading to disease progression, metastasis and marginal survival improvements. The low survival of metastatic cancer patients and the high relapse rates highlight the importance of clarifying intrinsic mechanisms that dictate response or resistance to any of these treatments and the development of new strategies is in urgent need. One mechanism that contributes to this resistance is therapy-induced senescence (TIS), where a subset of tumor cells enter a state of growth arrest. Despite the fact that these senescent cells do not proliferate, they remain metabolically active secreting factors known as the Senescence Associated Secretory Phenotype (SASP) able to shape the tumor microenvironment.

This project investigates the unexplored role of chemical RNA modifications, as a dynamic regulator of this stress response. We have generated cellular models where we have induced senescence in primary tumor cells and circulating tumor cells (CTCs) by platinum and taxane treatment. We have validated TIS induction through different assays. Nucleotide mass spectrometry of fragmented RNA from control and senescent cells revealed significant shifts in the modification landscape, notably one of the strongest effects is the decreased levels of m1A in senescence. Dot blot validation confirmed a dynamic regulation of m1A levels not only in primary tumor senescence but also in CTC senescent cells suggesting that specific RNA modifiers are dynamically changing during the transition to senescence. By depleting the m1A machinery we have observed that several of the methyltransferases involved in this modification are required for tumor growth and are able to specifically target the senescent cells. We are currently performing experiments to elucidate the molecular mechanism and we are performing in vivo experiments in metastatic lung cancer mouse models in order to investigate the therapeutic potential of targeting these enzymes.

Abstract submission – RiboRed 2026 RNA meeting

Title: (Max 20 words) Engineering circular RNAs for selective protein expression in dysfunctional endothelial cells.

Authors: Nerea Hernández Egido [1,2], Fatma Betul Dincaslan [1,2], Meaghan E. Richardson [1,2], Lianne M. Mulder [1], Malgorzata Krajewska [3], Piotr S. Kowalski [1,2]

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Abstract: (Max 350 words)

Introduction & Objectives

The success of the messenger RNA (mRNA)-based vaccines against COVID-19 proved the clinical potential of RNA technology for protein production in vivo. Circular RNA (circRNA) is a new class of single-stranded RNA with a closed-loop structure that improves its stability. In contrast to mRNA, circRNA is translated via Internal Ribosome Entry Site (IRES)-mediated translation that often relies on the assistance of factors known as Internal Ribosome Entry Site Trans-Acting Factors (ITAFs) which may vary in their expression depending on the cell type and cell state. IRES engineering could provide an avenue for tissue-specific expression by making circRNA the preferred RNA for the ribosome in a cell under stress conditions such as hypoxia and inflammation, relevant in high-medical-need diseases such as sepsis or cancer.

Materials & Methods

Here, we investigated a library of 40 viral and cellular IRES sequences for their ability to drive robust and specific circRNA translation. CircRNAs containing the 40 viral and cellular IRES candidates and the *Gaussia* luciferase reporter gene were synthesised, purified and assessed in vitro.

Results

We found that Group IV viral IRES displays robust secreted luciferase activity in primary human endothelial cells (ECs) at 24 h after transfection with comparable protein expression levels to m1ψ-modified mRNA. Furthermore, we observed statistically significant differences in translation efficiency between circRNA and mRNA in ECs under hypoxia and other stress conditions (endoplasmic reticulum stress, oxidative stress, inflammation, viral infection and nutrient deprivation).

Conclusion

These results demonstrate the great potential for circRNA in therapeutic applications to overcome the challenges that mRNA technology poses. Therefore, circularisation of coding RNAs could be used as an approach to fine-tune expression levels, extend the duration of protein translation and achieve tissue-specificity.

Keywords

circRNA, IRES, tissue-specific, hypoxia

Insights into the spatial organization and dynamics of Cajal bodies by studying TCERG1-dependent RNA–protein assemblies.

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Transcription Elongation Regulator 1 (TCERG1) is a nuclear protein implicated in transcription elongation and pre-mRNA alternative splicing. TCERG1 is enriched at nuclear speckles, membraneless compartments of the nucleus where transcription and splicing machineries are concentrated. The FF4/FF5 domains of TCERG1 constitute a localization signal that targets the protein to the periphery of nuclear speckle. Recent work of our group showed that TCERG1 also localizes to the periphery of another nuclear compartment known as Cajal Bodies (CBs). CBs are dynamic nuclear microenvironments that host the transcription, assembly, and maturation of small nuclear ribonucleoprotein particles (snRNPs) that constitute the spliceosome. CBs are enriched in Small Cajal body specific RNAs (scaRNAs) that play a crucial role in guiding base modifications of snRNPs. Upon its overexpression, TCERG1 shifts localization from a diffuse nuclear pattern to form several aggregates per nucleus. Under these conditions, the Cajal bodies scaffold protein coilin mis-localizes and both the number and size of Cajal Bodies are reduced. In contrast, overexpression of TCERG1ΔFF4/FF5 displays the same diffused localization as the endogenous protein, and does not affect CBs number or size. RNA interactome analysis revealed that TCERG1 associates with approximately 1,600 RNAs, comprising 80% mRNAs and 20% non-coding RNAs, including scaRNAs. Notably, TCERG1ΔFF4/FF5 exhibits a drastic reduction in RNA interaction across all RNA categories, including scaRNAs. Knockdown of TCERG1 similarly results in a decrease in CB number and size. Furthermore, the localization of scaRNA-binding proteins is altered, suggesting that TCERG1 plays a critical role in the recruitment of scaRNAs to Cajal bodies. Our main objective is to elucidate the mechanisms by which TCERG1 contributes to the integrity and function of CBs, particularly in the proper assembly and maturation of spliceosomal snRNPs. Investigating the interaction between TCERG1 and CBs specific RNAs (scaRNAs) will allows us to gain insights into the spliceosome biogenesis.

Abstract RiboRed 2026

Title: Application of bulk RNA-Seq in FFPE samples enables reliable transcriptomic profiling and tumor microenvironment characterization in Diffuse large B-cell lymphoma

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Abstract:

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma and exhibits marked clinical and molecular heterogeneity. While the standard R-CHOP regimen is effective for many patients, 30–40% experience relapse or refractory disease. Advances in next-generation sequencing are key to identifying new biomarkers enabling risk stratification. Although some molecular signatures like Cell of Origin (COO) classify patients by risk, the role of the tumor microenvironment (TME) in disease progression is yet to be fully undertood. Until recently, RNA research has been limited to fresh or frozen tissue, due to the highly degraded RNA from formalin-fixed paraffin-embedded (FFPE) samples, limiting comprehensive analysis of the TME in real-world clinical samples.

Here, we evaluated QuantSeq 3' mRNA-Seq for transcriptomic profiling and TME characterization in retrospective FFPE DLBCL samples. We analyzed 50 FFPE tissues, including 5 reactive lymph nodes and 45 diagnostic DLBCL samples. RNA extraction was performed with truXTRAC® FFPE total NA Kit (Covaris), libraries prepared using QuantSeq 3' mRNA-Seq (Lexogen), and sequenced on Illumina NextSeq500. Alignment was performed using the Kangaroo platform (Lexogen) against the hg38 reference genome. Differential expression and pathway analysis were performed with DESeq2, Gene Set Enrichment analysis (GSEA), and single sample GSEA (ssGSEA), deconvolution with MUSIC, and COO classification with geneSeqCOO.

Bulk RNA-seq transcriptomic analyses identified DLBCL-specific gene expression patterns and pathways, including increased proliferation, immunoglobulin production, alterations in oxidative phosphorylation, and increased glycolysis and gluconeogenesis compared to reactive lymphoid tissue. These analyses also revealed distinct cellular compositions characterized by an increased proportion of B cells and previously reported markers

associated with the COO (like IRF4 and germinal center pathways). These findings highlight the specific transcriptional and metabolic features that characterize DLBCL.

Title: Hippocampal small RNAs from patients with schizophrenia induce specific cognitive-like symptoms in mice

Authors: **Marcos Galán-Ganga** (1, 2, 3), Anna Guisado-Corcoll (1), Anna Sancho-Balsells (1, 2, 3), Marina Herrero-Lorenzo (1, 4), Iván Ballasch (1, 2, 3), Lisa Patterer (1, 2, 3), Belén Arranz (4), Albert Giralt* (1, 2, 3, 5) and Eulàlia Martí* (1, 2).

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Abstract Body:

Schizophrenia is a neuropsychiatric syndrome that affects around 1% of the world population. It is characterized by psychotic or positive symptoms (e.g. hallucinations and delusions), negative symptoms (e.g. social withdrawal and anhedonia) and cognitive deficits (e.g. social cognition deficits, impaired executive functions and working memory). Currently, antipsychotic treatments can help to manage psychotic symptoms in acute episodes of schizophrenia. However, there is an unmet medical need for the treatment of those cognitive symptoms that appear since the premorbid phase and affect almost 98% of patients. Despite the identification of some molecular pathways that are impaired in schizophrenia, little is known about the possible contribution that small non-coding RNAs (sRNAs) may have in its aetiology and pathophysiology.

To define the contribution of sRNAs to the cognitive symptoms of schizophrenia, we have developed a novel translational model based on the injection of sRNAs from the brain of schizophrenic patients or non-affected individuals into the brain of wild-type mice.

Wild-type mice receiving sRNAs from schizophrenic patients showed an impairment in spatial working memory measured by the T-maze test, in comparison with mice receiving sRNAs from healthy controls or vehicle. Golgi staining revealed that sRNAs from schizophrenia patients induced a decrease in the spine density of the pyramidal neurons from the CA1 hippocampal region in mice. RNA-seq data showed an altered hippocampal expression of synaptic genes related to dendritic spine development and function. Furthermore, characterization of sRNA profiles by sequencing of patients' samples has allowed us to define potential candidates responsible for these effects, specifically related with some microRNAs (miRNAs) species.

In summary, our results suggest that sRNAs might be sufficient for inducing cognitive symptoms and could play a role as important contributors in schizophrenia.

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Presentation: Preferentially Oral Presentation (if it is not possible, we would be grateful to present this work as a Poster Presentation).

Keywords: Small RNAs, Schizophrenia, micro RNAs, Hippocampus, Cognition.

Search for new drugs for the treatment of Aicardi-Goutières syndrome

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Human cells face everyday challenges, in the forms of physical or chemical alterations of the DNA (DNA lesions). Thus, they have evolved a complex family of mechanisms, collectively known as the DNA Damage Response (DDR), to be able to deal with such DNA lesions to avoid, or at least minimize, their impact. The repair of DNA double strand breaks (DSBs) is a complex process that requires multiple molecular machineries, among them, Homologous Recombination (HR) is the most error free way to repair a broken DNA molecule.

One rare disease that has been loosely associated with impairment in the DDR is Aicardi-Goutières Syndrome (AGS). AGS is a genetically inherited rare disease characterized for an increase in type I interferon activity in the cerebrospinal fluid (CSF). It causes an early-onset encephalopathy associated with increased numbers of white cells in the CSF, suggestive of an inflammatory process. Genetically, it is an heterogenous disease that has been associated with mutations in up to nine different genes: TREX1, RNASEH2A, B, and C, SAMHD1, ADAR1, IFIH1, LSM11 and RNU7-1.

We have recently described that ADAR1 depletion caused a defect in HR. Follow up analysis showed that ADAR1 was required for DNA end resection, as downregulation of the protein caused a defect in RPA foci formation. Moreover, recently we have been able to identify a physical interaction with resection proteins, specifically with BRCA1. Thus, we concluded that ADAR1 is a bona fide member of the resection machinery, and we are currently exploring the relationship of the other AGS-related genes with this process and using this as a readout to look for new possibilities for treatments of this disease.

Targeting ADAR RNA editing proteins to overcome PARP Inhibitor resistance in BRCA-deficient cells

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The DNA damage response (DDR) is a critical determinant of cancer progression and therapeutic escape. While many anti-cancer agents function by inducing lethal DNA lesions, tumors frequently develop resistance mechanisms that bypass these effects. Emerging evidence suggests that RNA-specific adenosine deaminases (ADAR)-mediated RNA editing plays an influential yet poorly understood role in tumorigenesis and DDR regulation.

We recently established a direct physical and functional interaction between ADAR proteins and BRCA1, identifying both as essential components of the homologous recombination (HR) pathway. Given that HR deficiency is a hallmark of nearly 40% of cancers and a key predictor of response to platinum-based chemotherapy and Poly (ADP-ribose) polymerase inhibitors (PARPi), we investigated the ADAR-BRCA1 axis as a modulator of chemoresistance.

Using a comprehensive panel of non-tumor and tumor cell lines (encompassing BRCA1 WT, KO and PARPi-resistant cells), we demonstrate that ADAR1 or ADAR2 depletion significantly sensitizes cells to a broad spectrum of clinically relevant genotoxic agents. Most notably, we show that targeting ADARs successfully reverts acquired PARPi resistance, restoring sensitivity in resistant lines to parental knockout levels.

These findings identify ADARs as novel therapeutic targets. By characterizing this RNA-DNA repair crosstalk, our study provides a tractable strategy to overcome acquired resistance, effectively exploiting synthetic lethal vulnerabilities in *BRCA*-mutant patients progressing on current clinical protocols.

The role of vitamin B_{12} in the regulation of gene expression and cellular methylome during animal development

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Vitamin B_{12} is solely synthesised by bacteria and Archaea, but it is vital in humans and animals for the activity of methionine synthase and the regeneration of methionine from homocysteine. Methionine is subsequently converted into S-adenosylmethionine (SAM), a major methyl donor in the cell. In *Caenorhabditis elegans,* vitamin B_{12} deficiency leads to developmental delay due to reduced SAM synthesis (3). However, how vitamin B_{12} deficiency affects RNA modifications, particularly the RNA methylome, is unknown.

We investigated how vitamin B_{12} deficiency and reduced SAM levels affect *C. elegans* RNA modifications and subsequent gene expression changes using Illumina short-read sequencing and Oxford Nanopore direct tRNA sequencing. Our data showed that prolonged vitamin B_{12} deficiency leads to upregulation of antioxidant and immune stress responses. Additionally, we found an altered expression of SAM-dependent methyltransferase enzymes. Finally, we will present our latest data on tRNA sequencing, suggesting that vitamin B_{12} deficiency alters multiple tRNA modifications and potentially tRNA stability.

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2. Bito et al., *FEBS Open Bio* 3, 112-117 (2013)
3. Watson et al. *Cell* 156, 759-770 (2014)

TITLE: **Understanding stress-triggered biogenesis and function of small RNAs derived of transfer RNAs in plants**

AUTHORS: **Joan Marquez-Molins** and German Martinez

AFFILIATION: Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences

ABSTRACT:

Transfer RNAs (tRNAs) are no longer viewed merely as passive adapters in the translation machinery. Emerging evidence suggests they are a source of regulatory molecules known as tRNA-derived small RNAs (tsRNAs). While these fragments are known to accumulate under stress in multiple organisms, the specific mechanisms governing their biogenesis and their downstream functional roles in plants remain poorly defined. In this study, we demonstrate that diverse abiotic stresses trigger a significant overproduction of tsRNAs in the model plant *Arabidopsis thaliana*. Our results indicate that this stress-induced biogenesis is mainly due to RNS1 upregulation. However, constitutive overexpression of this enzyme did not increase the tsRNAs levels as much as that of RNS3, pointing towards additional factors regulating tsRNA biogenesis. Furthermore, we explored the physiological relevance of these fragments using loss-of-function and overexpression transgenic lines of RNS1,2 and 3. Phenotypic analysis in salinity and oxidative stresses revealed a slight increase in fresh weight in the lines over accumulating more fragments, while the triple mutant showed a reduced chlorophyll content. Subsequently, active translation assays in these lines showed the effect of tsRNA fragment overaccumulation/depletion in modulating translation, highlighting their functional role. Overall, our results indicate that high levels of tsRNAs do not merely represent degradation products; rather, they actively regulate the stress response in plants.

lncRNA *NEAT1* is a key regulator of autophagy in multiple myeloma

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Multiple myeloma (MM) afflicts the global population as the second most common hematological malignancy, accounting up to 10% of all blood-related cancers. Current treatment approaches often lead to disease relapses and multidrug resistance. MM cells exert survival amid high protein turnover through hyperactive autophagy and proteasomal degradation. In recent years, ncRNAs are increasingly relevant for their subtle roles in cellular physiology and metabolism. We investigated the function of lncRNA *NEAT1* in its ability to determine mRNA fate through RNA-RNA interactions. We leveraged the recent findings from our lab, relating *ATG16L2* sequestration through *NEAT1:ATG16L2* interaction in maintaining normal cellular physiology.

Proteasome inhibitor bortezomib (BTZ) treatment in MM cell reveals the upregulation of alternate *ATG16L2* paralog against the conventional *ATG16L1*. This dynamic interaction was confirmed by performing a crosslinking and RNA pulldown of *NEAT1* and detecting its interaction with *ATG16L2* by comparing BTZ treated vs untreated samples. At molecular scale, we employed antisense oligonucleotides (ASOs) to disrupt the *NEAT1:ATG16L2* interaction. To ensure its translational potential, ASOs were specifically designed to be stable from nucleases and prevent potential off targets. Thus, this approach successfully inhibited downstream *ATG16L2* protein production in MM cells. Combinatorial aspect with ASOs along with current treatment strategies will ameliorate MM disease outcomes. Altogether, the *NEAT1* mediated regulation of autophagy widens the door to cancer therapeutics and ncRNA understanding.

TERRA functions are driven by their loci of origin

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Abstract

Telomeric repeat-containing RNAs (TERRAs) are long non-coding RNAs transcribed from subtelomeric regions toward telomeres. Despite extensive *in vitro* characterization, their physiological roles *in vivo* remain elusive. Here, we generate mouse models lacking the prominent TERRA loci on chromosomes 18 and 9 to dissect their locus-specific functions. We find that TERRA activity is governed by its genomic origin, as Chr18- and Chr9-TERRA *knock-outs* exhibit distinct and non-overlapping phenotypes. Chr18-TERRA deficiency leads to telomere elongation, progressive obesity, divergent sex-associated disease susceptibility, and reduced lifespan, particularly in males. Proteomic and metabolic profiling reveal impaired fasting-induced fatty acid β-oxidation, uncovering TERRA as a novel player in hepatic energy homeostasis. In contrast, Chr9-TERRA *knock-out* mice display telomere shortening and a mild increase in DNA damage without affecting overall survival—possibly offset by the striking upregulation of the mitochondrial ADP/ATP translocator SLC25A31. These findings establish that TERRA molecules exert locus-specific roles in organismal physiology, unveiling a novel regulatory axis linking telomere biology to systemic homeostasis.

Modified or not? Functional consequences of single m6A sites in breast cancer lncRNAs

N6-methyladenosine (m6A) is a dynamic and reversible RNA modification that shapes multiple aspects of RNA metabolism. Although transcriptome-wide m6A patterns have been extensively characterized, the functional contribution of site-specific methylation within long noncoding RNAs (lncRNAs) remains poorly understood. In particular, the extent to which single-molecule heterogeneity in m6A occupancy influences lncRNA behavior in cancer cells is unclear.

Here, we combined Oxford Nanopore direct RNA sequencing with site-specific 4SedTTP-based m6A mapping to identify and validate individual m6A residues in breast cancer-associated lncRNAs. Direct RNA sequencing enabled analysis of native transcripts at single-molecule resolution, uncovering heterogeneous modification patterns across individual RNA molecules. Orthogonal 4SedTTP reverse transcription assays confirmed high-confidence methylation sites.

To investigate the functional relevance of m6A, we examined lncRNA localization and stability following depletion of the m6A methyltransferase METTL3. Nuclear-cytoplasmic fractionation and transcript turnover assays were used to assess how global reduction of m6A influences lncRNA distribution and half-life. Together, our study integrates single-site epitranscriptomic mapping with perturbation of the m6A machinery, providing a framework to dissect how modification heterogeneity contributes to lncRNA regulation in breast cancer cells.

SPSignal: Web Tool for Structural Prediction of NLS and NES in Proteins

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Protein function is tightly linked to subcellular localization. Many regulatory proteins need to shuttle between the nucleus and the cytoplasm, and their specific subcellular localizations are essential for their biological role. However, identifying functional Nuclear Localization Signals (NLS) and Nuclear Export Signals (NES) remains challenging: conventional computational methods often exhibit high false-positive rates, constrained by limited training data, an incomplete understanding of consensus patterns and a reliance exclusively on sequence-based approaches. Furthermore, these predictors frequently lack accessible, user-friendly interfaces, creating a significant gap between computational prediction and practical application in biological research.

Here we present SPSignal, a web-based tool designed for the integrative analysis of nucleocytoplasmic localization signals in eukaryotic proteins. SPSignal combines a manually curated database of experimentally validated NLS and NES with machine-learning models, motif detection, intrinsic disorder estimation, solvent accessibility calculations, and structure-based exposure scoring. The tool accepts either protein sequences or structures and provides an integrated confidence score together with interactive 2D and 3D visualizations, enabling intuitive exploration of candidate motifs within their structural context. Case studies across diverse eukaryotic proteins demonstrate that SPSignal identifies structurally exposed NLS and NES candidates and facilitates discrimination between simple motif matches and biologically plausible localization signals. Additionally, conservation analysis of predicted motifs enables comparative exploration of localization signals across homologous proteins. By bridging sequence and structural information within an accessible web framework, SPSignal provides a robust platform for exploring the regulatory logic of nucleocytoplasmic protein localization and for guiding experimentally testable hypotheses.

Biosynthetic Persister States and Metabolic Plasticity Fuel Tumor Recurrence

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Tumour expansion ultimately depends on sustained biomass production. Ribosomes play a central role in this process because they generate the cellular proteome and determine the anabolic capacity of the cell. Accordingly, increased ribosome biogenesis is a universal requirement for tumour progression and a critical step that converts small transformed cell clusters into fully developed tumours.

In colorectal cancer (CRC), 5-fluorouracil (5-FU)-based therapy is a cornerstone of first-line regimens that effectively eliminate rapidly proliferating tumour cells. However, resistance to 5-FU represents a major clinical challenge. Indeed, a subset of cells survives treatment and can re-establish tumour biomass, ultimately leading to disease recurrence. Increasing evidence indicates that relapse is frequently driven by drug-tolerant persister cells (DTPs), a small population of tumour cells that survive therapy without acquiring stable genetic resistance and can later regenerate tumour growth. However, how persister cells maintain the biosynthetic capacity required to restart tumour growth remains poorly understood.

We previously discovered that the small ribosomal subunit forms a complex with LARP1 that stabilizes the anabolic transcriptome of the cell. Under conditions of chronic mTOR inhibition—a determinant of DTP state entry and a consequence of 5-FU treatment—the 40S–LARP1 complex preserves mRNAs encoding ribosome biogenesis factors, translation machinery, and metabolic pathways in a translationally inactive but stable state. This mechanism allows cells to rapidly resume protein synthesis and growth once mTOR signalling is restored.

Using colorectal cancer models of chemotherapy exposure and recovery, we found that LARP1 is required for DTP state entry and for the translational preservation of biosynthetic programs necessary for efficient tumour regrowth following 5-fluorouracil treatment. Our data indicate that this phenotype does not arise from the acquisition of genetic resistance but rather reflects a reversible adaptive state that can be repeatedly elicited upon treatment. These observations support the existence of a population of relapse-competent persister cells, which we termed Biomass Fueling Cells (BFCs), whose ability to regenerate tumour biomass depends on LARP1-mediated preservation of anabolic potential. By defining the molecular identity of these states and understanding how LARP1 coordinates the maintenance of the anabolic translational program with entry into drug-tolerant persister states, this study identifies a previously unrecognized therapeutic vulnerability underlying tumor relapse in CRC and beyond.

Title: **Long non-coding RNAs in *Fussarium*: Unraveling new levels of regulations of secondary metabolism.**

Presenting author: **Adrián Perera-Bonaño** (US)

All authors and their affiliations: Javier Avalos (US) and M. Carmen Limón (US)

Max. 350 words (no references included)

Indicate if you want to give a talk or present a poster: TALK

Fusarium fujikuroi is a filamentous fungus that causes bakanae disease in rice, widely used in studies of secondary metabolism. Our group is using this fungus as a model for studying the regulatory mechanisms involved in carotenoid biosynthesis. Carotenoids are lipophilic terpenoid pigments with antioxidant activity and, in many cases, with roles as vitamin A precursors. Although carotenoid biosynthetic pathways are well described, regulatory mechanisms in non-photosynthetic organisms remain poorly understood.

In *F. fujikuroi*, carotenoid synthesis is negatively regulated by the ubiquitin ligase encoded by the gene *carS*, whose expression is modulated by the upstream long non-coding RNA *carP*. Deletion of *carP* results in an albino phenotype; this is explained by an increase of the mRNA levels of *carS* gene that causes a downregulation, under illumination, of the expression of carotenoid biosynthetic genes (*carRA*, *carB, carX*). These data support a *carP-carS* negative regulatory circuit. To gain a deeper understanding of the role of *carP*, a strain overexpressing the *carP,* OE*carP*, was generated controlling this lncRNA under the strong promoter P*gpdA*. Molecular characterization of several OE*carP* candidates will be shown as well as their phenotype under illumination conditions.

Adjacent to *carS* there is another photoinducible gene, *carF*, previously identified in *Fusarium oxysporum*, which encodes a small protein of unknown function. The gene *carF* is probably also regulated by an upstream putative lncRNA, *carN*, located between *carF* and *carP*. Deletion mutants of *carF* and *carN* were generated in *F. fujikuroi* using CRISPR-Cas9. Δ*carF* mutants showed reduced carotenoid accumulation whereas Δ*carN* mutants exhibited increased carotenoid production both in light and darkness, suggesting a possible regulatory interaction between *carF* and *carN*.

The studied genomic region contains two light-regulated lncRNAs (*carN* and *carP*) that share a promoter region and appear to play important roles in the regulation of carotenoid production. These findings underscore the involvement of lncRNAs in fungal secondary metabolism and provide new insights into the transcriptional control of carotenoid biosynthesis.

miRNA-mediated cell-to-cell communications boost DNA repair during the Radioadaptative Response in human fibroblasts

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DNA is the molecule that carries the information necessary for the proper development and functioning of living organisms. This underscores the need for mechanisms that protect and repair DNA when it is exposed to damaging agents. Double-strand breaks (DSBs) are considered the most cytotoxic type of lesion because they compromise the integrity of both DNA strands, and one of the main sources of DSBs is ionizing radiation (IR). Over time, this fact has been exploited therapeutically to treat cancer, using IR to eliminate tumor cells, albeit at the cost of causing damage to surrounding healthy tissue. It is in this context that the concept of the Radioadaptive Response (RAR) emerges. The RAR is a phenomenon in which low doses of radiation protect cells against subsequent exposure to higher doses. Remarkably, this protective capacity can be transmitted to neighboring cells that have not been previously irradiated, causing them to behave as if they had been exposed, a process known as the Radiation-Induced Bystander Effect (RIBE). In this thesis, we have determined that the RAR is not a universal phenomenon, and that the observed increase in cell survival is a consequence of accelerated DNA repair via homologous recombination (HR). This enhancement in repair kinetics is communicated from cell to cell through signaling molecules packaged within small extracellular vesicles (sEVs), which exhibit altered RNA and protein content following exposure to a low dose of radiation. Specifically, we observed reduced expression of two microRNAs, miR-126-3p and miR-451a, and elevated levels of a MAP kinase, MAPK1. This suggests that the molecular cargo released from the cell can influence nuclear processes. These findings represent a novel perspective on the systemic effects of radiation and may have important implications for optimizing radiotherapy protocols in cancer patients.

CPEB1 translation repression is mediated by a crosstalk between the 5’CAP and the 3’UTR. Eulàlia Belloc, Chiara Castellazzi, Verónica Chanes, Raúl Méndez.

The Cytoplasmic Polyadenylation Element Binding (CPEB) family of proteins play a pivotal role in post-transcriptional gene regulation. CPEBs can either activate or repress mRNA translation. While the mechanism of CPEB1-mediated activation—driven by the recruitment of a poly(A) polymerase —is well characterized, the molecular basis for its role as a translational repressor is controversial. In this study, we use a genome-wide approach to identify Gemin5 as a critical co-factor in CPEB1-dependent silencing. Our findings reveal that CPEB1 interacts with Gemin5 that in turn binds to the cap structure promoting a repression complex that sterically inhibits the binding of the initiation factor eIF4E to the cap structure, thereby preventing the assembly of the translation initiation complex. These results define a novel mechanism for CPEB-mediated repression, uncovering a molecular crosstalk between 3' UTR-bound regulatory proteins and 5' cap-dependent translation initiation.

Role of ASF1 in Topoisomerase II-associated DNA damage response

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RNA polymerase II (RNAPII) suffers a promoter-proximal pause during early elongation. The negative elongation factor NELF is one of the main factors implicated in promoting pausing and therefore, regulating transcription elongation and gene expression. NELF has also been implicated in regulation of DNA repair although its function in this process is not yet well established. Here, we performed a proteomic assay to identify NELF interactors in response to with topoisomerase II (TOP2)-associated DNA damage (TOP2-breaks). Notably, we have identified ASF1, a histone chaperone, as a NELF partner that promotes TOP2-breaks repair through the regulation of NELF stability. We propose a model in which NELF plays a central role in the regulation of chromatin structure at promoters to promote the DNA damage response.

Targeting RNA Structures for Broad-Spectrum Orthoflavivirus Inhibition

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Orthoflaviviruses include major human pathogens, such as dengue, Zika and yellow fever viruses, as well as other less-characterized emerging members. Collectively, they cause hundreds of millions of infections and thousands of deaths worldwide. Considerable efforts have been devoted to antiviral drug discovery targeting viral proteins. However, no specific antiviral drugs have been approved for the treatment of any orthoflavivirus infection.

Essential RNA elements for viral replication represent attractive targets for antiviral discovery, particularly those that are conserved across the genus since they provide opportunities for broad-spectrum therapeutic strategies. Using infectious clones and reporter viruses, we show that the well-characterized dengue virus RNA synthesis promoter, an RNA element located within the viral genome, can be functionally substituted by promoters from any pathogenic orthoflavivirus, indicating a conserved mechanism of promoter-polymerase recognition. Structure-function and computational analyses reveal that, although sub-elements of promoters have diverged in distinct orthoflavivirus host environments, co-evolution of these elements preserves polymerase binding competence for viral replication. Guided by this conservation, we performed a high-throughput screen and identified small molecules that bind the promoter in vitro and inhibit replication across multiple orthoflaviviruses in cell culture. Together, these findings provide proof of principle that the RNA promoter can serve as a pan-orthoflavivirus antiviral target. Moreover, other conserved RNA elements may offer analogous antiviral targeting opportunities. We seek to develop integrated computational and experimental approaches to discover novel conserved RNA structures that can be screened for small-molecule binders to identify new broad-spectrum inhibitors.

This work highlights conserved viral RNA structures as a viable foundation for broad-spectrum antiviral strategies. RNA viruses can drive sudden epidemic waves, as illustrated by the 2020 SARS-CoV-2 pandemic and the 2015 Zika virus epidemic. Developing broad-spectrum treatment strategies represents a fundamental preparedness measure for future outbreaks.

Impact of Gemin5 protein status in RNA-binding and translation regulation of mitochondrial genes

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Gemin5 is a predominant cytoplasmic RNA-binding protein involved in spliceosome assembly and translation control. Depletion of this essential protein results in embryonic lethality. Gemin5 contains a TPR-like dimerization domain at its central region. Patients carrying mutations in this domain present cognitive and motor developmental delay, hypotonia, ataxia, and cerebellar atrophy. However, the mechanism through which alterations in Gemin5 lead to disease remain poorly understood. In this work, we show that the activation of signaling pathways upon poly I:C stimulus led to protein phosphorylation, enhancing Gemin5 RNA-binding affinity. Given the relevance of the dimerization domain, we examined the role of a stretch of potentially phosphorylatable residues (S-STS-S) located in the first helix of the dimerization domain. Mutations in these residues altered protein functions in translational control, ribosome binding, and protein stability. In parallel, we analyzed how increased Gemin5 levels affect alternative splicing (AS) in human cells. Remarkably, the AS transcript variants are enriched in the polysomal fraction, likely leading to the synthesis of defective proteins. Conversely, reduced Gemin5 levels cause widespread transcriptomic alterations, including the downregulation of multiple mRNAs encoding mitochondrial proteins, besides ribosomal proteins and histones. We show that Gemin5 silencing results in decreased mitochondrial respiration and reduced mitochondrial ATP production. Taken together, these findings provide new insights into how post-translational modifications of Gemin5 influence its activity, and how changes in its cellular levels affect key RNA metabolism processes, such as splicing and translation of fundamental genes.

PARP1-Induced Nuclear RNA Condensates Link DNA Damage to Translation Arrest

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While the DNA damage response is known to coordinate transcription and protein degradation, its role in regulating translation remains poorly understood. Here, we uncover a function for PARP1 in suppressing cellular mRNA translation when hyperactivated in response to specific DNA lesions. We show that APE1-dependent cleavage of abasic sites triggers PARP1 hyperactivation and translation arrest. Mechanistically, PARP1 promotes the formation of large nuclear RNA-containing condensates enriched in nuclear speckle factors and RNA-binding proteins involved in RNA splicing and export. These condensates disrupt RNA homeostasis by mislocalizing RNA-binding proteins and sequestering RNAs within the nucleus, thereby reducing the cytoplasmic mRNA pool available for translation. As a result, PARP1 limits the expression of DNA damage-induced stress response factors by blocking the export of their transcripts. Together, our findings reveal an unexpected role for PARP1 in uncoupling mRNA abundance from protein production during the DNA damage response, restraining gene expression programs in cells experiencing excessive genotoxic stress.

ATRX safeguards rDNA transcription under amonafide-induced nucleolar stress

Ribosomal DNA (rDNA) is among the most transcriptionally active and structurally fragile regions of the genome, requiring specialized mechanisms to maintain its integrity. ATRX, a chromatin remodeler associated with repetitive DNA, has been implicated in safeguarding genomic stability, although the role of ATRX in nucleolar stress remains poorly understood.

We investigated the cellular response to amonafide, a DNA intercalator that prevents TOP2 binding to chromatin, and found a pronounced inhibition of RNA polymerase I-mediated transcription of ribosomal genes. Amonafide triggered a robust nucleolar stress response characterized by the formation of UBF-marked nucleolar caps. Strikingly, ATRX was recruited to these nucleolar caps upon amonafide treatment, suggesting a chromatin-topology-dependent mechanism.

Our findings reveal a novel role for ATRX in protecting rDNA integrity during transcriptional stress. By facilitating chromatin repair at ribosomal genes, ATRX mitigates nucleolar stress and genome instability. In line with this, ATRX-deficient cells are significantly more sensitive to amonafide, highlighting its therapeutic potential for ATRX-deficient tumors.

Discovering novel plant RNA viroids through planetary data mining

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Plant viroids are the tiniest known infectious genomes, consisting of ~300 nt of circular RNA with uncertain evolutionary origins. Since their discovery in 1971, viroids have been considered rare biological entities restricted to angiosperms with only 45 species formally recognized. Recent studies have challenged this view by revealing thousands of new viroid-like agents, which appear to replicate in simpler organisms such as fungi and bacteria rather than plants. Here we developed a planetary-scale exploration of plan viroids and identified 21 novel viroid species, increasing the known diversity of this family by ~50%. Most of these new viroids are widespread in previously unreported plant crops like black pepper, Sichuan peppers, bamboos, poplars or magnolias, among others.

Bioassays in experimental host plants confirm replication and spreading of representative examples, validating our approach to uncover bona fide viroids. This expansion represents a substantial leap for classic plant viroids but also suggests their limited genetic diversity when compared with obelisks and other ribozyme-containing circular RNAs.

Scalable pipeline for RNA structural comparison

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Long non-coding RNAs (lncRNAs) have emerged as central regulators in various biological functions. Dysregulation of lncRNAs is implicated in complex human diseases, including cancer, neurological disorders, and immune responses. LncRNAs often form **complex secondary structures**, which tend to be more conserved than primary sequences and are thought to mediate their molecular functions. Most RNA structural comparison methods rely on sequence-based alignments or computationally heavy algorithms, making them unsuitable for high-throughput studies. Here, we present a **sequence-agnostic pipeline for large-scale RNA structural characterization**, offering rapid and scalable structural similarity measurements across thousands of RNAs.

Our model utilizes **contrastive learning and graph neural networks** trained on in silico-generated triplets to produce **robust structural embeddings** from RNA secondary structures. Using data from diverse non-coding RNA families in the Rfam database, we demonstrate that our model **effectively clusters RNAs by family based on their structural features, despite high sequence divergence**. Furthermore, the framework **accommodates RNA structure ensembles**, which better represent the dynamic conformational variability of lncRNAs compared to single static structures.

Our pipeline represents a significant step toward overcoming current limitations in RNA structural comparison. Its development aligns with the growing need for efficient tools to explore the structural landscape of lncRNAs in biology and disease. Beyond lncRNAs, our method can be applied to other **highly mutable RNA classes, such as viral genomes or riboswitches**, where structural elements are critical for function. This approach provides a solid foundation for the large-scale structural characterization of RNAs, offering new perspectives on RNA-mediated regulation and evolutionary conservation.

Staufen2 modulates the temporal dynamics of human neurogenesis *in vitro*

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Abstract

RNA-binding proteins (RBPs) are central to post-transcriptional gene regulation during brain development, yet their specific functions in coordinating human neural lineage decisions remain poorly understood. In this work, we investigate for the first time the role of the double-stranded RBP Staufen2 (STAU2) in human neurogenesis. Characterization of STAU2 knockout iPSC derived cells using scRNA-seq shows that loss of STAU2 disrupts neuroepithelial cell identity and accelerates neural differentiation by altering the activity of key transcription factors and driving early metabolic transitions. Additionally, STAU2 regulates the expression of miRNA host genes and alters miRNA-mediated post-transcriptional control in progenitor cells, which exerts additional effects on STAU2 regulated gene regulatory networks. These changes result in neural progenitor exhaustion, unstructured neural rosettes, and reduced organoid size. Together, our work uncovers a previously unrecognized role for STAU2 as a central regulator of early human neurogenesis, acting through both miRNA-mediated and transcriptional pathways to coordinate progenitor maintenance and neuronal fate specification.

Host tRNA Epitranscriptome Remodeling Supports Arbovirus Persistence in *Aedes aegypti*

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Persistent infection of arboviruses in mosquito cells is essential for their transmission and propagation. Yet, how these viruses maintain viral protein expression and infectious virus production without compromising mosquito fitness remains poorly understood. To address this, we used *Aedes aegypti* mosquitoes persistently infected with chikungunya virus (CHIKV), as a model to conduct a comprehensive analysis of virus-induced changes in the transcriptome, translatome, and tRNAome, the main players in protein expression. We found that CHIKV infection alters tRNA modifications pattern, notably increasing queuosine (Q)-derived modifications (manQ and galQ) and decreasing 5-formylcytidine (f5C), both associated with oxidative stress and mitochondrial function. Integrated mRNA-seq and ribosome profiling analyses uncovered a striking uncoupling of transcription and translation: while genes involved in oxidative stress and mitochondrial function were transcriptionally upregulated, they were translationally repressed. This repression was codon-specific, affecting C-ending codons decoded by Q-modified tRNAs. Importantly, both antioxidant genes and the CHIKV RNA were enriched in these codons and showed reduced translation efficiency, indicating that Q modification mediates their selective translational repression. Among mitochondrial genes, only the one requiring f5C for efficient decoding showed significant repression, further linking modification dynamics to codon-specific regulation. Together, our results support a model in which CHIKV-induced oxidative stress drives tRNA modification reprogramming, leading to codon-specific translational repression of both host oxidatve stress genes and viral genes. This selective repression balances the energetic cost of viral replication with stress control, ultimately supporting long-term viral persistence in the mosquito host.

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The Maternal-to-Zygotic transition (MZT) orchestrates the reprogramming of early vertebrate development, encompassing zygotic genome activation (ZGA) and the clearance of maternally-provided RNAs. While some regulators of MZT have been identified, the vast majority of maternal RNAs remain functionally uncharacterized. Using our optimized CRISPR-RfxCas13d/CasRx technology, we performed a screening targeting maternally provided mRNAs encoding protein kinases and phosphatases in zebrafish, uncovering Bckdk as a novel post-translational regulator of MZT. Depletion of *bckdk* mRNA led to epiboly defects, global ZGA deregulation, reduced H3K27ac levels, and partial impairment of miR-430-dependent maternal RNA degradation. Phosphoproteomic upon Bckdk depletion revealed the main target of Bckdk, Phf10, a chromatin remodeling factor also involved histone acetylation and ZGA.

Despite our CRISPR-RfxCas13d optimizations *in vivo*, the activity of CRISPR-RfxCas13d can be further enhanced *in vivo*, and its application has generated controversy due to the potential collateral activity. In our lab, we have continued to improve the CRISPR-RfxCas13d system for an enhanced RNA targeting *in vivo* using zebrafish embryos as animal model and by different and compatible approaches. Indeed, we demonstrated that i) chemically modified gRNAs increase and maintain mRNA knockdown during early development, ii) specific nuclear RNA targeting can be more efficient using optimized nuclear localization signals, and iii) we show that *in vitro*-based computational models can predict gRNA efficiency *in vivo* but with a relatively modest accuracy. Furthermore, we showed that transient CRISPR-RfxCas13d approaches such as ribonucleoprotein complexes or mRNA-gRNA effectively deplete mRNAs in zebrafish embryos without inducing collateral activity, except when targeting extremely abundant and ectopic RNAs. To circumvent this potential issue, we have implemented alternative RNA-targeting CRISPR-Cas systems such as CRISPR-DjCas13d or CRISPR-Cas7-11 with reduced or absent collateral activity upon highly expressed RNA knockdown in zebrafish embryos.

Altogether, our findings i) demonstrate the potential of CRISPR-RfxCas13d to uncover novel early zebrafish developmental factors shedding light on the role of Bckdk as post-translational regulator of MZT and ii) contribute to optimize CRISPR-Cas technology for RNA targeting in zebrafish through transient approaches, promoting the development of in vivo CRISPR-Cas knockdown therapies.

Disruption of nuclear proteostasis in 40S ribosomal protein deficiencies

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Defects in ribosome synthesis cause a group of human disorders known as ribosomopathies, but the role of malformed ribosome precursors (preribosomes) in their pathogenesis is poorly understood. Diamond-Blackfan anemia (DBA) is one such disorder and is caused by haploinsufficiency of one of about 20 ribosomal proteins, with *RPS19* being the most frequently mutated gene. Different DBA-linked mutations can give rise to various types of abortive preribosomes, including immature or near-mature 40S or 60S subunit precursors formed in distinct cellular compartments, yet these abnormal intermediates have not been systematically explored as a unifying pathogenic mechanism or as a possible explanation for the high prevalence of *RPS19* mutations. Using imaging-based screenings and biochemical analysis in human cells, we systematically examined how the individual depletion of all 40S ribosomal proteins affects the dynamics of early preribosomes. We found that all 40S protein deficiencies, independent of the specific step of ribosome assembly affected, trigger the nucleoplasmic accumulation of malformed preribosomes. These defective particles arise in the nucleolus and can appear either as dispersed subcomplexes throughout the nucleoplasm or as numerous stable abnormal condensates that form during nucleolar reassembly in late mitosis. Their persistence disrupts the normal turnover of key nucleolar factors, generating a proteostasis imbalance between the nucleolus and nucleoplasm. Distinct ribosomal protein deficiencies produce characteristic patterns of abnormal preribosome condensation and dispersion, with RPS19 loss causing some of the most severe defects. Altogether, our findings identify the formation of nucleoplasmic aberrant condensates and preribosome subparticles as a common consequence of RPS deficits, and suggest that their accumulation may contribute to the pathogenesis of ribosomopathies such as DBA.

A novel circular RNA-based therapeutical platform for infectious and non-infectious diseases

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Proper folding of RNA molecules is critical for their regulation and functionality. Consequently, alterations in this process can lead to genetic diseases such as Amyotrophic Lateral Sclerosis (ALS) or Myotonic Dystrophy type 1 (DM1) where aberrant secondary structures produce RNA-mediated toxicity through the production of toxic peptides and splicing alterations. Additionally, viral RNA transcripts often contain highly conserved structures that play crucial roles in viral propagation. Thus, secondary RNA structures within cellular mRNAs and viral RNA transcripts present untapped potential as therapeutic targets for a wide range of infectious and non-infectious diseases. At noctuRNA Therapeutics, we have developed an innovative circular RNA (circRNA)-based strategy that effectively targets such RNA structures, opening new possibilities for therapeutic interventions. As a proof-of concept, we have demonstrated the efficacy in cell culture of circRNAs designed to correct genetic-related aberrant RNA structures — repairing splicing defects (DM1) or inhibiting RAN translation (ALS) — and to inhibit multiple viral infections, including those caused by hepatitis C virus, dengue virus, west Nile virus, influenza virus and SARS-CoV-2.

Key advantages of noctuRNA circRNAs include: (i) exceptional stability *in vivo*, without the need for chemical modifications, ensuring a sustained therapeutic effect; (ii) versatility to specifically target a single RNA structure or to generate broad-spectrum circRNAs by incorporating multiple hybridization regions targeting different structures within a single circRNA molecule; (iii) an ultrafast response to novel viral threats, with the capacity to generate highly efficient antiviral circRNAs within 4 weeks after viral sequence release; and (iv) increased resistance to escape mutants by targeting highly conserved secondary structures.

In conclusion, our circRNA-based therapeutic platform holds promise in addressing a wide range of infectious and non-infectious diseases pushing the boundaries of RNA-based therapeutics to bring significant advancements in the field of medicine.

A framework for the design of personalized antisense oligonucleotide therapies for rare neurological diseases

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Personalised antisense RNA therapies have evolved rapidly in last years, after the development of the first individualized splice-modulating antisense oligonucletide (ASO) drug in 2019 (1). Following the same strategy, our group has designed personalized ASOs for two neurological diseases caused by rare deep intronic mutations.

In **Spastic Paraplegia Type 7 (SPG7)**, an incurable neurodegenerative disorder with a phenotype ranging from pure spastic paraplegia to ataxia, we identified 11 patients with a deep intronic mutation in *SPG7* gene (c.286+853A>G) in homozygous or compound heterozygous with another pathogenic variant. The mutation activates the inclusion of a cryptic exon with a premature stop codon, leading to SPG7 loss of function (2). We successfully designed and validated synthetic ASOs which restored *SPG7* mRNA processing, normalized protein levels and rescued downstream mitochondrial alterations. Upcoming work includes evaluating selected ASOs in iPSC-derived motoneurons and cerebellar organoids, characterising haplotypes using Oxford Nanopore long read sequencing, conducting longitudinal clinical assessments of amenable patients, and identifying biomarkers that predict therapeutic response.

In parallel, we are developing an ASO drug for a pediatric patient with ***SUCLG1*-related mitochondrial DNA (mtDNA) depletion syndrome**, caused by a deep intronic mutation in *SUCLG1* gene, which creates a novel 3´splice-site and leads to the inclusion of a frameshifting exon. The patient is currently in a stable condition with a severe generalized dystonia and ongoing cognitive progress. ASO screening has identified good candidates that block cryptic exon inclusion.

For both conditions, our goal is to establish a robust preclinical and regulatory framework to obtain AEMPS approval for intrathecal treatment under compassionate use and named-patient exemption programs. Moreover, we are integrating short and long-read whole-genome sequencing with specialized bioinformatic pipelines to **identify additional patients** with neurological conditions who may benefit from this personalised therapeutic strategy.

How development is regulated by an m^{6}A-YTHDF module: Lessons from plants

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Post-transcriptional regulation by modifications of internal nucleotides in mRNAs is of paramount importance in biology. These chemical marks can modulate the affinity between the modified mRNAs and specialized mRNA-binding proteins that act as 'readers' of the modification. The best characterized *N6*-methyladenosine (m^{6}A) readers are YT521-B homology (YTH) domain proteins. In mammals, cytoplasmic m^{6}A-mRNA regulation by YTH domain proteins of the YTHDF clade regulates pluripotency factors in a process that is indispensable for embryo development, and is associated with neurodevelopmental diseases and cancer. The plant YTHDF homologs, called ECTs in arabidopsis, promote growth by boosting the proliferation of primed stem cells in the primordia of lateral organs such as leaves, flowers and roots. However, the specific genes whose regulation by this pathway is responsible for the stimulation of the growth program remain ill-defined, a difficult problem given that over 6000 genes are targets of the m^{6}A-ECT2/3/4 module. For that reason, genetic ablation of ECT2/3/4 or m^{6}A-deposition factors results in a complex syndrome that so far has precluded a deeper understanding of this process. I will present novel results from isolated plant stem cells that reveal, with striking simplicity, how the growth boost is achieved in plants by this fascinating regulatory system.

Chemically modified RNAs for enhanced biomolecular targeting and biomedical applications

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RNA therapeutics are emerging as promising treatments for a wide range of diseases, showcased by the mRNA-based COVID-19 vaccines and established siRNA drugs. Within the field, RNA aptamers are also garnering more attention due to their ability to provide precise targeted therapy by binding protein targets with high affinity and specificity. Despite their relevance, clinical application of RNA therapeutics faces challenges due to low stability, immunogenicity and potential off-target effects.
To tackle these challenges, we aim to integrate innovative bioconjugation strategies, in vitro selection techniques, and RNA nanotechnology to develop multivalent chemically enhanced RNA molecules for drug discovery and biosensing applications. For this, we have developed a novel method for the co-transcriptional incorporation of chemically modified nucleobases, which is fully compatible with SELEX-based in vitro selection strategies, thereby enhancing RNA chemical diversity and stability.
A key goal of this technology focuses on developing RNA aptamers decorated with different saccharides for carbohydrate-protein recognition. We are currently focused on targeting mannose-binding lectins such as Langerin and other sugar-binding lectins to study their mode of action and explore their potential therapeutic applications.
This method also allows the incorporation of locked nucleic acids (LNAs) into RNA, enabling the selection of LNA-aptamers via SELEX. We have implemented this strategy for selecting aptamers targeting CD40 ligand, an important immune checkpoint protein expressed in T-cells that regulates B-cell activation. Recently, we characterized some of these RNA:protein complexes using Cryo-EM structural analysis. This provided new insights into their interaction modes and multimerization strategies. These advancements pave the way for developing efficient alternatives to antibody-based therapies. Overall, our strategy holds significant potential for creating new RNA-based tools for biomedicine and diagnostics.

RNA regulatory networks in nitrogen-fixing legume symbionts

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Small non-coding RNAs (sRNAs) are emerging as central regulators of bacterial adaptation, yet their roles in the *Rhizobium*-legume symbiosis remain poorly understood. In the alfalfa symbiont *Sinorhizobium meliloti*, we characterized two major RNA-based regulatory circuits that orchestrate metabolic and physiological shifts during early root colonization and nodule formation.

During pre-infection stages, the homologous *trans*-sRNAs AbcR1 and AbcR2 drive extensive post-transcriptional remodelling of nutrient uptake and metabolism. Their expression is differentially controlled by the redox-responsive regulator LsrB and the stress-responsive σ factor RpoH1. MS2 Affinity Purification coupled with RNA Sequencing (MAPS) revealed large and overlapping AbcR1/2 interactomes (~6% of coding genes), with broad repression of transport and metabolic mRNAs through independent anti-Shine-Dalgarno motifs. This regulation likely optimizes nutrient use in the rhizosphere. Functionally, AbcR1 is required for efficient root-surface colonization, underscoring its relevance during early symbiotic steps.

We also identified a nitrogen-responsive module centered on the sRNA NfeR1, which integrates nitrogen availability with the NtrBC two-component system. Antagonistic binding of NtrC and LsrB to the NfeR1 promoter generates peak NfeR1 levels under nitrogen starvation and in endosymbiotic cells. NfeR1 pairs with the translation initiation region of *ntrB*, attenuating NtrBC production and modulating operon autorepression. This mixed feedback design enables *S. meliloti* to fine-tune nitrogen metabolism throughout symbiosis. MAPS further revealed that NfeR1 regulates additional mRNAs related to motility, osmotolerance, and cell cycle progression, and forms a feedback loop with the dual-function sRNA SmelC549.

Together, these findings position AbcR1/2 and NfeR1 as key nodes within extensive RNA-centered regulatory networks that rewire *S. meliloti* metabolism and support an efficient symbiosis with legumes.

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SATR2 Satellite RNA Shapes Replication and Chromatin compaction through Nuclear and Cytoplasmic DNA-RNA Hybrids formation

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Although repetitive elements constitute a significant portion of the human genome, they still remain refractory to detailed molecular analysis. In this study we found SATR2, an abundant euchromatic satellite sequence transcribed by RNA polymerase III during S phase, resulting in the accumulation of DNA-RNA hybrids. These hybrid structures modulate the chromatin state, impeding replication fork progression. Furthermore, the processing and translocation of these DNA-RNA hybrids to the cytoplasm elicits the cell immune response. Finally, our results demonstrate that the SATR2-induced immune response triggers the PI3K/AKT/mTOR signaling pathway, allowing cells to evade cell cycle checkpoint associated with replication stress and keep proliferating. Collectively, these findings uncover a novel regulatory crosstalk between satellite DNA expression, immune signaling and chromatin state, highlighting the pivotal role of RNA-DNA hybrids in cellular adaptation to replication stress.

RNA Editing Repression by Dynamic Helicase Complexes During Trypanosome Development

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Abstract

Trypanosoma and Leishmania protists undergo post-transcriptional uridine (U) insertions and deletions in their mitochondrial mRNAs guided by antisense guide RNAs (gRNAs) to produce translatable sequences. Parasitic *T. brucei* experiences lifecycle-regulated editing linked to the use of glycolysis in the bloodstream stage and oxidative phosphorylation (OXPHOS) in the insect (procyclic) stage. We previously demonstrated that REH2C, a regulatory RNP complex containing the DEAH-Box RNA helicase KREH2, the zinc-finger protein KH2F1, and additional partners, partially suppresses editing of mRNA *ND7* specifically in procyclics. However, editing of cytochrome-encoding mRNAs in bloodstream-stage parasites is nearly absent due to unclear mechanisms. Here, we report approximately 1000-fold repression of *COX3* editing in bloodstream-form parasites mediated by REH2C proteins. This repression mainly occurs at an early checkpoint and involves multiple novel regulatory gRNAs ("terminators") that are activated while canonical gRNAs associated with editing maturation are inhibited. *COX3* editing repression is highly sensitive to REH2C protein levels: misregulation—particularly of KH2F1—partially restores early *COX3* editing and maturation. Using systematic mutagenesis, genetic complementation, and AlphaFold3 multimer modeling, we are exploring how REH2C RNP architecture supports editing repression, substrate recognition, complex assembly, and interactions with other factors in the editosome holoenzyme. These findings reveal a previously unrecognized phenomenon of RNA editing repression that regulates mitochondrial gene expression in *T. brucei.*

Deciphering the role of pseudouridine synthase PUS10 in intestinal inflammation.

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Pseudouridine synthases are a family of epitranscriptomic writers that catalyze the uridine isomerization into pseudouridine (ψ) in all kinds of RNA molecules. One of those synthases is pseudouridine synthase 10 (PUS10), which has recently been linked to the inflammatory response and is genetically associated with the development of intestinal inflammation. Considering the converging roles of genetic susceptibility and epitranscriptomic regulation in complex inflammatory disorders, we hypothesized that this disease-associated genetic variant exerts its functional effect by modulating PUS10-mediated pseudouridylation**.** Therefore, our objective is to elucidate the role of PUS10 and the associated SNP in the development of intestinal inflammatory disorders.

In this study, we used a CRISPR-Cas9 approach to generate a mutant epithelial cell line, hemizygous for the risk allele. Using this cell line, we observed that cells harboring only the risk allele had an exacerbated inflammatory response towards IFNγ exposure in comparison with wild-type heterozygous cells. One of the main upregulated pathways was the antigen processing and presentation pathway. We confirmed that some of the genes in this pathway, such as TAP1, are negatively regulated by PUS10. Interestingly, overexpression of PUS10 can reverse the increase of TAP1 upon IFNγ stimulation. Moreover, in order to know which RNA molecules had a ψ catalyzed by PUS10 that could be affected by the associated SNP, we used a bisulfite-induced deletion and sequencing technique. We identified PHGDH, a serine metabolism enzyme, and YBX1, a transcription factor involved in

inflammation, as PUS10 ψ mRNAs. Interestingly, antigen presentation pathway genes did not seem to be modified by PUS10.

Collectively, our data identifies novel PUS10 mRNA targets and demonstrates that the inflammation-associated SNP contributes to the inflammatory response in intestinal epithelial cells. This work establishes a mechanistic link between genetic risk and epitranscriptomic dysregulation in intestinal inflammation, highlighting PUS10 as a potential modulator of epithelial homeostasis.

This abstract is for oral presentation.

I will attend in-person.

Structural characterization of a U1 snRNP riboswitch allows rational design of novel riboswitches for polyadenylation regulation in mammalian cells.

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U1 snRNP is a crucial ribonucleoprotein (RNP) that orchestrates spliceosome assembly and inhibits premature polyadenylation (pA). We have guided U1 snRNP to mRNAs of interest to decrease their pA and, therefore, specific gene expression with therapeutic interest. In a previous work, we employed a novel Capture-SELEX-based technique (SEREX) to engineer novel tetracycline (TC)-responding U1 snRNP riboswitches such as rsON9.

In the absence of the TC, rsON9 binds U1 snRNP leading to gene expression inhibition. When present, TC shifts rsON9 conformation blocking U1 snRNP binding and allowing gene expression. This riboswitch demonstrated improved performance in cell culture and *in vivo* (150-fold inhibition and 13-fold induction in mice).

We have now performed an in-depth structural characterization of the rsON9 riboswitch employing P^{32}-radiolabelled RNA, EMSAs, microscale-thermophoresis (MST), SHAPE and HMX on the rsON9 RNA and a battery of mutants both in the presence and absence of TC or a U1snRNA mimic. Interestingly, rsON9 3D structure shows a flat surface in which the U1 binding region (U1BS) lays partially stacked and exposed to the solvent. This increases U1 binding affinity 1000-fold compared to that of a plain antisense linear RNA sequence. TC binding forces U1BS to interact with an inner core motif, thus drastically decreasing U1 binding and ultimately switching on gene expression. Importantly, mutations in this motif and in additional selected domains can alter TC-mediated regulation and increase U1 binding affinity even further, by augmenting solvent exposure of the U1 binding region.

These results allowed us to identify the key sequences and structures that drive the U1 binding and the switching mechanism of rsON9, which can be employed as a module for rational design of riboswitches, where the TC aptamer might be replaced by another ligand-sensing domain of interest leading to highly efficient polyadenylation riboswitches in mammalian cells.

Finally, ongoing overexpression experiments of rsON9 to act as a U1snRNP sequester, show strong phenotypic impact on cell proliferation, mitochondrial function and unexpected accumulation of polyadenylated non-coding RNAs.

NELF Facilitates Topoisomerase II-associated DNA Damage Response

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DNA topoisomerase II (TOP2) is essential for maintaining DNA topology during transcription. Our previous work established a link between TOP2 activity and promoter-proximal pausing in gene regulation. In this study, we identify the Negative Elongation Factor (NELF) as a critical mediator of the DNA damage response triggered by TOP2 poisons. Upon etoposide treatment, NELF shows enhanced recruitment to chromatin and correlates with elevated RNA polymerase II (RNAPII) occupancy near transcription start sites. Notably, NELF depletion impairs DNA damage repair, evidenced by reduced γH2AX signaling. To further elucidate the role of the NELF complex, we conducted proteomic analyses to characterize its interaction network under etoposide exposure. Based on these findings, we propose a model in which NELF modulates the chromatin landscape and facilitates the recruitment of specific repair factors, thereby promoting both signaling and repair of TOP2-induced DNA damage.

Decoding the role of the non-essential yeast ribosomal protein eL22 during cytoplasmic maturation of 60S ribosomal subunits and its impact on translation dynamics.

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Ribosome biogenesis is an essential, evolutionary conserved, and highly coordinated process that couples pre-rRNA processing with ribosomal protein assembly. In the yeast *Saccharomyces cerevisiae*, this complex pathway involves around 300 *trans*-acting factors and 79 r-proteins. Although many r-proteins have well-defined roles, the function of some non-essential proteins remains unclear. We investigated the non-essential r-protein eL22 from the 60S r-subunit, which reported roles beyond ribosome synthesis and whose human ortholog is frequently dysregulated in cancer. Loss of eL22 causes mild, cold-sensitive growth defects, a reduced pool of 60S r-subunits, and a modest impairment in pre-60S nuclear export. Moreover, the absence of eL22 compromises efficient processing of 27S pre-rRNAs. Our data further indicate that correct eL22 assembly is required for the activity of distinct assembly factors acting during late 60S cytoplasmic maturation. To assess downstream functional consequences, we performed ribosome profiling in the eL22-null mutant and uncovered widespread alterations in translation efficiency (TE) that exceed transcriptional changes, including mutant-specific TE up/down gene sets and a shared core of predominantly TE-downregulated, functionally clustered genes. Beyond expected defects in early translation steps, the common signature detected was the enrichment of the mRNA surveillance (e.g., nonsense-mediated decay) and the co-translational SRP-dependent targeting to membranes processes, suggesting that perturbations linked to eL22 impact nascent-chain behavior, promote proteostasis stress, and remodel secretory pathway homeostasis.

Biosynthetic Persister States and Metabolic Plasticity Fuel Tumor Recurrence

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Tumour expansion ultimately depends on sustained biomass production. Ribosomes play a central role in this process because they generate the cellular proteome and determine the anabolic capacity of the cell. Accordingly, increased ribosome biogenesis is a universal requirement for tumour progression and a critical step that converts small transformed cell clusters into fully developed tumours.

In colorectal cancer (CRC), 5-fluorouracil (5-FU)-based therapy is a cornerstone of first-line regimens that effectively eliminate rapidly proliferating tumour cells. However, resistance to 5-FU represents a major clinical challenge. Indeed, a subset of cells survives treatment and can re-establish tumour biomass, ultimately leading to disease recurrence. Increasing evidence indicates that relapse is frequently driven by drug-tolerant persister cells (DTPs), a small population of tumour cells that survive therapy without acquiring stable genetic resistance and can later regenerate tumour growth. However, how persister cells maintain the biosynthetic capacity required to restart tumour growth remains poorly understood.

We previously discovered that the small ribosomal subunit forms a complex with LARP1 that stabilizes the anabolic transcriptome of the cell. Under conditions of chronic mTOR inhibition—a determinant of DTP state entry and a consequence of 5-FU treatment—the 40S–LARP1 complex preserves mRNAs encoding ribosome biogenesis factors, translation machinery, and metabolic pathways in a translationally inactive but stable state. This mechanism allows cells to rapidly resume protein synthesis and growth once mTOR signalling is restored.

Using colorectal cancer models of chemotherapy exposure and recovery, we found that LARP1 is required for DTP state entry and for the translational preservation of biosynthetic programs necessary for efficient tumour regrowth following 5-fluorouracil treatment. Our data indicate that this phenotype does not arise from the acquisition of genetic resistance but rather reflects a reversible adaptive state that can be repeatedly elicited upon treatment. These observations support the existence of a population of relapse-competent persister cells, which we termed Biomass Fueling Cells (BFCs), whose ability to regenerate tumour biomass depends on LARP1-mediated preservation of anabolic potential. By defining the molecular identity of these states and understanding how LARP1 coordinates the maintenance of the anabolic translational program with entry into drug-tolerant persister states, this study identifies a previously unrecognized therapeutic vulnerability underlying tumor relapse in CRC and beyond.

Targeting Nonsense-Mediated mRNA Decay (NMD) potentiates radiotherapy and promotes the abscopal effect

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Nonsense-mediated mRNA decay (NMD) is a regulatory pathway responsible for transcriptome surveillance, degrading transcripts harboring premature termination codons (PTCs) and preventing the expression of aberrant proteins. In the context of cancer, NMD has gained increasing attention, as several studies suggest that its inhibition sensitizes tumor cells to first-line therapies. Radiotherapy can occasionally induce the abscopal effect, a rare phenomenon in which tumor regression occurs not only at the irradiated site but also in distant, non-irradiated lesions. The development of novel therapeutic strategies targeting NMD has therefore emerged as a promising approach in oncology.

Our results demonstrate that NMD inhibition through SMG1 impairment significantly enhances the efficacy of radiotherapy. In vitro assays revealed a sustained reduction in cancer cell proliferation following irradiation combined with SMG1 blockade, suggesting that NMD functions as a protective mechanism against radiation-induced DNA damage. In multiple tumor models, radiotherapy efficacy was increased when NMD was inhibited. Importantly, the antitumor effect was partially dependent on the immune system, as the therapeutic benefit was reduced in immunodeficient mice. Further evidence supporting immune system involvement was the potentiation of the abscopal effect, with a marked reduction in distal, non-irradiated tumors.

The therapeutic potential of NMD inhibition in combination with radiotherapy could be further harnessed through the development of RNA aptamer-based RNA interference (RNAi) targeting strategies.

Control of retrotransposon-driven activation of the interferon response by the double-stranded RNA binding protein DGCR8

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Abstract

The type I interferon (IFN) response is the main innate immune pathway against viruses in mammals. This pathway must be tightly regulated to prevent viral spread while avoiding excessive immune responses. Here, we show that inactivation of the double-stranded RNA (dsRNA)-binding protein DGCR8 unleashes the IFN response in human cells. We demonstrate that DGCR8 restricts the accumulation of endogenous dsRNA originating from protein-coding mRNAs that harbour transposable elements (TEs), primarily LINE and SINE elements. We propose that DGCR8 binding to TE-rich mRNAs is essential to resolve dsRNA structures, and in its absence, accumulated dsRNA signals through MDA5-MAVS pathway triggering the IFN response. This mechanism is relevant to conditions where DGCR8 expression levels are altered, including the 22q11.2 deletion syndrome (22qDS). Supporting this, we show that 22qDS-derived cells exhibit an exacerbated type I IFN response which inversely correlated with DGCR8 levels. All these together demonstrate the importance of suppressing endogenous TE-dsRNA accumulation to prevent unwanted immune activation and associated disease pathogenesis.

snRNA modification heterogeneity as a novel alternative splicing mechanism

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Alternative splicing allows a single gene to produce multiple messenger RNAs and expands the diversity of proteins that cells can generate. The extent of alternative splicing is closely associated with the complexity of organisms, with multicellular organisms showing diverse patterns of alternative splicing that can be cell- and tissue-specific and developmentally regulated. Similarly, many diseases, including human cancers and developmental disorders, are associated with altered transcript splicing patterns. RNA splicing is carried out by the spliceosome, which consists of over a hundred proteins and five U-rich small nuclear RNAs (snRNAs) named U1, U2, U4, U5, and U6. All spliceosomal snRNAs are highly structured and carry multiple RNA modifications; however, the function of these modifications remains largely unknown.

We recently showed that the m6A modification of U6 snRNA is essential for recognising 5' splice sites with adenosine at the +4 position (+4A) in *C. elegans* and humans. In the absence of m6A modification, 5' splice sites shift from a +4A position to a +4U position. Our results highlight the power of snRNA modifications in sequence-specific splicing regulation.

Furthermore, our analysis indicates that this shift in splice site positions is not random. We found that 5' splice sites of transcripts with +4A and +4U positions can be conserved across millions of years, raising the possibility that the spliceosome uses both modified and unmodified snRNAs for alternative splicing. We identified alternative splicing events that affect protein function and subcellular localisation, all regulated by the U6 snRNA m6A modification. We propose a novel mechanism of alternative splicing that uses snRNA modifications, and we will present our latest data on uncovering this regulatory process.

The regulation of the orthoflaviviruses translation is mediated by the RNA elements in the 3' UTR of the genome

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The genus *Orthoflavivirus* comprises a large number of vector-borne positive-sense single-stranded RNA viruses, including Dengue virus (DENV), Yellow fever virus (YFV), Zika virus (ZIKV), and West Nile virus (WNV). Their genome is a compact entity of approximately 11,000 nt that contains all the information required to complete the viral life cycle. It encodes a single open reading frame flanked by untranslated regions (5' and 3' UTRs). To achieve the storage of all genetic information, highly conserved structural RNA elements have evolved beyond the protein-coding sequence to encode essential information. These RNA elements play essential functions in the regulation of viral processes. In orthoflaviviruses, most of the functional elements described to date are located within the 5′ and 3′ UTRs. In particular, the 3′ UTR plays a crucial role in translation control through the combined enhancer and repressor activities of distinct structural elements. We have demonstrated that this function can be extrapolated to heterologous contexts; however, full activity is only recapitulated within its native molecular context. This observation suggests that the 3′ UTR operates through the direct recruitment of cellular and/or viral protein factors, as well as through the establishment of complex RNA–RNA interaction networks. Our results indicate that the specific recruitment of the 40S ribosomal subunit by structural elements of the 3' UTR of WNV, together with the formation of a complex network of intra- and intermolecular RNA–RNA interactions, represents key mechanisms controlling the efficiency of viral translation and, potentially, the transition between different stages of the viral life cycle.

Antisense RNA controls phycobilisome degradation in heterocyst-forming cyanobacteria

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Extensive antisense transcription occurs in the heterocyst-forming cyanobacterium *Nostoc* (=*Anabaena*) sp. PCC 7120. We are currently investigating antisense RNAs that may regulate the expression of genes encoded on the opposite strand.

Phycobilisomes are the major antenna complexes for photosynthetic light-harvesting in cyanobacteria. Their degradation under non-optimal growth conditions requires the NblA protein. *nblA* transcription is activated in the absence of a nitrogen source or under strong illumination. This activation triggers phycobilisome dismantling and recycling of phycobiliprotein-derived amino acids. A noncoding antisense RNA (*as_nblA*) is transcribed opposite to the *nblA* mRNA in *Nostoc* and is conserved among other heterocyst-forming cyanobacteria. Inactivation of *as_nblA* expression leads to increased *nblA* transcript levels and increased NblA protein accumulation, resulting in excessive phycobiliprotein degradation even when nitrogen is available or under normal light conditions. Missexpression of *nblA* due to absence of *as_nblA* impairs cell growth and promotes rapid accumulation of mutations that suppress *nblA* expression. Thus, *as_nblA* transcription, together with regulation by the small RNA NsrR1, acts as a brake on *nblA* expression. These combined regulatory mechanisms counteract basal leaky *nblA* transcription and ensures that phycobiliprotein degradation occurs only when *nblA* expression surpasses a threshold in response to environmental cues such as nitrogen limitation or excess light.

To elucidate the mechanism of *as_nblA*-mediated regulation, we examined whether RNase III-dependent codegradation of mRNA–asRNA duplexes, a common antisense RNA regulatory pathway in bacteria, plays a role. Our analysis found no evidence supporting this mechanism. Instead, a detailed quantitative study of mRNA degradation kinetics following rifampicin treatment revealed that *as_nblA* acts through transcriptional interference. Specifically, *as_nblA* blocks *nblA* transcription arising from leaky termination of upstream gene transcription. Induction of *nblA* under nitrogen deprivation or high-light conditions overrides this interference, allowing proper expression of *nblA* when required.

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Virus-Induced Noncanonical Translation of Non-Coding RNA: A Potential Source of Neoantigens in Type 1 Diabetes

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Type 1 Diabetes (T1D) is a chronic autoimmune disease that develops in genetically predisposed individuals following exposure to environmental triggers. The timing and mechanisms by which such environmental exposures contribute to disease onset remain uncertain. Among the proposed environmental triggers of T1D, viruses have emerged as relevant candidates, particularly Coxsackievirus B1 (CVB1). In parallel, most genetic variants associated with T1D localize in non-coding regions of the genome, including loci encoding long non-coding RNAs (lncRNAs). By acting on these non-coding regions, viral infections may induce the noncanonical translation of micropeptides, some of which could function as neoantigens that escape immune tolerance in T1D. The aim of this study is to investigate how CVB1 infection alters the RNA translation landscape in pancreatic beta cells and its potential role in neoantigen generation.

A pancreatic beta cell model (EndoC-βH1) infected with CVB1 was analyzed in biological triplicates against controls. To characterize virus-induced changes in RNA translation, we performed ribosome profiling (Ribo-seq), enabling a global analysis of actively translated RNAs. Using multiple bioinformatic approaches, we focused on detecting open reading frames (ORFs) with potential translation derived from noncoding regions and compared them between conditions.

Ribo-seq analysis reveals that CVB1 infection induces increased translation of genes involved in the splicing machinery, chromatin remodeling, and activation of TP53-associated pathways. Moreover, we detect active translation across a wide variety of ORFs annotated as non-coding, including lncRNAs. Among the most strongly differentially translated lncRNAs, relevant candidates are identified, including *POLG-DT*, previously identified in beta cells as a potential coding lncRNA after exposure to a synthetic analog of viral dsRNA. Our results highlight the impact of CVB1 infection on the translatome of beta cells.

Taken together, these preliminary findings remark the importance of investigating the non-coding genome in the context of Coxsackievirus infection. Our study proposes a novel mechanism by which viral infections may generate neoantigens derived from noncoding regions, providing new insights into the initiation of autoimmune diseases and the role of viral infections in conditions such as T1D.