# BORATORY MANUAL

RIO • ARES • HANNON • NILSEN

# Non-coding RNA Biology

**Genomica Funzionale** 

AA 2025/2026

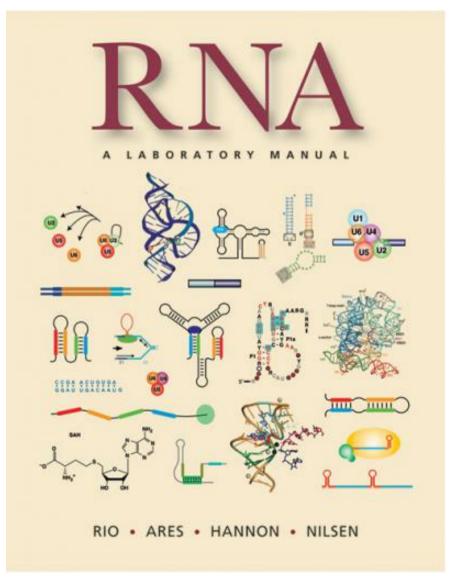
## **Prof. Schoeftner**

(Docente di riferimento, BIOS-6A Incontro con docente: preavviso per mail - online)

MS TEAM CODE: 863n8kv

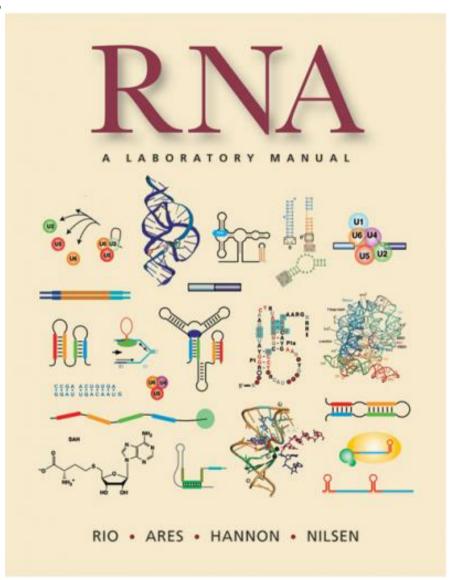
### **Program**

- 1. Overview on research focus of the Laboratory for non-coding RNA and genome stability
- 2. Organisatorial aspects of lecture "ncRNA Biology"
- 3. Goal of the lecture: "ncRNA Biology"



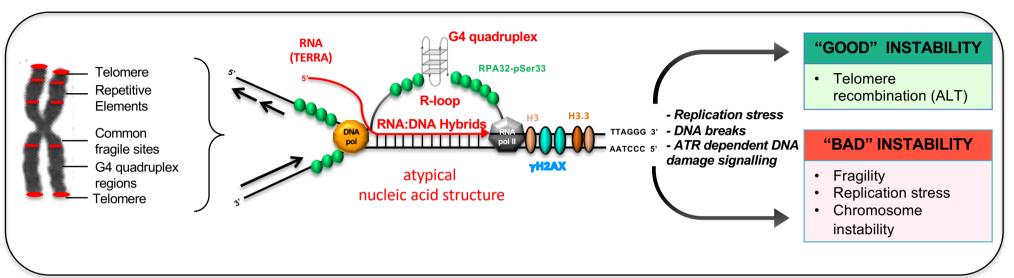
### **Program**

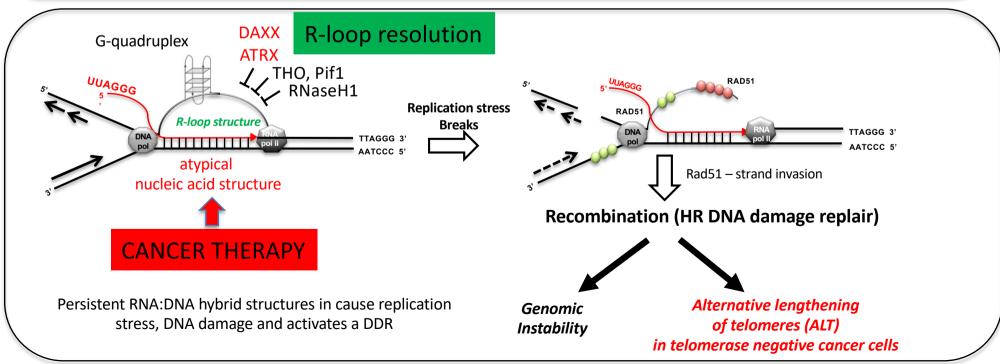
- 1. Overview on research focus of the Laboratory for non-coding RNA and genome stability (master thesis position(s) available)
- 2. Organisatorial aspects of lecture "ncRNA Biology"
- 3. Goal of the lecture: "ncRNA Biology"



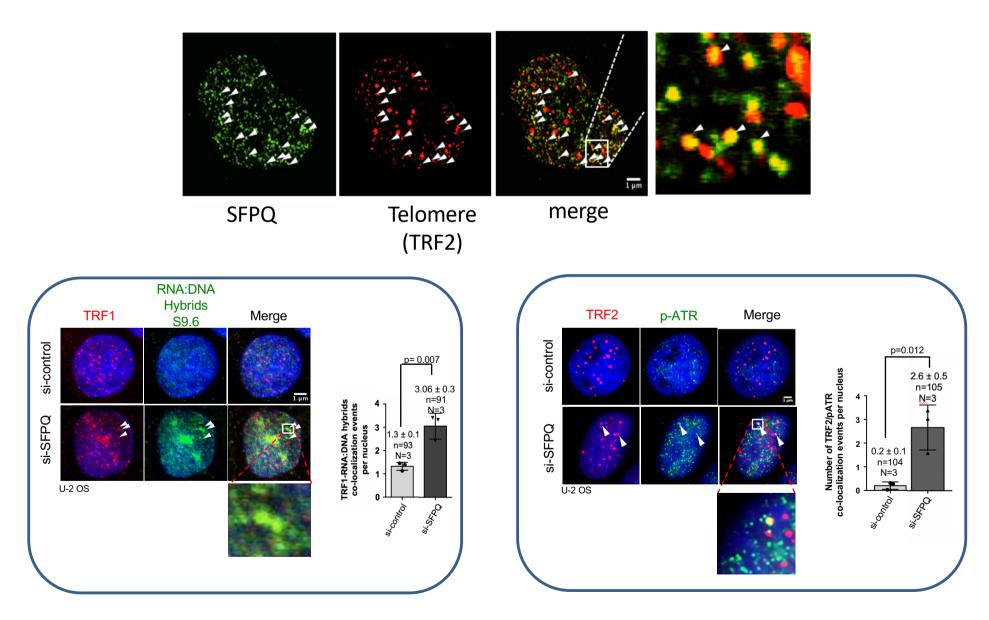
### Laboratory for non-coding RNA and genome stability

### 1. Control of genome stability by RNA:DNA hybrid management machineries

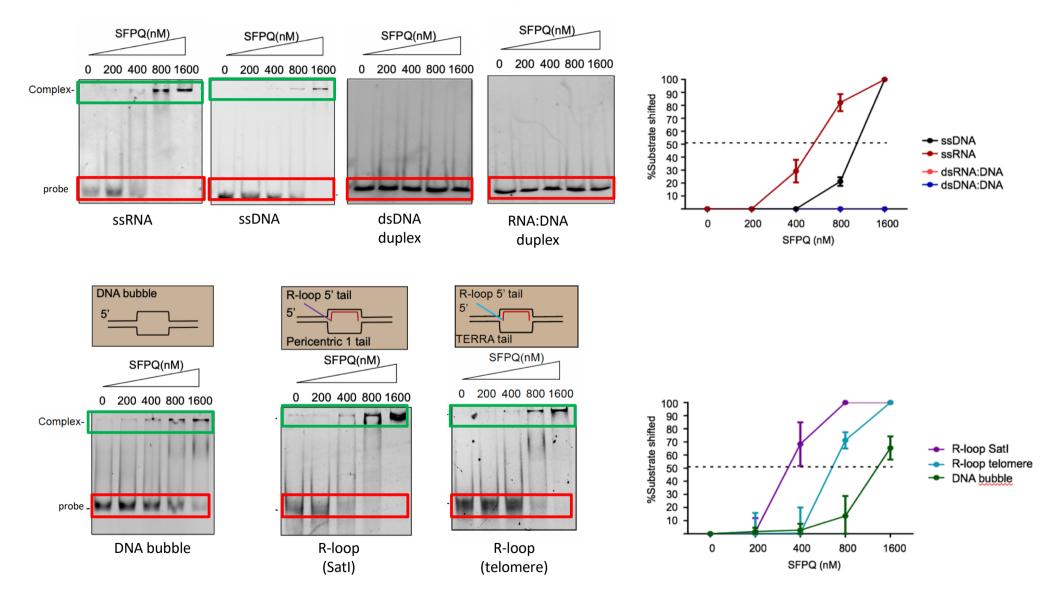




### SFPQ is associatated with telomeres to suppress R-loop formation

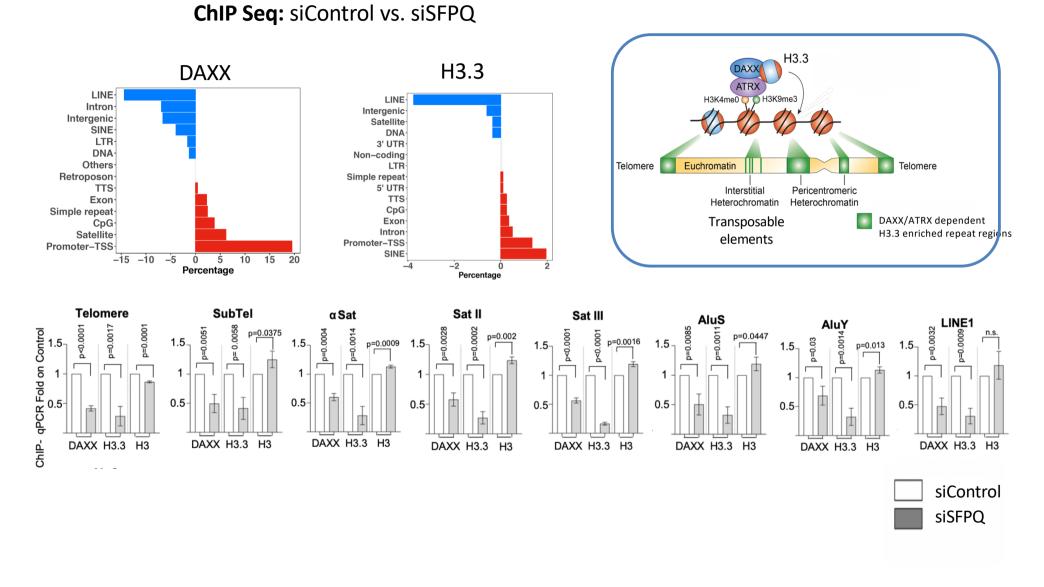


### SFPQ shows prefered binding to R-loop structures in vitro



Does SFPQ interact with R-loop resolution machineries?

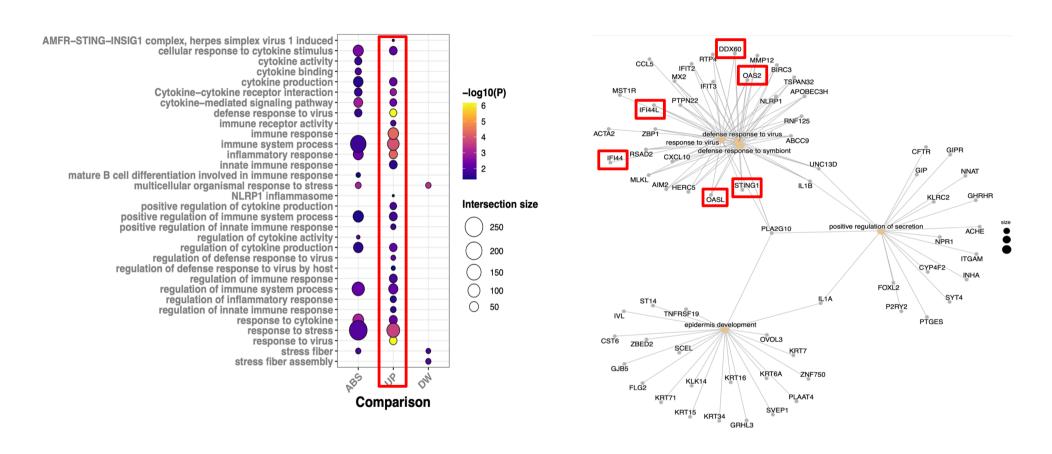
### SFPQ recruits DAXX dipendent H3.3 chaperon activity to repetitive elements



What about replication stress and DNA damage?

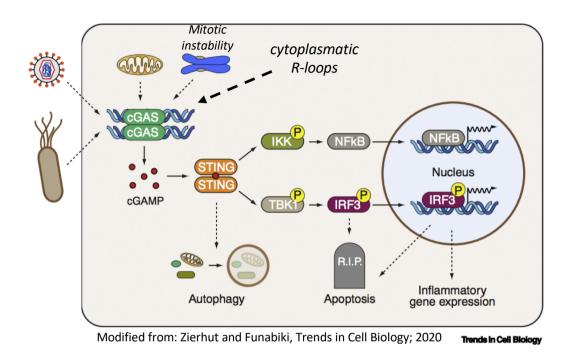
### SFPQ suppresses innate immunity pathways

RNASeq: siCon vs. siSFPQ



Does loss of SFPQ promote the formation of cytoplasmatic DNA and activation of the cGAS/STING pathway?

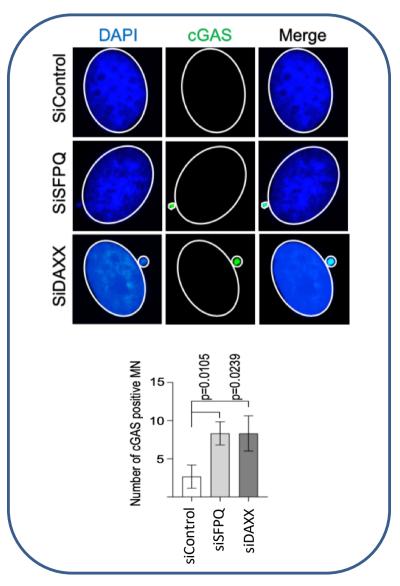
### Loss of SFPQ promote micronuclei formation and activation of cGAS



- Tumor-suppressive effect (Therapy)
- Tumor-promoting effect

**MODIFICATION OF THE TUMORMICROENVIRONMENT??** 

**REGULATION OF SFPQ (LLPS, PTMs)??** 









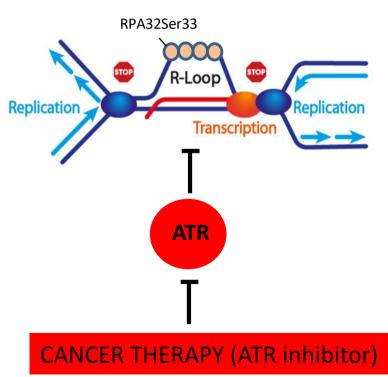




### 2. R-loops and cancer therapy

### ANTI-NEOPLASTIC DRUGS DRIVE R-LOOP FORMATION

### A ROLE IN THERAPY AND RESISTANC TO THERAPY?



### **Goal of the project:**

- 1. Relevance of R-loops in cancer therapies and therapy resistance focussing on ATRi
- 2. Identify drugs that boost R-loops to re-sensitizes cancer cells to ATRi therapy
- 3. Identify and evaluate potent R-loop forming RNAs
- 4. Explore the modification of TME mediated via genome instability

Modified from: Rinaldi et al. 2021 Frontiers in Cell and Developmental Biology



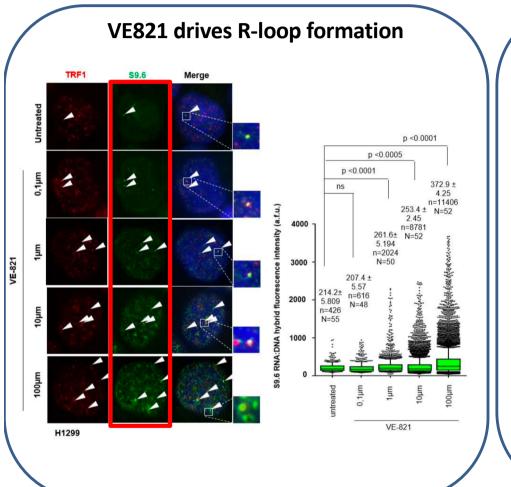


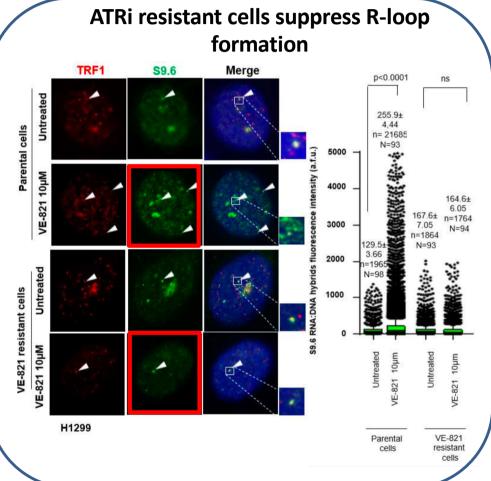






### Cells with adaptive resistance to ATRi activate pathways to suppress R-loops





Modified from: Rinaldi et al. 2021 Frontiers in Cell and Developmental Biology

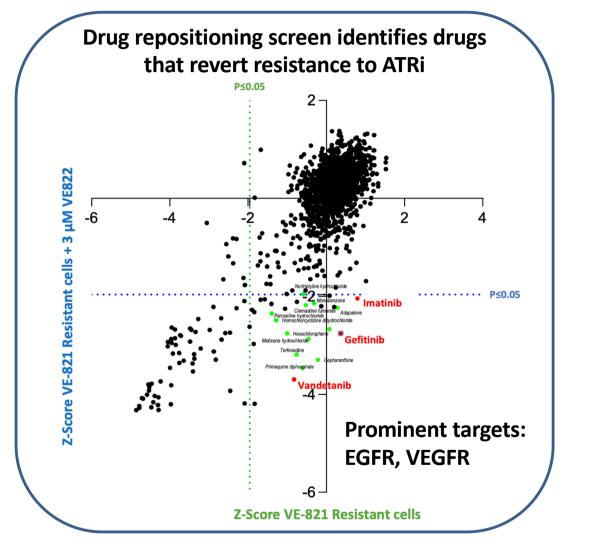


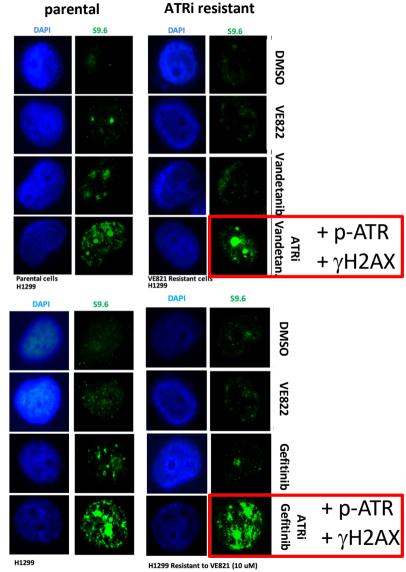




















### **FUTURE OUTLOOK:**

- DRIP seq under repositioning conditions to map R-loop landscape in resensitizised cells
- Identify RNAs that engage in R-loop formation to drive damage
- Characterization of singalling pathways that connect Vandetanib/Gefitinib to R-loop resolution machineries
- Investigate impact on pathways that imact on the TME: main target cGAS/STING

R-loop-derived cytoplasmic RNA–DNA hybrids activate an immune response		
https://doi.org/10.1038/s41586-022-05545-9	Magdalena P. Crossley <sup>1,8</sup> , Chenlin Song <sup>1,8</sup> , Michael J. Bocek <sup>1</sup> , Jun-Hyuk Choi <sup>1,6,7</sup> ,	
Received: 30 June 2021	<ul> <li>Joseph Kousorous<sup>1</sup>, Ataya Sathirachinda<sup>1</sup>, Cindy Lin<sup>2,3,4</sup>, Joshua R. Brickner<sup>1</sup>, Gongsh</li> <li>Hannes Lans<sup>5</sup>, Wim Vermeulen<sup>5</sup>, Monther Abu-Remaileh<sup>2,3,4</sup> &amp; Karlene A. Cimprich<sup>1</sup></li> </ul>	
Accepted: 8 November 2022		

### Eventi organizzato per le studentesse e gli studenti del DSV

### 15.10.2025, mattina: Caffè delle Matricole – Evento del Dipartimento di Scienze della Vita (DSV)

- Lezioni cancellati
- Aperto a tutte le studentesse e tutti gli studenti
- Funzionamento della Segretaria didattica
- Presentazione "Sistema Ricerca Trieste"
- Sistema Innovazione FVG (SiS FVG)
- Incontro Aziende (Biomed, Psicologia, Ambiente)

### 12.11.2025, pomeriggio: Fiera dell DSV

- Presentazione progetti di ricerca
- Poster session preparato da studenti di dottorato e assegnisti del DSV

### 18.12.2025, mattina: AIRC – Lecture – Fabrizio d'Adda di Fagagna, IFOM Milano (organizzato dal CdS in Genomica Funzionale)

- Lezione
- Round table discussion con studentesse e studenti su tematiche (emerging topics in biomedical research, develoment of competences, percorsi professionali nella ricerca biomedica; national/international curriculum

### Venerdì, una vota per mese: Cheers to Science, organizzato dagli studenti delle scuole di dottorato del DSV

- Incontro tra studenti, assegnisti, colaboratori e docenti del DSV (Ed M, Aula Magna)
- Presentazioni scientific ambiente Biomed, Psicologia, Ambiente
- Social get together

### **Events organized for DSV students**

### 1. October 15, 2025 – Morning: Coffe delle Matricolo: Event organized by the Department of Life Sciences (DSV)

- Classes canceled
- Open to all students
- •Didactic Secretariat will be operational
- Presentation: "Trieste Research System"
- •FVG Innovation System (SiS FVG)
- •Meeting with companies (Biomedicine, Psychology, Environment)

### 2. November 12, 2025 - Afternoon: DSV Fair

- Presentation of research projects
- Poster session prepared by PhD students and DSV fellows

### 3. December 18, 2025 - Morning: AIRC Lecture Speaker: Fabrizio d'Adda di Fagagna, IFOM Milan

(Organized by the Degree Program in Functional Genomics)

- •Lecture
- •Round table discussion with students on:
- Emerging topics in biomedical research
- •Skills development
- •Career paths in biomedical research
- National/international academic paths

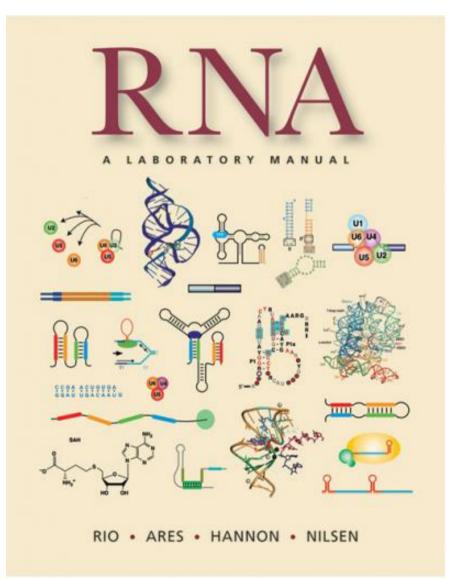
### 4. One Friday a month: Cheers to Science

(Organized by PhD students from DSV schools)

- •Meeting between students, fellows, collaborators, and faculty (Ed M., Aula Magna)
- •Scientific presentations in:
- •Biomedicine
- Psychology
- Environment
- Social gathering

### **Program**

- 1. Overview on research focus of the Laboratory for non-coding RNA and genome stability
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### ncRNA Biology - AA2024-2025



### **PART 1: Classic Lectures**

### **PART 2: Student seminar presentations**

Starting on the selection of a high profile paper students will elaborate in **teamwork** (3 collegues) a scientific seminar in english language:

- Introduction on background and relevance
- Presentation of scientific study in professional manner
- Discussion and future outlook
- QUESTIONS FROM COLLEGUES
- Totale time: 70-80 min

# SUPPORTING MATERIAL FOR THE LECTURES AND SEMINAR ACTIVITY WILL BE PROVIDED ON TEAMS

- Team code: 863n8kv

- Lecture slides, seminar material, recordings
- Other supporting material

# ncRNA Biology Schedule lectures – Seminars – AA 2025/2026

WEDNESDAY Building C9, Aula A; 10:00 – 12:00 THURSDAY Building D, Aula 3B; 12:00 – 14:00 FRIDAY Building C1, Aula I; 11:00 – 13:00

PPT SLIDES:
MS TEAMS

Teams code: 863n8kv

Prof. Stefan Schoeftner E-mail: sschoeftner@units.it

I only reply to official students' emails: @units.it (no @gmail; @libero....)

### ncRNA Biology - AA2025-2026



### **Lecture Program:**

- 3 lectures per week (3x2 hours)

24.09.2025

25.09.2025

- Lecture break for ERASMUS week and seminar preparation

Lecture 1

Lecture 2

Draft
schedule

26.09.2025	Lecture 3
01.10.2025	Lecture 4
02.10.2025	Lecture 5
03.10.2025	Lecture 6
08.10.2025	Lecture 7
09.10.2025	Lecture 8
10.10.2025	Lecture 9
15.10.2025	caffe delle matricole
16.10.2025	Lecture 10
17.10.2025	Lecture 11
22.10.2024	ERASMUS WEEK
23.10.2024	ERASMUS WEEK
24.10.2024	ERASMUS WEEK
29.10.2025	Lecture break
30.10.2025	Lecture break
31.10.2025	Lecture break
05.11.2025	Lecture break
06.11.2025	Lecture break
07.11.2025	Lecture break
12.11.2025	START WITH SEMINARS

### **Student seminar presentations**

- 2 seminars per week
- ca. 13 seminar presentations
- Until 19.12.2025
- Presence at seminars is compulsory

1	BALZAN	RICCARDO
2	BARBIERI	LETIZIA
3	BARDELLOTTO	AMANDA LUCIA
4	BODINI	DANIELE
5	BOUJMIL	SELMA
6	CANTONE	SAMUELE
7	CEBIN	RICCARDO
8	COLLINA	FABIO
9	COSTELLA	NORA
10	DURANTE	TOMMASO
11	FRANZIN	SARA
12	GHEORGHITA	MARIA ELENA
13	KLINGER	MAXIMILIAN
14	KUZMANOVIC	STEFANIJA
15	MANCINELLI	CLAUDIA
16	MARCIANO	VALENTINA
17	MION	CHIARA
18	MURRU	LORENZO
19	RAVALICO	PETRA
20	SANA	ALICE MARIA
21	SCHETTINO	MICAELA
22	TARANTINO	LORENZO
23	ZAMBONI	MATTEO
24	ZAT	ALICE
25	QUINET	Eléa
26	<b>EVERTS</b>	Lara
27	HYARDET	Anne-Lou

### ncRNA Biology

### **My Lectures:**

- 1. Introduction Non coding RNA revolution, classification of ncRNA classes
- 2. IncRNA in Physiology:
- Introduction IncRNAs
- Pseudogene derived IncRNAs
- Pseudogene IncRNA function: *mOct4P4* IncRNA and ancestral gene regulation
- 3. Controlling the action of miRNAs (ceRNAs)
- miRNA biogenesis review and practical advice
- ceRNAs in cancer
- circular RNAs (circRNAs)
- endosiRNAs in stem cell biology
- 4. IncRNA and the control of epigenetics in disease
- 5. Control of regulatory elements by ncRNAs
- eRNAs and enhancer control
- 6. DNA Damage and ncRNA
- DNA damage response RNAs

### STUDENTS' PPT PRESENTATION

### Your seminars: 60-70min:

- **1. First part of seminar** (max. 20 min): general introduction into the topic using the selected reviews (**10-15 powerpoint slides**).
- 2. Second part of the seminar (ca. 40 min): Students present the results of a key scientific publication on the topic (25- 35 powerpoint slides)

Publications to be selected are available on MSTeams

- 3. Third part (max. 5 min): Integrative model of research paper, put into a larger contex (1-2 powerpoint slides)
- 4. Fourth part of the seminar (min. 15 min): Discussion: question by collegues. Important: 3 other student groups have to prepare at least 3 questions for the discussion (Total 9 questions + questions from other students)
- 5. The use of artifical intelligence tools for the designing the introduction and analysing additional research papers is encouraged (For example »Solve any biology problem»: <a href="https://bgpt.pro/">https://bgpt.pro/</a>) <a href="mailto:Indicate what AI tools used and for what tasks AI was used">https://bgpt.pro/</a>) <a href="Indicate what AI tools used and for what tasks AI was used">Indicate what AI tools used and for what tasks AI was used</a>

### **IMPORTANT:**

Seminars need to be prepared in collaboration – not as seperate fragments put together

Students of a group needs to cover the same amount of presentation time

Note: The use of artifical intelligence tools for the designing the introduction and analysing additional research papers is encouraged. For example »Solve any biology problem»: https://bgpt.pro/

PS: an example of a good seminar ppt will be available online on MSTeams

### ncRNA Biology - SEMINARS

### **HOW TO CHOOSE A TOPIC – AND HOW TO START**

Topics: Recent research papers are available on MSTeams
Check the excel file for main research fields

- **Step 1**: Students together with class representatives to provide a final list with all students and their e-mail (@units.it) that will attend the lecture AA 2025 2026 until **MONDAY 29.09.2025**
- **Step 2:** Students form groups and choose research papers and seminar slot until **FRIDAY 03.10.2025.** Send program per e-mail to me. Program will be published on Moodle together with assigned discussion groups. Folders for all group seminars will be deposited on Moodle.
- **Step 3:** Student groups select **2 appropriate reviews with high impact journals** on the respective topic and upload the files to Moodle until **WEDNESDAY 08.10.2025**
- **Step 4**: Prof. will confirm/reject the chosen reviews per e-mail
- Students start to prepare presentations and also READ ALL THE OTHER PAPERS IN THE WEEKS
   WITHOUT LECTURES
- ASSISTANCE WITH SEMINAR PREPARATION: DRAFT OF PRESENTATION WILL BE DISCUSSED WITH ME
   1 WEEK BEFORE ACTUAL SEMINAR: check the schedule online for meetings and deadlines

### ncRNA Biology - SEMINARS

### Some comments on the use of AI for preparation of scientific data to an audience of experts

All is used as a tool for out work but its the **presenter's responsability to provide audience with <u>correct</u> information** 

Al tools can help to identifie key points of a topic

Al tools can help to screen a higher number of paper and allow to efficiently sumerize the information and relevanct

Al tools can help you to improve scientific language and make a text more efficient

Al tools can help you creating images or graphs

Al tools can help get fast information on details on a method/topic/basic knowledge

....BUT: AI tools may use sources that can also not be trusted

....The professional use of AI requires the validation of obtained infromation (open link to reference information, read related reviews or papers)

### **SYNERGY TASK:**

Students can search and test AI tools and integrate the tools into the work of seminar preparetion At end of lecture: EXCHANGE OF EXPERIENCES BETWEEN COLLEGUES

### ncRNA Biology – STUDENT SEMINARS

### Topics for student seminar presentations – Already on moolde

	IncRNA aand genome organization		
T1	Genome organization around nuclear speckles drives mRNA splicing efficiency		
T1	Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments		
	Enhancer RNAs		
T2	Identification of a SNAI1 enhancer RNA that drives cancer cell plasticity		
T2	Long non-coding RNAs direct the SWI/SNF complex to cell type-specific enhancers		
	LINE - SINE		
Т3	LINE-1 regulates cortical development by acting as long non-coding RNAs		
T3	SINEUP long non-coding RNA acts via PTBP1 and HNRNPK to promote translational initiation assemblies		
	RNAbased immunity in bacteria		
T4	Retron-Eco1 assembles NAD+-hydrolyzing filaments that provide immunity against bacteriophages		
T4	Widespread RNA-based cas regulation monitors crRNA abundance and anti-CRISPR proteins		
	Circular RNAs		
T5	Exosomal circLPAR1 functions in colorectal cancer diagnosis and tumorigenesis through suppressing BRD4 via METTL3-eIF3h interaction		
T5	Circular RNAs drive oncogenic chromosomal translocations within the MLL recombinome in leukemia		
	Functional screenings on IncRNAs		
Т6	Transcriptome-scale RNA-targeting CRISPR screens reveal essential IncRNAs in human cells		
Т6	CRISPR-Cas13d screens identify KILR, a breast cancer risk-associated IncRNA that regulates DNA replication and repair		
Т6	CRISPRi screens identify the IncRNA, LOUP, as a multifunctional locus regulating macrophage differentiation and inflammatory signaling		
	Circulating miRNAs and anti-virus activity		
<b>T7</b>	Adipose-derived circulating miRNAs regulate gene expression in other tissues		
<b>T7</b>	An isoform of Dicer protects mammalian stem cells against multiple RNA viruses		
	Micropeptides		
T8	Micropeptide CIP2A-BP encoded by LINC00665 inhibits triple-negative breast cancer progression		
	X-inactivation		
Т9	Targeting Xist with compounds that disrupt RNA structure and X inactivation		
Т9	XIST directly regulates X-linked and autosomal genes in naive human pluripotent cells		
Т9	Xist spatially amplifies SHARP/SPEN recruitment to balance chromosome-wide silencing and specificity to the X chromosome		

### ncRNA Biology - EXAM - SEMINAR EVALUATION

### **Evaluation seminar (for entire group, max. 16 points):**

- Introduction: clearness, biological/scientific importance, quality of slides; prepares colleagues for scientific paper?
- Research paper: important data shown, quality of slides, clear structure of data presentation (Why?, How?, Result? Conclusion?)
- Summary and Outlook: quality of summary and conclusion, explanation or relevance, future outlook
- Discussion session: ability to answer questions; flexibility (all group members should give answers)
- Overall level of preparation for the seminar and motivation

Note: Quality of English will be not evaluated

### Oral exam (evaluation: max. 15 points):

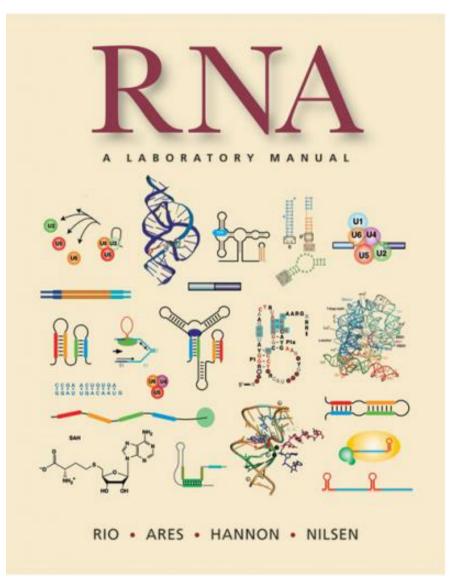
- An inscription into an "Appello" on Esse3 is necessary to perform the oral exam, participation only after completed seminar presentation
- → 1 question about own seminar presented
- → 1 question on seminar of colleagues
- → 1 question of Prof's lectures
- → Students need to show general knowledge on individual topics and discuss experimental approaches on how to address a particular problem related to the topics (scientific question experimental approach chosen result interpretation
- → Duration: 20-30 min per exam
- →Books, electronic devises or scripts are not allowed during the exam.

### Final grade (voto finale): max 31 = 30L

- Points Student's lecture + Points oral exam

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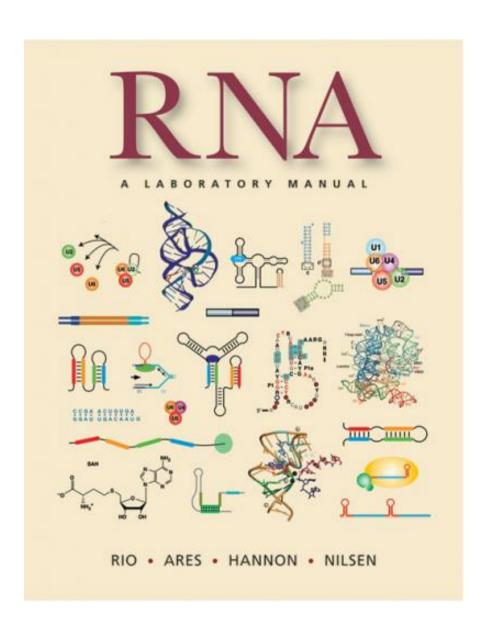
### WHAT IS YOUR EXPECTION ON THE LECTURE....

### WHAT DO YOU THINK YOU SHOULD LEARN....

Form groups, discuss 5-7 minutes, individuate 2-3 of your goals

Choose speaker of group

Present result



### WHAT IS MY EXPECTION ON THE LECTURE....

### WHAT I THINK YOU SHOULD LEARN....

General knowledge on ncRNA biology (classic lecture)

A joint elaboration of key topics in ncRNA biology focussing on basic research

Group seminar presentations top publications on modern topics

- 1. General overview on ncRNA function in development and diseases
- 2. Learning to extract general information on a larger field of ncRNA research
- 3. Learning to analyze-understand scientific data from a publication ("Journal club"); identify scientific question, understand experiments and interpret the data, make conclusions
- 4. Reliable use of AI for literature analysis and seminar presentation
- 5. Prepare a scientific talk (seminar)
- 6. Presenting most important information to an audiance in a comprehensive manner
- 7. DISCUSS DATA WITH AUDIENCE
- 8. Getting prepared for Master thesis reality
- 9. Get used to apply scientific language
- → Read the paper of your colleagues!!!
- → Participate in the discussion
- → BE PRESENT AT YOUR COLLEGUES' SEMINARS

FINAL GOAL: USE SCIENTIFIC PAPERS AS CASE STUDY TO OBTAIN GENERAL EXPERTISE