Meeting attendance link

Time	Host	Speaker 1	Title of Talk	Speaker 2	Title of Talk
March, 3rd, 2021	Sheng	Zhen Chen	Lab work overview		
April, 7th, 2021	Irene Wen	Yaron	Lab work overview		
May, 5th, 2021	Irene Wen	He Fang (Christine lab)	Lab work overview		
June, 2nd, 2021			4DN Phase2 collaborative projects discussion		
July 7th 2021	Huaiying	Sheng	Lab work overview		
August 4th 2021	Sheng	Huaiying	Lab work overview		
Sep 1 2021	Irene Wen	Wenbo Li	Lab work overview		
Oct 2021	Huaiying	all	Collaboration project discussion		
Nov 3 2021	Wenbo	all	Detailed plan on collaborative project		
Dec 1 2021	Huaying	All	More detailed plan on collaborative project		
Jan 5 2022	Sheng	Discussion			
Feb 2022	Wenbo	Xinzhao / Sheng			Shared mission: RNA vs. Technology
Mar 2022	Huaiying	Discussion	Collaborative		

			project		
April 2022	Yaron				
June 2022	Xinxian		Collaboration projects on RNA in 4DN	1-slide presentation on lab work related to RNA in 4DN from each group	
October 2022			Discuss RNA IG's presentation in annual meeting		

Date: 7/5/2023, 10PST, 1EST - HOST: Christine Disteche
(Zoom recording link)

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Date: 6/1/2022, 10PST, 1EST - HOST: Xinxian Deng
One-slide presentation per group on potential projects of RNA 4DN

Disteche/Deng: 2D linear (NPCs, cardiomyocytes) and 3D (cortical organoids) differentiation systems from human iPSCs (WTC11, additional normal XY and XX lines).

Mouse embryonic development system (F1 hybrid embryos every 24h from E6-14 and Lmna mutants), in vivo study for RNA in 4DN

Sheng: iMARCI to identify the location of RNAs (caRNA domains); caRNA domains cover 1-10Mb in three cell lines (H1, HFF, K562) and are associated with highly transcribed RNAs; there are cell-line specific and common caRNA domains; weak association between caRNA domains and TADs

This tool can be used to study the role of caRNA during differentiation/development.

HuaiYing; imaging with RNA FISH to study repetitive RNA localization and dynamics; 20% TERRA clusters with telomeres while the other 80% somewhere else; TERRA colocalizes with LSD1; siRNA knockdown deplete TERRA and localization of LSD1; TERRA in WTC unknown; can be applied to other repetitive RNAs, e.g. satellite RNAs

Wenbo: enhancer RNAs (eRNAs) in gene regulation; interplays between RNA sequence, RNA methylation and secondary structure; molecular mechanisms of eRNA in cancer and neurodevelopment (NPCs, i3N neurons from WTC11; cortical organoids); m6A of eRNAs interacts with RNPs to mediate condensation

Yaron: imaging with RNA FISH to map the location of mRNAs and lncRNAs; mRNA compaction for transportation via nuclear pores (5' end regions of mRNA exist first); MKI67, high-level mRNAs co-exist via nuclear pores?; large foci of RNA

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Date: 4/6/2022, 10PST, 1EST - HOST: Yaron Shav-Tal
Discussion of common project

Decide on 30 min meetings from now on but can be flexible if needed.

<u>Cell line</u>:
Xinxian: has grown WTC11 on plastic and Xist FISH. Grow on a monolayer and no MEF needed.

Christine: WTC11 knows of experiments on FISH. Probably will be used in the new imaging working group. H1, H9 are more comparable.

Huaiying: DNA FISH on WTC11

Wenbo: not much omics data out there. H1 has much more.

Yaron: Don't want feeder cells for imaging.

Sheng: 4D standard needs to be feeder free.

Yaron: <u>In conclusion, probably WTC11 will be first choice</u>, and then H1, H9.

<u>Differentiation</u>:
Xinxian - Allen Inst make cardiomyocytes, NPCs, cortical organoids. Sheng - NPCs would be good for the next 4DN second phase/mission.
Wenbo - each lab can focus on a certain differentiation direction and then omics can be shared as well as protocols.
Xinxian, Christine: study of titin a highly spliced large gene during differentiation.

Huaiying: What would be the first step? Move step-wise- undifferentiated, early and late differentiation.

Wenbo: uses a differentiation-accelerated cell line to neurons. Probably best to use the 'regular' cell line.
Yaron: Maybe we can get protocol from some other lab that uses the cells regularly.
Xinxian: Need the optimized cell line.
Yaron: Where to get them from - Coriell.
Sheng - difficult to obtain. Try using OH for help.

https://data.4dnucleome.org/biosources/4DNSR7GFFYBK/
Information about WTC11

Cardiomyocytes protocol: https://www.allencell.org/sops.html

Goal for now: get the cells.

Sheng: using Nature Protocols for organoids by H1 cells

Ask Suzzane for protocol and can discuss if there are common interests or plans. Tag RBPs…

Next meeting: May- we will be at meetings so will meet next for 1 hr on June 1st.

Think of RBPs that we would like to use. Each lab to think of an idea to present on one slide of what they wish to do and with help of others. For example, CTCF or HP1.

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Date: 3/2/2022, 10PST, 1EST - HOST: Huaiying Zhang
Discussion of common project

Christine and Sheng reported on the 4DN common project discussion they attended: there was a strong emphasis on brain and development, and there was also interest in RNA.
Thoru suggested that maybe a project focused on genome changes during antibody diversity could be highly relevant to development and RNA analyses, and could play a role in the common project.

We discussed an RNA interest group common project that could be integrated to the greater common goal focused on development: i.e. rules governing RNA localization in the nucleus during differentiation of a cell line and embryonic development.

Christine stressed the importance of including both male and female cell lines/ tissues in this project.

Discussion of cell lines that can be differentiated to model developmental changes:

1. **iPSCs**
   WTC11: iPSC, male, advantages: well studies, tons of **data on nuclear proteins location** by imaging at the Allen Institute.
   Female iPSC: the Allen Institute may derive a female iPS cell line, but we should not wait. Other iPSCs could be used but need to be monitored for X inactivation erosion.

2. **ESCs**
   Rues2: female ESC, can differentiate, available at WiCell. Xinxian will check on data obtained on this line.
   Human H1 or H9: male and female ES cells. Xinxian noted that they can be differentiated, have data from phase I and ENCODE, but are derived from later embryonic stages though. However, **ESCs are not easy to image.**

We concluded that we can start with **WTC11** and then move to other systems: e.g. male and female mouse embryo tissues for in vivo studies, once we have integrated RNA data on WTC11. This would work well with the new emphasis defined by the 4DN II common project on development.

We discussed the **scheduling of meetings** and Qiuyang will resend a new poll with earlier times, 8-11 am to accommodate overseas participants.

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Date: 2/2/2022, 10PST, 1EST - HOST: Wenbo Li
Xingzhao Wen (3rd Year student from Sheng Zhong lab)

Xing Zhao presented for 45mins on her work that examines chromatin RNA landscapes and features using iMARGI data.

This is followed by a lot of discussion.

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Date: 1/2/2022, 10PST, 1EST - HOST: Sheng Zhong
Discuss collaborative research ideas further.

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Date: 12/01/2021, 10PST, 1EST - HOST: Huaiying Zhang
Discuss collaborative research ideas in detail.

1.Impacts of RNA processing, RNA sequence, and all other nuclear components to the 3D spatial organization of RNA.
Rules governing RNA localization in the nucleus:
Comparison (what?) between imaging and omics data (locus-focused comparison?
Benchmark and functional study of caRNAs in the same cell line.
Cell line: K562 (female), already data, suspension, not good for imaging.

U2OS, good for imaging,

WTC11, lots of 4DN data, Allen Inst have cell lines. Too bad it's male. Can be expanded to differentiation, organoid (winner for now)

IMR90, female, 4DN tier 1 (also good)

Loci: lncRNA, enhancer RNA,

Omics:

Imaging: single molecular RNA FISH targeting different regions (40 probe per RNA), 10-100RNA. Spatial resolution: 100nm. Live with MS2 low throughput. RNA paint:

Perturbation: cell cycle, development**,**

RNA relevant localization to DNA and nuclear body, Different variant of RNA location (chromatin-association or nucleus vs cytoplasm) and function.

1/5/2022: Inclusion of mouse tissue, ability to look at both alleles. F121-9. Nominate WTC11. Splicing variants should be considered. Functionality beyond 1d RNA sequence. RNA recruiting protein complex. RBP. and proximity's role.

Initiate an RNA-technology contact matrix.

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Date: 11/3/2021, 10PST, 1EST - HOST: Wenbo Li
Discuss collaborative research ideas in details.

1. Standardizing / benchmarking caRNA.

a. Definition (caRNAs? Nascent RNAs? Chromatin-proximal RNAs?),

HUAYING: there can be RNAs not associated with chromatin but regulate chromatin architecture

Christine: Xist - an example of a RNA acting via assembly of protein complexes

Sheng: As an example of Huaiying's case: Nuclear mesh is a component with proteins and RNAs.

b. Methods of RNA detection and their differences
   --- (RNA-seq, chromatin isolation RNA-Seq, iMARGE etc., TT-Seq, PRO-Seq, mNET-Seq etc.)
   --- Comparison between imaging and omics data (locus-focused comparison?)

   Xinxiang: features of RNAs? RNA secondary structures, and/or chemical modifications.

c. Origin and biogenesis.
d. Variation of RNA expression across cell types, development, disease (environmental responses), species.

   A selected differentiation system - H1 to NPCs?

   A selected disease system - senescence? Or ?

e. Correlation with features of 3D nuclear/genome organization, including nuclear organelle and 3D genome structure.
f. Comparison between imaging and omics data (locus-focused comparison?)
g. Biological roles?

Priority: cell types to focus. H1, HFF, WTC-11, K562 (female), HEK293T, Endoderm, GM12828 (female), mouse ES

Some select loci to focus? Especially for imaging and biological roles etc.?

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Date: 10/6/2021, 10PST, 1EST - Huaiying Zhang
Discuss collaborative research ideas

Standardizing / benchmarking caRNA. Definition, origin and biogenesis, roles, methods, variation across cell types, development, disease (environmental responses), species. Impacts on 3D nuclear/genome organization, including nuclear organelle and 3D genome structure.

Priority: cell type. H1, HFF, WTC-11, K562 (female), HEK293T, Endoderm, GM12828 (female), mouse ES

Huaiying: 1. A review of how lncRNAs contribute to 3D nuclear organization.
2. Examples of specific RNA's role to the 3D nuclear organization with insight to principles. TERRA in **cancer** (also in **aging**).

3. Tools to **visualize** RNA and modulate RNA localization. How RNA contributes to **phase separation**, recruiting proteins, and bridge to chromatin?

Christine: X chromosome and **development**. Cell line and animal models on each allele. LINE repeats' role in X structure / silencing? RNA-chromatin interactions that potentially modulate epigenome?

Xinxian: mouse embryogenesis as a shared system. **Heart or brain development**.
LaminA and LaminB mutants. LncRNA's roles in heterochromatin formation / maintenance.

Zhen: disease process. Roles of enhancer transcribed caRNAs in inflammation in diabetes / aging.

Yaron: imaging of RNA, FISH, Ms2 system. Highly diverse nuclear RNA composition and deposition. Nuclear condensates and phase separation, nuclear organelles.

Wenbo: RNA modification mediated DNA-RNA chromatin organization **m6A Methylation** RNA MARGI? (e.g., Zhong lab, Chen lab)
•TERRA RNA RNP condensate and roles of m6A in there? (Huaiying)
•Repeats (LINE or ERV) RNAs, m6A on them, and 3D genome in development (Disteche and others)?

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Date: 9/1/2021, 10PST, 1EST - WENBO LI

Wenbo Li discussed his overall lab work.

Conclusion:
Next month may be a good time to discuss specific mission and collaborative projects for this RNA IG.

Some thoughts form Wenbo:

• RNA modification mediated DNA-RNA chromatin organization m6A Methylation RNA MARGI? (e.g., Zhong lab, Chen lab)

•TERRA RNA RNP condensate and roles of m6A in there? (Huaiying)

•Repeats (LINE or ERV) RNAs, m6A on them, and 3D genome in development (Disteche and others)?

•As a product for this 4DN RNA IG group – shall we write a joint PERSPECTIVE paper on RNAs and 4D Nucleome in the next year or two?C

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Date: 2/3/2021, 10PST, 1EST - the first meeting
Agenda:

1. **4DN Phase II, RNA Interest Group (RIG)**
   Topic statement: RNA's roles in nuclear organization. Chromatin-associated RNA and their roles in genome organization and gene regulation.

Overall interests of each lab that come to today's meeting:
Sheng Zhong: Roles of caRNAs in nuclear organization and gene regulation.

Wenbo: RNA's roles in gene transcription regulation and human diseases - e.g. cancer and neurodevelopment.

Rene: Main focus in the lab is a model of human pluripotent stem cell differentiation to endodermal derivatives. Hoping to learn more about caRNAs and roles in regulating stem cell differentiation and cellular state.

Yaron: Single molecule RNA dynamics with relevance to nuclear speckle formation and gene expression and RNA transport

Christine: X inactivation, FIRRE, polycomb related gene regulation

Huaiying: RNA mediated nuclear condensates; Telomere elongation in cancer.

Zhen: role of caRNAs in endothelial dysfunction in metabolic and cardiovascular disease

NOTE: The overall mission of interest group is to exchange ideas and expertise, and therefore, there are not very well-defined deliverables (this differs from working groups).

Current Co-chairs: Sheng Zhong, Wenbo Li, Huaiying Zhang, Xingzhao (Irene) Wen
*** Can be more dynamic. Trainees encouraged to take roles here.

Current Members (by 02-03-2021): Thoru Pederson, Yvonne Fondufe-Mittendorf, Jian Ma, Ying Liu, Xinxian Deng, Zhijun Duan, Yaron Shav-Tal, Jenn Phillips-Cremins, Yue Yang, Soo Lee, Rene Maehr.
*** Can be more dynamic. More trainees are encouraged to join.

2. Logistics

Leadership: Anyone else interested to be co-chairs?
Irene Wen (a student trainee from Sheng Zhong lab) volunteered to be a co-chair.

NOTES / questions:
Rene: What are the responsibilities for a co-chair (by Rene), typing and recording of the meetings, organizing meetings, or to invite other speakers, or to coordinate discussion with other interest/working groups.

Rene: Are we expected to submit any meeting minutes or progress reports etc.

Wenbo: Interest group is a new thing for 4DN phase II. So we may test run for a few times and see how things go.

Christine: 4DN encourages the formation of interest groups, which may be really helpful to forge new collaborations. Perhaps some sort of report (even not extremely detailed) to the 4DN is ultimately needed.

CONCLUSION: some responsibilities or exact duty of co-chairs still need to be unfolded as we move on.

Participation: Shall we include trainees as co-chairs or as only presenters in future Meetings? We should encourage trainees to be co-chair or presenters.

*** discussion needed. Vote: Yes.

Meeting time:
Meeting frequency: Shall we meet every month? Or every other month?

CONCLUSION: First Wednesday of the month, 10am PST / 1pm EST. Monthly meetings.

Huaiying: If we do it like a lab meeting (without institution boundary), then this can be done once a month.
Zhen: we can include trainees to talk about landmark papers or new techniques (not only just the primary ongoing work they are doing).
Rene: speak every other month (about own lab work), invite other speakers from 4DN (or present papers not in that lab). To impose less obligation on presentation frequency.
Christine: whoever we want to invite from outside of 4DN should be pre-approved. Meet every month might be demanding for some labs. Could adjust along the way.
Yaron: we will see how it goes, if one slot has no science, then good papers (like journal club), that is fine.
Wenbo: shall we try - (after the first 4-5 months of overview/introduction), in future, for one month we present unpublished new science form our labs, then the other month we can present a recent paper.

Additional thoughts about Meeting frequency: Shall we have some Joint Interest Group meeting (with the other interest groups e.g. repeats, or immunology etc. every two months?)?

Wenbo: i like this idea, but let us see how it goes.
Rene: this may happen by “invited speakers” from other groups.

Meeting duration: 1 hour?

*** discussion needed. Vote: Yes.

To ensure/maximize participation, any suggestions? We hope it did not start with enthusiasm, but ends with few participants.

*** DID NOT HAVE TIME TO DISCUSS.

Chances of joint training: can there be short term visit or joint work for a trainee?

*** DID NOT HAVE TIME TO DISCUSS.

Anything else?

3. Format of future RIG meetings.

*** Please add additional proposals (if any) as Proposal 2, Proposal 3 etc. ***

Proposal 1: Presentation formats: 2 of 20minutes short presentation, each followed by 10mins discussion? each from a different lab, like a virtual departmental workshop? Preliminary studies encouraged? “virtual lab meeting”? “how to foster collaboration, or joint-training opportunities for trainees”

*** discussion needed.

Zhen Chen: this can be more flexible, 20mins + 10mins or 40mins + 20mins. Discussion is really the key for this interest group.
Rene: unpublished work is encouraged.
Christine: keep the presentation short, longer discussion - less than 20mins presentation.

Proposal 2: Each meeting will have one trainee or PI present ongoing (or recently published) work. The presentation will be “lab meeting” style (QA both during and after presentation?) to encourage sharing of unpublished findings and informal exchange of ideas. As such, the presentation length should be under 30 minutes (?), to allow ample time for discussion.

*** discussion: For March to July 2021 meetings:
Sheng: what do people want to hear - Sheng wants to hear the overview of each lab.
Zhen: each PI gets 10mins to have a high-level overview of RNA-related work ongoing in the lab (3-5 slides).
Sheng: 10mins presentation + 10-20mins discussion. (Two presenters are more suitable).
Huaiying: may not be so much RNA focused, can be more broad.

*** Conclusion: the first few meetings will be for PIs to have some overview presentation of their ongoing work. So in 4-5 months all PIs can learn about each other’s work. This will also set up some examples for trainees to conduct future discussions.

ADDITIONAL INFORMATION:
OVERVIEW by 4DN on Working groups (WG) and Interest Groups (IGs):

Various working groups and interest groups are being formulated as part of the second phase of the 4D Nucleome Consortium. The purpose of this document is to solicit ideas from consortium members for what groups should be created, and to encourage members to sign up to lead or participate in the various groups.

Working groups are intended to meet regularly throughout the entire period of the project and are focused on primary science, policy, and logistical issues of interest to many consortium members. The working groups will regularly report back to the full consortium on the monthly 4DN call. Interest groups, in contrast, are less formal, self-organized and more dynamic. An interest group might be quite large or might be composed of just a few individuals focused on a particular question.

The 4DN Organizational Hub will provide logistical support for each working group and interest group, including maintaining a wiki page and corresponding Google Drive folder, setting up a Slack channel and email list for communication, maintaining an agenda/minutes/action items document, and tracking meeting dates on the Google Calendar.

Below is a list of proposed working groups and interest groups. Please add ideas for new groups, and add your name alongside groups you are interested in leading or participating in. Group leads will be responsible for scheduling meetings, setting meeting agendas, tracking attendance, recording minutes and action items during the meetings, and as necessary reporting back to the full consortium.

Note that non-PI members of 4DN are particularly encouraged to take on leadership roles in these groups. We will aim to include at least one non-PI as a co-leader of each group.

Minutes:

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Date: 3/3/2021, 10PST, 1EST - the Second meeting

Zhen Chen presented her past and ongoing work on lncRNAs in Endothelial cells. Nice talk and many stimulatory discussions. It turns out that one speaker per meeting is also good. We still plan to have two talks per meeting. We can test run a few more times to see.

There are some Zoom link issues this time that caused a few IG members not be able to join. Riccardo and OH have fixed these issues.

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Date: 4/7/2021, 10PST, 1EST - the third meeting
Yaron gave an overview of his lab research interest and shared many exciting results on nuclear speckle dynamics. He discussed several splicing factors and they affect on post-transcriptional RNA processing, speckle changes after viral infection and RNA nucleus export mechanisms.

There are many following questions and discussions. People agreed to have Christine's talk defer to the next meeting.

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Date: 5/5/2021, 10PST, 1EST - the fourth meeting
He Fang from Christine's lab talked about her work on lncRNA FIRRE and it's role in maintaining the H3K27me3 in Xi. 30min talk plus around 15-20min questions seem to fit one hour slots very well.