TEMPLATE FOR PROJECT OUTLINE – Due Oct 23

Student's name: Sebastian Howen Nesgaard Munk

Topic chosen: eRNA

SPECIFIC QUESTION:
How is eRNA involved in chromatin loop formation?

HYPOTHESIS:
eRNA contributes to chromatin loop formation by bridging structural factors and/or effector molecules between the enhancer and the target promoter to facilitate chromosomal loop formation.

EVIDENCE ON WHICH THE HYPOTHESIS IS BASED (INCLUDE REFERENCES):

Kim, Y. W., Lee, S., Yun, J., & Kim, A. (2015). Chromatin looping and eRNA transcription precede the transcriptional activation of gene in the β-globin locus. *Bioscience Reports*, *35*(2), 1–8. http://doi.org/10.1042/BSR20140126

Lam, M. T. Y., Li, W., Rosenfeld, M. G., & Glass, C. K. (2014). Enhancer RNAs and regulated transcriptional programs. *Trends in Biochemical Sciences*, *39*(4), 170–182. http://doi.org/10.1016/j.tibs.2014.02.007

PREDICTION(S):
If my hypothesis is right, it would be possible to isolate eRNA with bound structural looping factors known to interact with the enhancer and the promoter sequence in a chromatin loop. It could further be demonstrated that these eRNA-looping factors are necessary for loop formation by blocking/mutating the binding sites of the effector molecules involved in eRNA interaction.

EXPERIMENTAL APPROACH TO TEST PREDICTION (INCLUDE ANY DETAILS THAT YOU HAVE WORKED OUT SO FAR):

- Since eRNA production is required together with chromatin looping of the β-globin LCR and β-globin locus for transcriptional activation, the β-globin locus and its LCR are very well studied in cells derived from mice and would therefore appropriate for the experiment.
- The idea is to examine the structural proteins involved in chromatin loop formation of the β-globin (e.g. GATA1, TAL1, E2A, LMO2, and LDB1) in a mice cell line. Published results also

indicate that subunits of Mediator complex and cohesin could be of interest to examine for eRNA interaction, since they are known to contribute to chromosomal looping. It is not unlikely that these subunits co-interact with eRNA in chromatin loop formation.

- A CLIP (cross-linking immunoprecipitation) experiment would determine if some of these proteins could be cross-linked to eRNA.
- If just one of the target proteins immuniprecipitate cross-linked to the eRNA, non-target proteins might also be co-precipitated if bound to the eRNA. Possible non-targeted protein interactions could be revealed this way.
- The eRNA would afterwards be made into cDNA, or analyzed in a RNAse activity assay, to determine whether eRNA was present or not from the CLIP experiment.
- cDNA is also preferable due to greater stability relative to RNA.
- If present, eRNA (or derived cDNA) would be sequenced in order to determine if it truly is eRNA transcribed from the enhancer sequence of interest.

LIST OF RELEVANT PRIMARY AND REVIEW ARTICLES READ, AND SUMMARY OF RELEVANT INFORMATION FROM EACH (this is the start of an annotated bibliography):

(Hsieh et al., 2014; Kim, Lee, Yun, & Kim, 2015; Lai et al., 2013; Lam, Li, Rosenfeld, & Glass, 2014; Li et al., 2013; Plank & Dean, 2014; Smith & Shilatifard, 2014)

Hsieh, C.-L., Fei, T., Chen, Y., Li, T., Gao, Y., Wang, X., … Kantoff, P. W. (2014). Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proceedings of the National Academy of Sciences*, *111*(20), 7319–7324. http://doi.org/10.1073/pnas.1324151111

Relevant information:

- "Whether eRNAs function as molecular bridges that mediate spatial interactions of distal enhancers and target promoters, or directly configure a chromatin state that facilitate transcription factor binding at the target promoters is unclear"
- "Therefore, we propose that an eRNA may function as a scaffold that guides an AR- associated protein complex to target chromatin and selectively enhances DHT-stimulated transcription either intrachromosomally (cis activity) or interchromosomally (trans activity). This positive regulatory loop may provide new insight into RNA-dependent functional effects on the regulation of lineage- or tissue-specific gene expression."

Kim, Y. W., Lee, S., Yun, J., & Kim, A. (2015). Chromatin looping and eRNA transcription precede the transcriptional activation of gene in the β-globin locus. *Bioscience Reports*, *35*(2), 1–8. http://doi.org/10.1042/BSR20140126

Relevant information:

- " A study using siRNA shows that eRNAs regulate chromatin accessibility and RNApolymerase II occupancy at the target genes [12].Transcrip- tional repressors have been reported to function by inhibiting the transcription of eRNAs in distal enhancers"
- "The results show that these events, chromatin looping and eRNA transcription, precede the transcriptional activation of gene and take place together during transcriptional induction procedure."

Lai, F., Orom, U. A., Cesaroni, M., Beringer, M., Taatjes, D. J., Blobel, G. A., & Shiekhattar, R. (2013). Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature*, *494*(7438), 497–501. http://doi.org/10.1038/nature11884,

Relevant information:

- The article propose a new mechanism of action for a class of lncRNAs termed ncRNA-activating (ncRNA-a) involved in long-range tran- scriptional activation through their association with the Mediator complex. It is noteworthy that such Mediator– ncRNA-a-dependent chromatin loops extend beyond the transcrip- tion start sites of the target promoters, which may reflect the inclusion of promoter proximal regulatory elements in such associations.

Lam, M. T. Y., Li, W., Rosenfeld, M. G., & Glass, C. K. (2014). Enhancer RNAs and regulated transcriptional programs. *Trends in Biochemical Sciences*, *39*(4), 170–182. http://doi.org/10.1016/j.tibs.2014.02.007

Relevant information:

- "Three possibilities have been considered with respect to the physiological roles of en- hancer transcription. The first possibility considers enhancer transcription as ‘noise’ from the spurious en- gagement of RNA PolII complexes to the open chromatin environment of enhancers. The second possibility hypothesizes that it is the process of transcription, not the features of the eRNA transcript itself, that is necessary for the activating functions of enhancers. The third possi- bility is that the RNA transcripts per se functionally contribute to enhancer activity. These possibilities are not mutually exclusive."
- "Chromatin interaction studies demonstrated that enhancers engaged in looping with promoters of protein-coding genes possess higher expression of eRNAs."
- Their findings (experiments) suggest that both enhancer transcription and the resultant enhancer RNAs can contribute to enhancer function.

Plank, J. L., & Dean, A. (2014). Enhancer Function: Mechanistic and Genome-Wide Insights Come Together. *Molecular Cell*, *55*(1), 5–14. http://doi.org/10.1016/j.molcel.2014.06.015

Relevant information:

- Introduction to eRNA and some proposed mechanism by which eRNA may function to regulate target gene expression.

Smith, E., & Shilatifard, A. (2014). Enhancer biology and enhanceropathies. *Nature Structural & Molecular Biology*, *21*(3), 210–219. http://doi.org/10.1038/nsmb.2784

Relevant information:

- The structure of a chromatin loop and the factors involved in communication between enhancer-promoter of mammalian β-globin LCR and β-globin locus.

Li, W., Notani, D., Ma, Q., Tanasa, B., Nunez, E., Chen, A. Y., … Rosenfeld, M. G. (2013). Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature*, *498*(7455), 516–520. http://doi.org/10.1038/nature12210

Relevant information:

- "Because several studies have implicated a role for cohesin in chromosomal interactions and enhancer–promoter looping events, we investigated whether cohesin was involved in the observed eRNAfunctions."

- "Knockdown of specific eRNAs by LNA or siRNA resulted in a decrease of cohesin recruitment."

HOW DOES THE QUESTION FIT INTO THE BROADER PICTURE, AND WHAT IS ITS IMPACT?

The question is considering a new perception of enhancers role in chromatin loop formation.
It has been known for a long time that enhancers were important to activate and regulate transcription of genes. Newly published articles suggest that the mechanism of how enhancers promote gene transcription might be more complex than previously thought.
These results suggest a new kind of regulatory molecules to be involved: the enhancer RNA. Further studies of this research area is important to understand the complex function of enhancers and the derived effects.
Studying enhancers with knowledge of the mechanistic function of eRNA, and its involvement in gene regulation, can be applied to review unanswered enhancer-related questions from the past. It could further be applied to identify and understand new principles of enhanceropathies in order to develop treatments of these enhancer-associated diseases.

POTENTIAL WAYS TO MAKE YOUR QUESTION KNOWN TO THE PUBLIC AT LARGE (OR TO YOUR NON-BIOLOGIST FAMILY AND FRIENDS):

Throughout my education, especially in the first year of university, I have used many videos from e.g. Youtube to supplement teaching material in a more graphic and illustrative way. I found this to be a very effective way to be introduced to a new topic, and many of the videos would briefly summarize the necessary information to perceive the topic in the beginning. If I were to present my question, I

would do so through a video as well since it would probably have the largest reach through e.g. Youtube or social media. Since most people have a perception of what DNA is, a quick description of DNA structure would be an appropriate beginning of the video. Then the central dogma of molecular biology could be explained, followed by that not all RNA are translated into proteins, some could also be involved in regulatory interactions and hence the question.
ANY OTHER PARTS OF THE PROJECT COMPLETED SO FAR:

ANYTHING YOU WOULD LIKE SPECIFIC FEEDBACK ON: