BIOL463 – Fall 2016

TEMPLATE FOR PROJECT OUTLINE

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I included two different questions in this outline, since I can't decide which one is better (one is in red). You did twice the amount of work; you should get credit twice!

Topic chosen:
Effect of cis-regulatory element CNE14 on GLI3 expression in developing mice.
Hedgehog signalling is a pathway regulating the development of limbs and central nervous system in all bilateral animals. The transcription factor GLI3 (GLI family zinc finger protein 3) is a key player in the development of vertebrate limbs, and its tissue-specific expression and processing is tightly regulated. The intronic regions of the GLI3 gene has been shown to contain several conserved non-coding elements (CNEs) that have been implicated in the regulation of GLI3 expression. Last year, two novel CNEs (CNE13 and CNE14), conserved in all tetrapods, were described in a paper by Anwar *et al.* [1], The individual CNEs seem to have distinct activities and tissue-specificity, suggesting they play different roles in GLI3 regulation. The mechanisms of regulatory functions and tissue-specific activities remain unknown.

A putative GATA1 binding site has been identified in CNE14 in intron 3 by comparative genomics. This project revolves around the possible binding of GATA1 to CNE14, since it may be important for CNE14 function.

Or

The paper by Anwar *et al.* showed that CNE14 in intron 3 is capable of driving reporter gene expression in the developing pectoral fin of zebrafish, but does not induce gene expression in nervous tissue, contrary to other CNEs.
The regulating actions of CNE14 in mammals/tetrapods *in vivo* is yet to be investigated. An *in vitro* assay with NIH3T3 cell line (by Anwar *et al.*) shows that CNE14 can induce reporter gene expression in a mammalian system, but does not address the tissue-specificity.

Both questions are good.
I like the "red" one better, but that is just a personal preference

SPECIFIC QUESTION:
Does GATA1 bind to CNE14 in the intronic region 3 of the GLI3 gene?

Or

Is CNE14 capable of inducing reporter gene expression in the developing limbs of mice? (Is CNE14 implicated in the tissue-specific regulation of GLI3 expression in mice in the same way as in zebrafish?)

HOW IS THIS QUESTION NOVEL AND ORIGINAL?
The recent discovery of CNE14 suggests that our otherwise well-characterized picture of GLI3 expression is still incomplete.

Studying the function of this cis-regulatory element could reveal more details of the complex regulation patterns of GLI3 during limb development, leading to a better understanding of the morphogenesis of vertebrate limbs.

POTENTIAL IMPACT OF THE PROPOSED QUESTION (WERE IT TO BE ANSWERED BY YOUR PROPOSED EXPERIMENT):

Proper patterns of GLI3 expression is crucial for normal limb development in humans as well as other vertebrates. Dysregulation can lead to polydactyly and syndromes such as Greig Cephalopolysyndactyly Syndrome and Pallister-Hall Syndrome.
Knowledge on the regulating components of GLI3 expression can broaden our view on the process underlying these syndromes, as well as refine our understanding of GLI3 regulation in general.

In your final draft you will have to address why it is important/relevant/useful to understand GLI3 regulation

HYPOTHESIS:

GATA1 binds to CNE14, driving GLI3 expression in developing limbs.

Or

Combined with your evidence, these are good hypotheses.

CNE14 is involved in the limb-specific expression of GLI3 in the developing limbs of mice.

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

CNE14 is conserved in all tetrapods [1].
CNE14 contains putative binding sites for many known transcription factors (including GATA1) [1].
CNE14 promotes reporter gene expression in the developing limb buds of zebrafish, and in NIH3T3 cells [1].

PREDICTION(S):

GATA1 will bind to CNE14…

Or

CNE14 will induce reporter gene expression in a site-specific manner in the developing limbs of mice.

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

For the first question, I would do either ChIP or DNase Footprinting.

The second question requires a transgenic mouse assay.
CNE14 would be inserted into a vector, containing a promotor and a reporter gene (e.g. GFP). In the transgenic zebrafish assay (from the article by Anwar *et al.*) a Tol2 assay based on a transposon system was used – I would research to see if this is relevant to use in a mouse model.

You will need details in your final draft, but I know you have a very good sense of how these techniques work and you will do a great job.

LIST OF RELEVANT PRIMARY AND REVIEW ARTICLES READ, AND SUMMARY OF RELEVANT INFORMATION FROM EACH:

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1. Anwar *et al.* Identification and functional characterization of novel transcriptional enhancers involved in regulating human GLI3 expression during early development. *Develop. Growth Differ.* (2015) 57, 570–580 doi: 10.1111/dgd.12239

Relevant information: GLI3 is a transcription factor important for mediating Shh signalling in development of CNS and limbs. Recently, two novel CNEs (conserved non-coding elements) were discovered in the intronic regions of the GLI3 gene. One of them, CNE14, was shown to induce reporter gene expression in the developing pectoral fin of zebrafish, suggesting an important role of this cis-regulatory element for GLI3 expression in limb development.
CNE14 contains a putative GATA1 binding site.

I haven't taken the time to add the other articles I read on the topic – they contained mostly background information on GLI3 function and regulation. I will add them and embed citations before I submit the next draft!

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

ANY OTHER PARTS OF THE PROJECT COMPLETED SO FAR:
I admit I have not taken as much time to complete this outline as I wanted to (midterm-busyness). All the sections will be improved - don't worry!

ANYTHING YOU WOULD LIKE SPECIFIC FEEDBACK ON:
My specific question:
I included two questions, because I have not been able to decide on a final question yet (sorry if it is inconvenient!).
My original idea was investigating the putative GATA1 binding to CNE14.
Writing this outline, I got the idea of a transgenic mouse assay to show the tissue specificity of CNE14 in mammals. This seems like a natural follow up to the transgenic assay showing the tissue specificity in zebrafish. It is definitely an interesting study, but I am uncertain of the relevance. I especially struggled with writing the "potential impacts" section – it is difficult to imagine any significant impact from such a specific experiment.

I am also considering a third project: investigating whether CNE14 is transcribed. It has putative binding sites for different transcription factors (GATA1, CREB, NFKB) so it seems possible – and it is a topic I find really interesting after our class discussions about the transcription of KCNQ1OT1. However, the terminology confuses me a bit. When they call it a non-coding element, does that mean that it is definitely not a gene (i.e. not being transcribed)?

Very good question. "non-coding" is a term that is now becoming more and more confusing and inappropriate… it means they don't have an open reading frame (and don't code for a protein).

I would love to know which of the three questions you find more relevant/suitable!

Which question I like most… I definitely like the last one ("Is it transcribed?"), as you could also ask "Where/when is it transcribed". Out of the other two questions, I like the transgenic mouse most, but that's probably because I am a biologist (and the other question is more of a biochemical one:). For the impact, there is one thing that would be important: if the results in the mouse reflect those in zebrafish, this would then provide a justification to extrapolate the work done in zebrafish to mice and maybe mammals in general. If the results did not reflect what is seen in zebrafish… that would be really interesting!