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Robert Tjian's Lecture Part 2:

Gene Regulation: Why so complex?

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1. Keywords and Terms

RNA polymerase II, nucleosomes, mediator, TFIID, chromatography, protein purification, long distance enhancers, self-renewal, embryonic stem cells, cell fate, transcriptional network, cellular differentiation, promoter, enhancer, necessary and sufficient, pluripotent

2. Lecture Notes

All of the cells in an organism have the same DNA, yet by regulating the time and place of gene expression some cells will become muscle cells while others may become neurons or blood cells.

TAF3

Originally identified as a component of TFIID, TAF3 was recently discovered to also play a role independently of the other subunits of TFIID. In particular, it interacts with the protein CTCF to mediate long-range DNA looping, helping to bring distal enhancers into close contact with the transcriptional machinery at the core promoter.

3. Review Questions

1. Approximately how many polypeptides are required to transcribe a eukaryotic gene?
2. How is DNA compaction achieved in eukaryotes? Why might this be necessary in eukaryotes and not bacteria?
3. List four functions of the subunits in the TFIID complex.
4. True or False: Transcription in all cell types utilizes the same pre-initiation complex but is regulated by a diverse set of activators and repressors. Give an example to support your answer.
5. How did researchers know that TBP plus the core transcriptional factors (RNA pol II, TFIIA, TFIIB, etc.) were not sufficient to support Sp1-regulated transcription? What was the key piece of data that showed this?
6. How did researchers determine that TAF3 is important for proper germ layer formation? What experimental approach did they take?
7. In the lecture, a biochemical complementation assay is based on the premise of trying to recreate in a test tube the minimum requirements for a particular function to occur. This allows one to address the question of whether something is sufficient for a particular process. How can the counterpart to this approach, i.e. a genetic approach, be used to address whether or not something is necessary for a particular function?
8. What is one major property of an embryonic stem cell?

9. Enhancers have an unusual property that they are capable of functioning at long distances. What is one way, discussed in the lecture, that enhancers may interact with the core machinery, even at long distances? What proteins are involved?
10. In the two lectures, a couple of cases were described where previously identified proteins were found to have new, additional functions. What were some of these proteins/complexes? Explain how these new functions contribute to gene regulation.

4. Answers to Review Questions

1. ~85 polypeptides
2. DNA compaction is achieved in eukaryotes through the use of histones. This is necessary in eukaryotes as their genome size is significantly larger than that of bacteria; however, cell size does not scale accordingly. DNA compaction through histones allow eukaryotes to fit more DNA in a proportionally smaller space.
3. Recognizes and binds to the TATA-box in core promoters through its component TBP (TATA-box binding protein) False. Tbp-associated factor 7-related (Taf7l) is expressed highly in adipocytes compared to most other tissues in an organism and plays a role in transcriptional activation of fat tissue-specific genes.
 - a. binds to modified histones
 - b. acts as an enzyme (acetylase, kinase)
 - c. interacts with transcriptional activators
4. An in vitro biochemical complementation assay was used to find the minimal components necessary to support activated transcription. The researchers first tried using only TBP plus the core factors (RNA pol II, TFIIA, TFIIB, etc.) to transcribe a template DNA that included the promoter and the SP1 binding sites. The key piece of data was the gel of the transcription reaction showing that transcript production in this condition was the same with and without the SP1 activator. This means that the factors in the reaction were not able to support SP1-directed activation. If they were sufficient, then addition of SP1 should give more transcript. Thus, something else must be needed to allow SP1 to activate transcription.
5. Researchers were able to determine that TAF3 is important for the regulation of proper germ layer formation by mutating/ knocking out the TAF3 gene and trying to differentiate into different cell types. The researchers saw that TAF3 knockout led to

the lack of endoderm formation and caused more mesoderm and ectoderm to form. This experimental approach is called the genetic approach.

6. Alteration or removal of a component allows one to test whether a process can occur in the absence of a particular factor, and hence its necessity for that function to occur. It is sometimes the case that multiple factors perform the same or similar functions; this redundancy can be tested by combinatorially knocking down or knocking out various components.
7. Pluripotency, the ability to become all cell types in an organism such as neurons, skin cells, or muscle cells, is one of the two major properties of an embryonic stem cell.
8. Enhancers can interact with core machinery from a remote site by “looping”. Making a loop allows the cell to contact two specific sequences while disregarding the amount of DNA physically between the two sites. CTCF is one protein that is involved in this looping phenomenon. TAF3 cooperates with CTCF to carry out DNA looping in embryonic stem cells.
9. SCC-B (XPC, Rad23b, CETN2): Originally discovered as a DNA repair complex, SCC-B was purified and identified via a biochemical fractionation and complementation approach as a stem cell specific co-activator required for Oct4- and Sox2- directed transcription of pluripotency genes such as Nanog.

5. Discussion Questions

1. Describe at least two ways in which histone proteins contribute to transcription regulation. You may need to consult the literature to help you answer this question.
2. In the purification of Sp1, non-sequence-specific DNA-binding proteins had to be eliminated. What functions could some of these other DNA-binding proteins have?
3. You have the nuclear fraction from a cancer cell line that you suspect contains a transcription factor that activates a specific gene called Tumorin, whose sequence is known. Design a series of experiments to identify this transcription factor and confirm that it is indeed an activator of Tumorin expression. Use biochemical techniques described in these lectures.
4. Speculate as to why eukaryotic organisms might need a more complex transcription apparatus as compared to bacteria. (There are many possible answers to this

question, some of which are touched on in the lecture, however, searching the literature may be helpful)

5. Given your understanding of TAF's, both as they function as subunits of TFIID and also as independent proteins, speculate as to how cell-type specific expression of certain TAF's might mediate the transcription of genes particular to a given cell type.
6. In addition to binary on/off states, how might intermediate transcription levels or fine-tuning be achieved?

6. Answers to Discussion Questions

1. Histone proteins compact eukaryotic DNA by forming nucleosomes. This compaction can physically block access to the DNA, preventing the transcription machinery from transcribing a gene. Nucleosomes can also be modified with chemical groups that are recognized by transcriptional proteins and can function to activate or repress a gene by recruiting these proteins to the correct location along the DNA.
2. Other proteins that bind DNA include DNA repair machinery, DNA replication machinery, and histones and other DNA packaging proteins.
3. One approach could be to synthesize a DNA sequence that contains the promoter of Tumorin attached to a reporter gene that can be used in an in vitro transcription assay. Using purified components of the preinitiation complex plus the nuclear fraction from the cancer cell line, one could test whether the portion of the Tumorin promoter included in the assay is enough to support activated transcription. If it is, then using various purification and chromatography techniques, the fraction containing the transcription factor of interest can be tracked and iteratively enriched. Finally, this transcription factor can be identified by mass spectrometry of the purified fraction containing the transcriptional activity.
4. One answer discussed in the lectures is that eukaryotes use nucleosomes to compact their DNA, while bacteria have "naked" DNA. Thus, the eukaryotic transcription machinery must use additional factors to help it navigate the densely compact chromatin. Another example may be related to differences in transcriptional requirements between bacteria and eukaryotes. Eukaryotes often have more complex cellular processes. This may require additional transcriptional regulation to be sure that the precise gene networks are activated at the correct time.

5. Variation in the content and expression levels of TAF's in different cell types allows for additional combinatorial effects with sequence-specific transcription factors. In addition, because TAF's often bridge multiple sequence-specific transcription factors with the preinitiation complex, they can be thought of as nodes in transcription networks. Therefore, altering the levels of just one TAF can potentially affect the expression of a large number of genes.
6. There are many answers to this question. Possibilities include the utilization of different subsets of all possible combinations of transcription factors that are normally required for full activation, the modulation of the presence or absence of repressors versus activators, the selective exposure of certain enhancer elements by chromatin remodellers, or the combinatorial possibilities of different histone modifications that would result in various degrees of transcriptional activation.

7. Explain or Teach These Concepts to a Friend

1. Describe the 3 major classes of macromolecules involved in transcription and name two examples of each: sequence-specific transcription factors, coactivators, and the core machinery.
2. Compare and contrast the genomes (size, number of genes) and regulation of gene expression in prokaryotes and eukaryotes.
3. Explain how a protein could be necessary but not sufficient for activating gene expression. How could a protein be sufficient but not necessary for activating gene expression?
4. Describe how the biochemical complementation assay works.
5. Explain how an organism utilizes the same genome to make different tissues such as skin, blood, and muscle with distinct gene expression programs. How can this be achieved with limited numbers of transcription factors?

8. Research the Literature on Your Own

1. Find an example of a target protein for one of the enzymatic functions of TFIID; how does this example contribute to gene regulation?

2. What is the main difference between a promoter and an enhancer?
3. Of the three domains of life, Eukaryota, Bacteria, and Archaea, are nucleosomes unique to eukaryotes? Do any of the other domains utilize the same or analogous packaging molecules?
4. What is an example of a sequence-specific transcription factor not mentioned in the lecture that is involved in dictating a cell's fate?
5. Find an example of a cell-type specific long distance enhancer that regulates gene expression.
6. Find an example of a transcription factor whose misexpression is indicative of a diseased state.
7. In addition to cryo-EM, which provides relatively low resolution information but is well-suited for studying larger macromolecules, what other techniques can be used to obtain structural information about biomolecules? What is an example of a transcription factor or co-factor whose structure was solved by one of these techniques?
8. What is the role of Oct4 and Sox2 in establishing pluripotency? Why did researchers think that there might be stem cell specific cofactors that potentiate Oct4/Sox2 activated transcription in human embryonic stem cells?

9. Papers for Journal Club

The following papers exemplify some of the earlier work done to determine how a gene is transcribed and what proteins are involved. The first four papers focus on Sp1, one of the first identified mammalian transcription factors. The paper by Dynan et al. shows how Sp1 recognizes the GC box in the SV40 early promoter to activate transcription, while the Briggs et al. paper shows how Sp1 was purified to homogeneity using the DNA affinity purification approach discussed in lecture 1. The following two papers by Pugh et al. and Ryu et al. show how researchers discovered that more than just TBP and the core machinery were required to obtain activated transcription, while the paper by Chen et al. teases out the roles of individual components of TFIID. Lastly, the paper by Landschulz et al. shows the identification of a class of transcription proteins containing a structural motif called the leucine zipper.

- Dynan, W. S. & Tjian, R. (1983). The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. *Cell* 35, 79.
- Briggs, M., Kadonaga, J., Bell, S., and Tjian, R. (1986). Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. *Science* 234, 47–52.
- Pugh, B.F., and Tjian, R. (1990). Mechanism of transcriptional activation by Sp1: evidence for coactivators. *Cell* 61, 1167–1197.
- Ryu, S., Zhou, S., Ladurner, A.G., and Tjian, R. (1999). The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature* 397, 446–450.
- Chen, J.L., Donatella Attardi, L., Verrijzer, C.P., Yokomori, K., and Tjian, R. (1994). Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* 79, 93–106.
- Landschulz, W., Johnson, P. & McKnight, S. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* 240, 1759–1764.

These two papers give examples of cell-type-specific roles for components of the preinitiation complex. Liu et al. describes the function of TBP-associated factor 3 (Taf3), a highly expressed protein in embryonic stem cells, in endoderm specification. Taf3 activates its downstream targets by looping DNA from distal enhancers to the promoters. Hart et al. shows that Tbp-related factor 3 (Trf3) is crucial in the development of blood cells--a process called hematopoiesis--in zebrafish embryos by activating blood lineage specific genes such as *mespa*. These papers provide examples of using genetic tools as well as modern sequencing techniques to describe cell type specific activities.

- Liu, Z., Scannell, D.R., Eisen, M.B., and Tjian, R. (2011). Control of embryonic stem cell lineage commitment by core promoter factor, TAF3. *Cell* 146, 720–731.
- Hart, D.O., Raha, T., Lawson, N.D., and Green, M.R. (2007). Initiation of zebrafish haematopoiesis by the TATA-box-binding protein-related factor Trf3. *Nature* 450, 1082–1085.

This paper by Fong et al. describes the identification of a new coactivator complex that is required for expression of embryonic stem cell specific genes. This paper is a good

example of a biochemical complementation approach and shows how one complex can have multiple, potentially unrelated, functions in specific cell types.

- Fong, Y.W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131.