NF-κB Interaction with Nucleosomes

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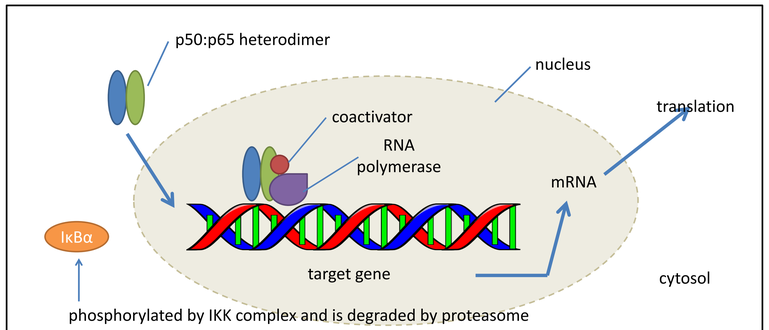
Abstract

Past studies have shown that *in vitro* experiments that tested if NF-κB will bind to DNA can work. The purpose of this experiment was to determine if NF-κB would continue to bind to DNA even with the histone octamer present. Since this models more realistic conditions, the results of this experiment would shed more light on NF-κB interactions with nucleosomes. This could then lead to the prevention or cure of diseases caused by NF-κB misregulation.

Background



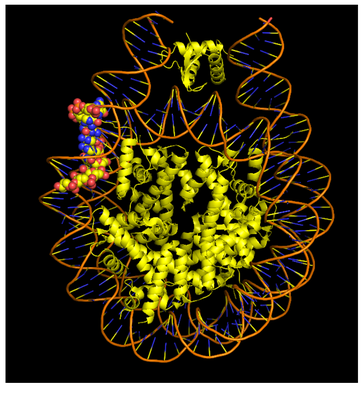
NF-κB is a family of transcription factors involved in immune system regulation, and is present in almost all multicellular organisms. The five members of this family (RelA or p65, RelB, c-Rel, p50, p52) form dimers and remain in the cytosol due to inhibition by IκB. Inflammatory stimuli activate the IκB kinase complex, releasing the dimers from IκB and letting them activate transcription in the nucleus.

All the different dimer combinations activate transcription of different sequences (and depending on if they have a TAD/Transcriptional Activation Domain, might not activate anything at all), but the above diagram is a general representation of NF-κB activity in the nucleus. DNA figure from http://www.zorgictzorgen.nl/gen-patenten-en-medische-big-data-analyse/

Methods

Homodimers and heterodimers made from the NF-κB/Rel family of proteins generally can bind to a DNA sequence that follows the form GGGRNNYYCC (R is any purine, Y is any pyrimidine, and N is any base). Near the end of the 147 bp 601 nucleosome positioning sequence, there is a potential NF-κB binding site that follows that form (GGGATTCTCC).

After purifying the proteins and refolding them into octamer form, the 147 bp 601 DNA surrounding it is labeled with Cy3 dye (which fluoresces a greenish-yellow) at the end closest to the potential NF-κB binding site. The NF-κB heterodimer p50:p65 (the most commonly occurring dimer) is labeled at one end terminal with B42 Alexa dye (which fluoresces red).



Nucleosome with a histone octamer core and 601 DNA. The potential binding site is depicted as spheres. (visualized with PyMOL)

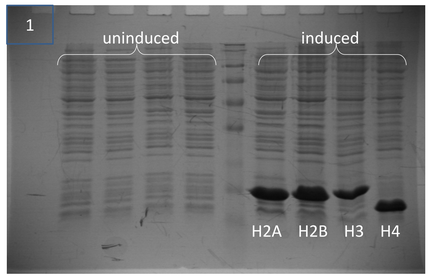
If the heterodimer bound to the site correctly, it should be less than or equal to 50Å away from the dye on the DNA. So, when we excite the electrons of the dye on the dimer, the electrons of the DNA dye should be excited as well and we should see a green color (from the Cy3).

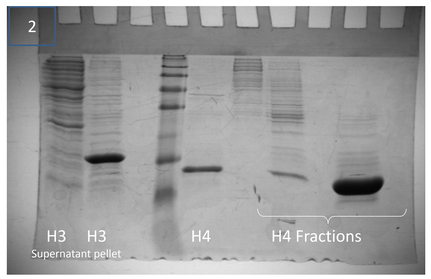
Protein Purification

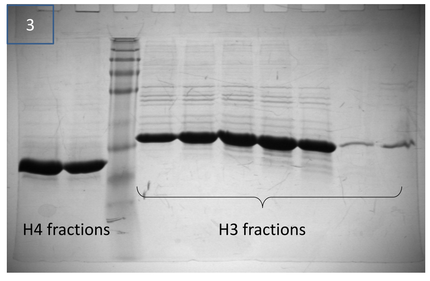
The histone proteins were expressed by BL21 competent E. coli after being induced by IPTG (Isopropyl β-D-1-thiogalactopyranoside). The difference before and after the addition of IPTG can be seen in the SDS-PAGE gel to the right, where the expression of the specific proteins appears as the thicker bands to the right.

Gels two and three differ from gel one because they show H3 and H4 histones after purification using FPLC (Fast Protein Liquid Chromatography).

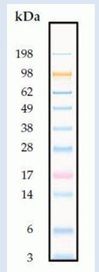
Fractions were collected as UV peaks emerged on the FPLC monitor. Especially clear in gel three, the thick bands occur as proteins are eluted from the column, with the maximum occurring in the middle band.



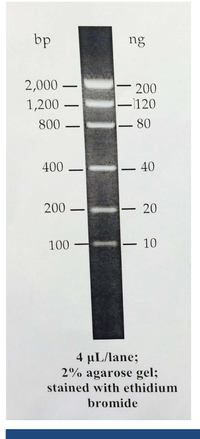




The SeeBlue Plus2 Protein Standard is used in SDS-PAGE to figure out the size of the proteins run in a particular gel. H4 is usually around 11 kDa and H3 is usually around 15 kDa.

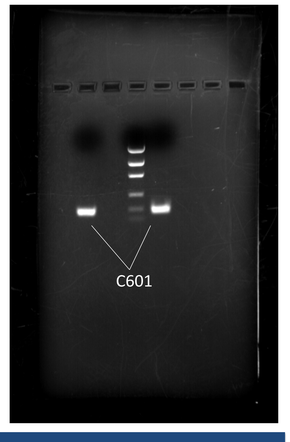


Polymerase Chain Reaction



Using PCR, we amplified the C601 sequence. The agarose DNA gel that we ran after this is on the right.

Using the DNA marker (on the left and in the middle well on the photo on the right), we can confirm that C601 is a little fewer than 200 base pairs long. This matches up with what we know: that C601 is 147 base pairs long, with 12 extra base pairs at the end (for a final sum of 159 base pairs).



References

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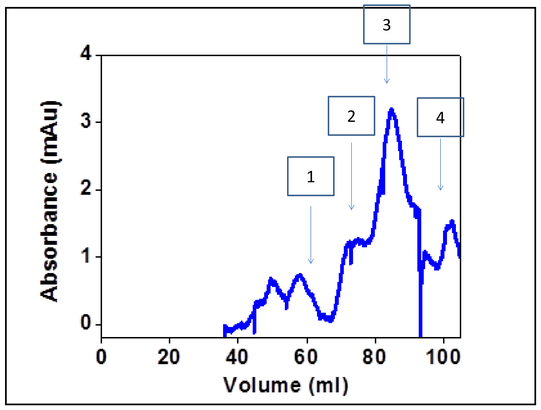
3. Luger, K., Rechsteiner, T. J., Flaus, A. J., Waye, M. M. Y., & Richmond, T. J. (1997). Characterization of Nucleosome Core Particles Containing Histone Proteins Made in Bacteria. *J. Mol. Bio.*, 272, 301-311.

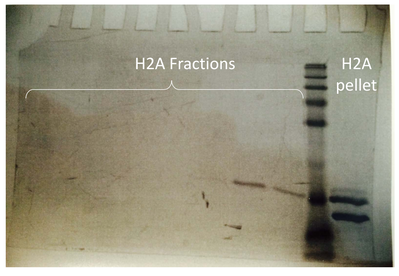
Octamer Reconstitution

A histone octamer and the DNA wound around it, form a nucleosome. The octamer is located at the center of the nucleosome, and is made out of a tetramer (two copies of H3 and H4) and two H2A/H2B dimers. To allow the proteins to refold into octamer form, reconstitution buffer (2 M NaCl, 10 mM Tris pH 7.4, 1mM EDTA, 5mM BME) was used.

In the below graph, two peaks representing dimer and tetramer formation emerged, but no octamer formation occurred. So, more protein purification is needed.

1. Octamer formation was supposed to occur at around 63 mL but did not.
2. A peak representing tetramer formation occurred.
3. A peak representing dimer formation occurred.
4. A peak representing the leftover monomers occurred.





The proteins represented in the graph above were run through the SDS-PAGE gel to the left. Since Size Exclusion Chromatography ejects proteins of the smallest size last, it makes sense that only a clear dimer peak appeared in the graph and that most of the protein was in the last few fractions and the pellet.

Discussion and Future Research

After purifying the proteins, they should form octamers in the refolding/reconstituting buffer. Then we can label the DNA and the NF-κB dimers. After exciting the electrons, seeing "green" florescence means that the p50:p65 dimer attached to the nucleosome. If the dimers attach to the potential binding site, then we will know that NF-κB will bind to that sequence and the presence of the histone core won't disrupt that from happening.

If we see red florescence, the dimer did not bind, and the presence of a protein must have prevented its binding to DNA. So something must have been different between the previous studies (which tested NF-κB binding only in the presence of DNA) and our trial. The next step would be to figure out how the histone core changed NF-κB interactions with DNA in nucleosomes.

Acknowledgments

Sincerest thanks to the Academic Connections program for providing this learning opportunity, to Professor Komives for directing the Research Scholars program and letting me learn in her lab, to Dominic Narang for taking the time to teach me and being patient despite my mistakes, and to Wei Chen for answering all of my questions, no matter how random.

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