**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.zengzirong Corpus: Patenter-en-medicinske-lig-data-analys](http://www.zengzirong).

**Methods**

Homodimers and heterodimers made from the NF-κB/Rel family of proteins generally can bind to a DNA sequence that follows the form GGGNNYYYCC (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 (GGGATTTCC).

After purifying the proteins and refolding them into octamer form, the 147 bp 601 sequence, there is a potential NF-κB dimer binding site (GGGATTCTCC). 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.

**Protein Purification**

The 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).

**Polymerase Chain Reaction**

Using PCR, we amplified the C601 sequence. The agarose gel that we ran after this is on the far right. The SeeBlue Plus2 polymerase chain reaction (PCR) program and letting me learn in her lab, to Dominic Narang for taking the learning opportunity, to Professor Komives for directing the Research Scholars program and answering all of my questions, no matter how random.

**References**


**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" fluorescence 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 fluorescence, 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.

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