Purification and Comparison of Human and Mouse Homologs of DNA-binding Proteins p50/RelA

Jackie Chan, Anahad Judge, and Hannah Baughman*

Please Scan for References!

Introduction & Background

- The Nuclear Factor-kappa B (NF-κB) is a signaling pathway regulating genes involved in immune response.
- RelA and p50 are key subunits in this pathway, together forming heterodimers that functions as transcription factors
- The p50/RelA heterodimer plays a crucial role in NF-κB signaling, however, most research relies on mouse models, raising questions about how accurately they reflect human health.

Research Objective

- **Objective:** Investigate species-specific differences in the stability and function of the human and mouse p50/RelA heterodimer within the NF-κB pathway.
- **Significance:** Both human and mouse p50/RelA proteins are conserved, but understanding differences reveal functional variation between species
- **Approach:** A comparative study of the human and mouse p50/RelA heterodimers through two experimental stages.

Protein Sequence Comparison

Sequence alignment of Human and Mouse RelA

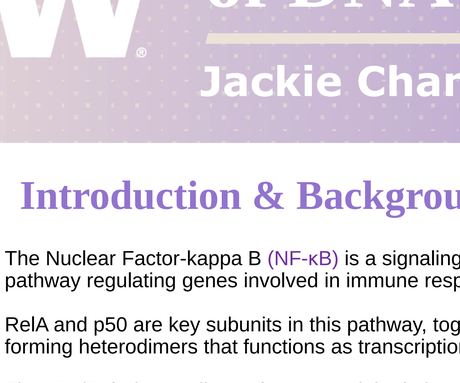
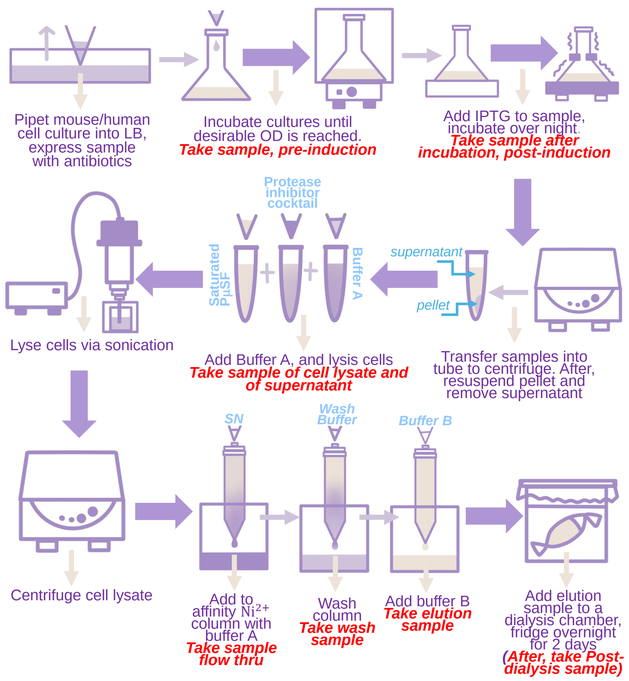


Figure 1: Sequence alignment of human (blue) and mouse (purple) RelA proteins generated using BLASTX.
This alignment shows 91% positives and 1% gaps, indicating high similarity with some regions that have diverged between species. **Red boxes** indicate mismatched residues, **yellow boxes** highlight structurally similar residues, and **green boxes** denote gaps in the alignment.

Sequence alignment of Human and Mouse p50

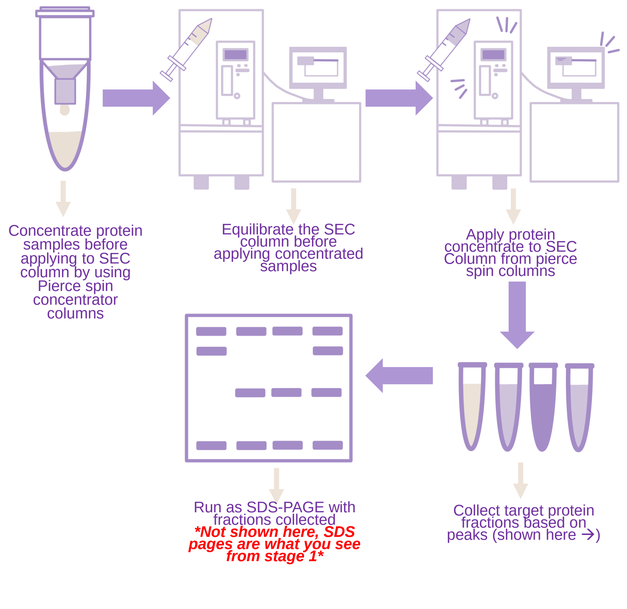
Figure 2: Sequence alignment of human (blue) and mouse (purple) p50 proteins generated using BLASTX.
This alignment shows 99% positives and 0% gaps, indicating a very high degree of similarity between the two species. **Red**, **yellow** and **green boxes** are denoted the same as *Figure 1.*

Methods for Stage I



Stage I Objective: Isolate and purify human and mouse p50/RelA heterodimers by transforming competent *E. coli* in LB broth with plasmids, monitoring optical density (OD) to ensure cells reach the optimal growth phase, inducing protein expression with IPTG, lysing the cells to release the expressed proteins, isolating them through Ni^{2+} affinity purification, and removing impurities via dialysis

Methods for Stage II



Stage II Objective: Assess purity, size, and integrity of human and mouse p50/RelA heterodimers after purification
Size Exclusion Chromatography (SEC): Determines molecular weight (MW) and yield by separating proteins based on size.
SDS-PAGE: Analyze purity and integrity of proteins for future research work.

Results from Stage I

Mouse SDS-PAGE

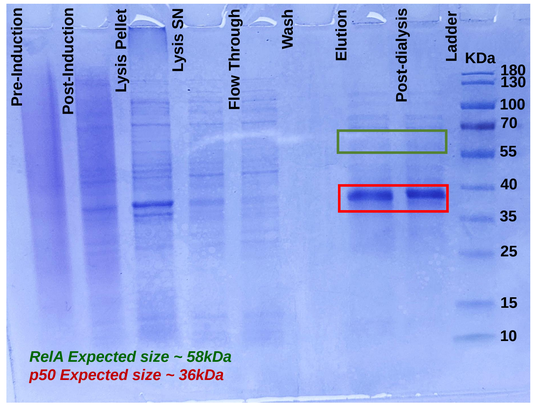


Figure 3. Mouse SDS-PAGE from Stage 1: Expected bands for *p50* were captured in the expected region of mouse (red). However, *RelA's* expected MW, measured as kilodaltons, (KDa) showed little-to-no visible bands (green).

Human SDS-PAGE

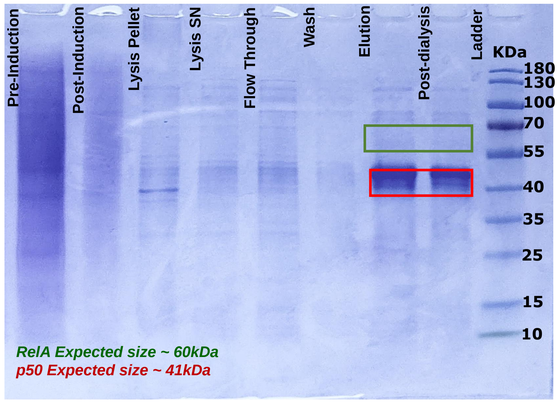


Figure 4. Human SDS-PAGE from Stage 1: Similarly to Fig1, the expected bands for *p50* were visible in the expected regions for human *p50* (red), and *RelA* did not show bands in expected regions (green).

Results from Stage II

p50/RelA Mouse vs Human SEC chromatogram

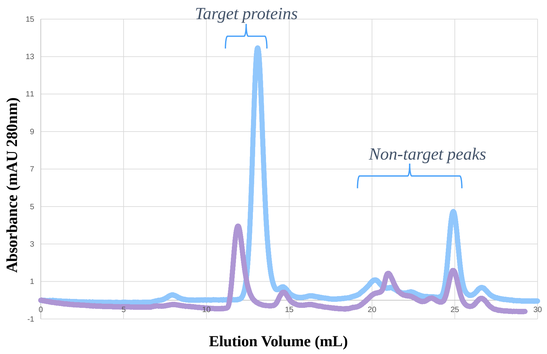


Figure 5: p50/RelA Mouse (blue) vs Human (purple) SEC chromatogram.
This chromatogram shows the elution of human p50/RelA at 12 mL (estimated molecule weight:101 kDa) and mouse p50/RelA at 13 mL (estimated molecular weight: 95 kDa). Non-target peaks represent degradation products, and other impurities. The higher absorbance of mouse proteins indicates greater yield compared to the human protein.

Results from Stage II

Final Purified Amount of p50/RelA

Table 1. Final Concentrations/Amounts After SEC Chromatogram

	Human	Mouse
Absorbance	0.037	0.119
Concentration (μM)	1.48	2.5
Volume (μL)	187	205
# of Mols	2.76×10^{-10}	5.13×10^{-10}

Table 1: Final protein amount measured and calculated after SEC chromatogram for human and mouse.
Calculations were made to determine the final concentration collected after purifying p50/RelA in Human and mouse from stage II. The mouse protein was nearly 2x more concentrated than human protein after using SEC chromatogram.

Conclusions

- **SDS-PAGE:** Stronger p50 bands indicate higher stability and yield; weaker RelA bands suggest degradation or post-translational modifications.
- **SEC Chromatography:** Human p50/RelA dimer eluted earlier (12 mL,101 kDa) than mouse dimer (13 mL,95 kDa) indicating size and structural differences.
- Results highlight species-specific stability and degradation differences, emphasizing the need to optimize conditions for translating mouse models to human health

Future Work

- **Optimize RelA Expression:** Adjust IPTG concentrations to improve yield
- **Analyze PTMs:** Use mass spectrometry to assess if post-translational modifications contribute to RelA degradation.
- **Study binding Interactions**: Explore p50/RelA binding affinity and interactions to better understand their roles in species-species immune responses and inflammation

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