Purification and Comparison of Human and Mouse Homologs of DNA-binding proteins P50/RelA

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The p50/RelA dimer is an essential part of the NF-κB signaling pathway, which is responsible for regulating inflammation and immune responses. Most prior biochemical research focused on the mouse version of the p50/RelA dimer. While the findings are useful, its implication to human health remains unclear. This raises the question, how effective do experiments involving mouse proteins reflect those involving humans? We used protocols to express and purify human and mouse p50/RelA dimers, aiming to generate proteins for structural and functional analysis. In the first stage, recombinant protein expression and affinity chromatography techniques were used for purification of both proteins, followed by an SDS-PAGE to assess molecular weight and stability. We found that mouse proteins showed higher intensity bands compared to human proteins, indicating a higher yield. This suggests stability factors as well as potential differences in degradation rates between species. In the second stage, ion exchange and size exclusion chromatography were used to further purify the proteins. During ion exchange chromatography, neither protein bounded as effectively as expected, highlighting the need for protocol optimization. Improving the chromatography conditions will help increase stability and yield of both proteins allowing for more accurate comparisons between the mouse and human p50/RelA dimers. These optimizations are important because it will improve our ability to compare NF-κB pathway functionality between species and ultimately make it easier to translate findings from mouse models to human health.