

## Complexes of Cellular Protein HAX-1 and Protein PA of the Influenza RNA Polymerase

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The influenza A virus is a global contagion causing five pandemics since the beginning of the twentieth century, moreover, the cumulative mortality of the intervening years far exceeds the mortality rates of the pandemics. Research of the virus has reduced its impact on humans. Unique among RNA viruses, the influenza A virus transcribes and replicates its genome inside the nucleus of the host cell. The RNA polymerase responsible for transcription and replication consist of subunits PB1, PB2, and PA. These subunits are produced in the cytoplasm and must be transported into the nucleus to be assembled into an active RNA polymerase trimer, a process in which cellular proteins play an important role. The Human HAX1 protein has been reported to bind to the subunit PA—inhibiting its translocation into the nucleus. This study aimed to characterize the interplay between HAX1 and PA in vitro and to visualize their interaction by means of structural biology. This study was conducted at the European Molecular Biology Laboratory from 1 June 2016 to 15 August 2016. We constructed a plasmid containing the RNA polymerase subunit PA gene and the HAX1 gene for expression in competent BL21 *E. coli*. The cells were cultured for protein expression using Isopropyl  $\beta$ -D-1-thiogalacopyranoside (IPTG) and incubated for 3 hours at 37C. We purified the proteins using Ni-Affinity Chromatography, however, the mass spectrometry data showed that the isolated proteins were other *E. coli* proteins. We repeated the process yet we were unsuccessful in isolating a PA:HAX1 complex for X-ray crystallography. This study shows that the expression of these proteins are limited to insect or mammalian cells as they too complicated for *E. coli* protein production. The literature characterized their interactions via human cells suggesting that there is an important human co-factor necessary for their interactions that must also be characterized.