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**Mediator Med25 Subunit Hijacking by Rsv Ns1 Protein**

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Respiratory syncytial virus (RSV) is a major cause of bronchiolitis in infants worldwide. RSV elicits a weak innate immune response and low type-I interferon (IFN-I) levels. IFN-I antagonism is partially mediated by the non-structural RSV NS1 protein. In the nucleus of infected cells NS1 interacts with Mediator, a transcriptional coactivator of RNA polymerase II, and modulates host transcription. We and others identified the MED25 Mediator subunit, more precisely its ACID domain, targeted by transcription factors (TFs), as an NS1 interaction partner. However, since NS1 lacks a DNA binding domain, it cannot act like a TF, and its mechanism remains puzzling. We analyzed the high affinity NS1−MED25 complex by mutational analysis and a combination of biophysical techniques, including AlphaFold prediction, NMR, ITC and BLI. The two subdomains of NS1 bind to a dual site, occluding the two TF-binding sites of MED25 ACID. We showed that NS1 disrupts the complex formed between MED25-ACID and ATF6α TAD, indicating how NS1 could outcompete MED25-mediated transcription. To investigate the impact of the NS1−MED25 interaction on viral infection, we engineered NS1 mutants of recombinant rRSV with decreased MED25 affinity. These rRSV mutants were attenuated and induced increased production of interferon-stimulated genes compared to WT rRSV. MED25 knock down confirmed that RSV hijacks MED25.