

NOTE: MAXIMUM 350 WORDS AND 30 LINES, FONT 12.

FUNCTIONAL INTERACTION MAP OF THE LYSSAVIRUS PHOSPHOPROTEIN P

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Lyssaviruses (rabies and rabies-related viruses) infect neurons *in vivo*. After receptor recognition and penetration by endocytosis, they use axonal retrograde transport to reach the perikarion where the viral ribonucleocapsid complex (RNP) is transcribed. The RNP is composed of the RNA genome encapsidated by the nucleoprotein (N), the polymerase L and the phosphoprotein (P). Using two-hybrid and GFP/reverse two-hybrid in yeast, co-immunoprecipitation, and a reverse genetic assay in mammalian cells, we are currently establishing a functional interaction network between lyssavirus proteins, and look for their cellular partners.

Screening a human brain cDNA library by two-hybrid, the cytoplasmic dynein light chain (LC8), was found to interact strongly with P of the two phylogenetically distant rabies virus (genotype 1) and Mokola virus (genotype 3), indicating that P/LC8 interaction is a common property of lyssaviruses. Co-localisation in infected cells and co-immunoprecipitation strengthened the observation, and the LC8 binding domain was mapped around a.a. 140-150. LC8 is a component of both cytoplasmic dynein and myosin V which are cellular motors involved in axonal retrograde microtubule-based transport, and actin-based transport, respectively. This provides support for a model of RNP axonal transport in which RNP (through P) interacts successively with actin filaments in the early step of viral entry, then with the microtubule network for fast axonal transport. As LC8 is also an inhibitor of the neuronal nitric oxide synthase (ncNOS), P/LC8 interaction could also sign a physiopathological aspect of infection.

Within the RNP complex, the P protein also has a central role at the interface between L protein and N-RNA template. Functional dissection of lyssavirus P proteins reveals one multimerization domain (a.a. 52-189), one L-binding domain (a.a. 1-19) and two N-binding domains: a strong one (a.a. 176-Cterm) and a weak one (a.a. 1-189). Random mutation of the strong domain identified 3K crucial for N/P interaction and transcriptional activity of RNP. However, this transcriptional activity is not significantly altered by removing most of the weak N-binding site (deletion aa 61-175) which encompasses the LC8 binding domain (140-150). Thus, the lyssavirus P appears as a multifunctional protein composed of autonomous domains for viral and host-related functions.