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VIRAL INFECTION

Propelling vaccinia virus to the neighbours

To spread successfully from cell to cell, vaccinia virus downregulates signalling by the host RHO GTPase RHOA to weaken cortical actin, which acts as a physical barrier to exocytosis. Here, Way and colleagues identify the mechanism by which the virus inhibits RHOA signalling, revealing F11 as the first known viral protein to have a functional PDZ domain.

PDZ domains are protein–protein interaction motifs that are involved in a range of cellular processes. Way and colleagues found that the highly conserved portion of F11 contains motifs that are characteristic of PDZ domains, and structural predictions suggested that it would adopt a PDZ-like fold. Interestingly, vaccinia virus with mutated F11 PDZ motifs formed fewer actin tails (which enhance viral spread), showed reduced cell–cell spread and released fewer infectious virions. These effects are indicative of failed RHOA inhibition, which suggests that the F11 PDZ domain regulates RHOA signalling.

Like other GTPases, RHO proteins are regulated by RHO guanine exchange factors (RHOGEFs; which activate them) and RHO GTPase-activating proteins (RHOGAPs; which inactivate them), both of which are known to have PDZ-binding motifs (PBMs). Through an *in vitro* pull-down assay, the authors identified two RHOGAPs, β-chimaerin and myosin IXa, that interacted with the F11 PDZ domain.

Focusing on myosin IXa (which is known to regulate RHOA and cortical actin), they further observed that deletion of the myosin IXa PBM abolished its interaction with F11. Moreover, no virus-induced drop in active RHOA was observed in myosin IXa-depleted HeLa cells, which also had fewer actin tails and lower production of active virions than wild-type cells. Analysis of plaque formation in myosin IXa-depleted adherent cells also indicated less vaccinia virus cell–cell spread. By contrast, there was no significant difference between myosin IXa-depleted cells and controls when cells were infected with F11-null vaccinia virus. These findings, together with the observation that fewer actin tails were formed in cells carrying GAP-mutant myosin IXa, suggest that myosin IXa GAP activity inhibits RHOA following interaction with the F11 PDZ domain.

Importantly, depletion of myosin IXa did not affect the size of the plaques formed by a vaccinia virus carrying a mutant form of F11 that cannot bind RHOA, suggesting that myosin IXa acts as a GAP for RHOA only when RHOA is bound to F11. Consistent with this, the three proteins were shown to form complexes in infected cells. Thus, the authors conclude that F11 acts as a scaffold, using its PDZ domain to bring myosin IXa and RHOA together and ultimately promote vaccinia virus spread.

This study identifies the first viral PDZ domain-containing protein; as F11 is present in other poxviruses, it is possible that this mechanism of promoting viral cell–cell spread is conserved.

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