EXISTENCE OF AN OPERATIVE PATHWAY FROM THE ENDOPLASMIC RETICULUM TO THE POXVIRUS MEMBRANE

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Origin of the initial lipoprotein membrane of poxviruses has perplexed investigators, even leading to suggestions that it may be synthesized *de novo*. The membrane is formed by an unknown mechanism within a specialized region of the cytoplasm called as *virus factory* where viral DNA replication, transcription and virion assembly occur. We previously showed that endoplasmic reticulum (ER) to Golgi transport is unnecessary for transport of viral proteins to factory and formation of mature virions. The present study was designed to determine whether an operative pathway exists between the ER, viral factory and membrane of vaccinia virus, the most studied member of *Poxviridae*. Initial experiments indicated that the highly conserved A9 viral membrane protein was localized to ER of uninfected cells with the same topology as in viral factory. Next, we found that the addition of a cytoplasmic tail containing ER exit motifs to A9 reduced its transport to viral factory and resulted in the accumulation of chimeric protein in the Golgi apparatus, implying that A9 was inserted into the ER and then diverted from its natural path. Most importantly, we demonstrated cleavage of a heterologous signal peptide fused to the N-terminal region of A9 and localized the truncated protein to factory and mature virions. Additionally, immunoelectron micrographs showed A9 in tubules containing protein disulfide isomerase, an ER luminal protein, near viral membranes. The present data, together with our previous report provide compelling evidence for an operative pathway from a sub-domain of ER within factory to the viral membrane.