The role of flavivirus E protein stem anchor domains in endoplasmatic reticulum retention

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The Flavivirus morphogenesis occurs in association with endoplasmic reticulum (ER) membranes. For viral assembly, the virion envelope (E) protein accumulates in this organelle suggesting the presence of ER retention signal. Towards the identification of such mechanism we constructed recombinant YF 17D viruses expressing an enhanced green fluorescent protein (EGFP), with sequential deletions of E protein stem anchor (SA) domains or with substitution of transmembrane domain (TM1) of 17D with TM1 of human Lamp-1 protein. A change in cellular localization of the EGFP protein expressed from these constructs would highlight the functional elements involved in E protein retention in the ER. Viruses were regenerated from cDNA by transfection of Vero cells with invitro transcribed RNA. Recovered viruses were employed to study the expression of EGFP in Vero cells by fluorescence microscopy. Co-localization with organelle trackers suggested EGFP retention in the ER and showed that this protein is not in lysosomes or dispersed in the Golgi system. EGFP was not detected by immunoprecipitation of [S35]-metabolically labeled proteins from culture supernatants, providing further evidence for its intracellular location. Therefore, it is suggested that the YF virus E protein SA elements do not contain ER retention signals in contrast to those mapped for Hepatitis C virus (Op de Beeck *et al*., 2004). The mechanism by which the flavivirus E protein remain associated to the ER membranes, where virus assembly takes place, remains largely unknown.