Enterovirus replication membranes are enriched in all isoforms of small cellular GTPases Arf

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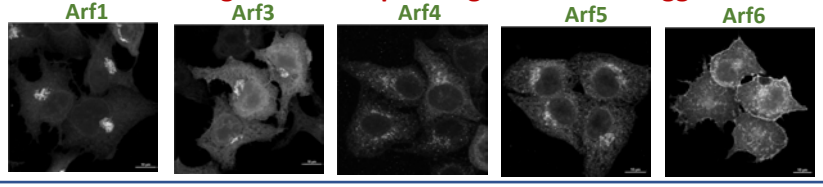
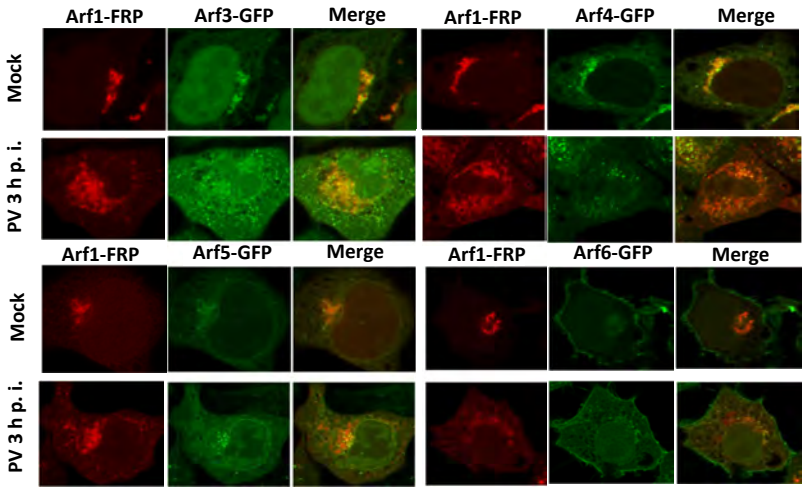
ABSTRACT

The small GTPases Arf regulate multiple steps of the cellular membrane metabolism. GBF1 catalyzes Arf-GDP to GTP exchange and is a known key host factor required for enterovirus replication, but the role of individual Arfs in the replication process is not understood. Here, we investigated the dynamics of activation of all human Arf isoforms upon enterovirus infection and identified those important to support the replication process. Arf1 appeared to be the first to associate with the replication organelles (ROs), followed by other Arf isoforms. Once activated and recruited to the ROs, all Arfs except Arf3 were no longer sensitive to inhibition of GBF1, suggesting that they do not actively cycle between GTP- and GDP-bound states in infected cells. siRNA targeting of individual Arfs revealed that depletion of Arf1 and to a lesser extent Arf6 caused a drastic inhibition of virus replication. Thus, our work reveals important details about the involvement of Arfs in the replication process and underscores the unique membrane metabolism of infected cells.

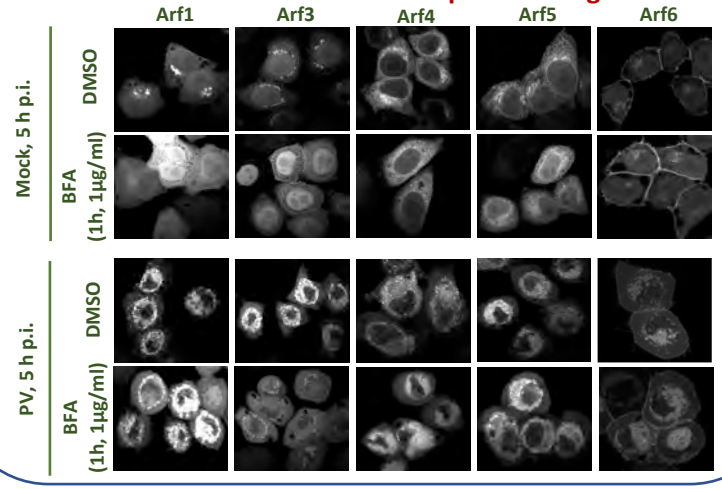
A. Establishing cell lines expressing human GFP-tagged Arfs



B. Arf1 is the first to associate with functional replication organelles.



C. Only Arf3 requires constant GEF activity to remain associated with the replication organelles.



D. Arf1 depletion strongly increases the sensitivity of viral replication to GBF1 inhibition.