

## Letter to the Editor

### Endogenous Virus of BHK-21 Cells Complicates Electron Microscopy Studies of Foamy Virus Maturation

Baldwin and Linial previously reported that in the absence of the envelope glycoprotein (Env), foamy virus (FV) particles were not released from BHK-21-derived FAB cells as determined by immunoprecipitation of FV proteins from culture media (2). These results were similar to those of Fischer et al. (5) who utilized 293T cells. Budding of Env-deficient viral particles from the plasma membrane (PM) was not seen by electron microscopy (EM) in either report. However, these two reports differed regarding EM evidence for intracellular FV budding in the absence of Env. Baldwin and Linial found a few examples of intracytoplasmic budding of Env-deficient FV particles in BHK-21-derived FAB cells, whereas Fischer et al. reported no such budding in 293T cells. Is there an explanation for this apparent discrepancy?

FVs are known to bud both intracellularly and at the PM (9, 12), in contrast to other exogenous retroviruses which regularly mature solely at the PM. FVs are also unique among retroviruses in that they possess an endoplasmic reticulum (ER) retrieval signal in Env (6, 8, 16). When this ER sorting motif was disrupted by mutagenesis, increased budding at the PM was observed by EM relative to wild-type FV (7). This result indicated a role for Env in partitioning the site of FV budding to intracytoplasmic membranes.

The published EMs of Env-deficient FV mutants in BHK-21-derived FAB cells (see Fig. 4D and E of reference 2) revealed viral particles within, or in the process of budding into, intracellular compartments. These EMs were presented to support the conclusion that Env-deficient FV can bud intracellularly. However, on further examination, it was recognized that the morphology of the particles in these images differed from those of the FVs in all other panels (see Fig. 4A, B, C, and F of reference 2). The particles in panels D and E possessed an electron-dense, mature-appearing viral core, as opposed to a characteristic electron-lucent, immature-appearing viral core typical of FVs (1, 9).

The literature contains a number of reports that endogenous viruses are present in BHK-21 cell lines (3, 4, 11, 13). These particles were termed "intracisternal R-type particles" (IRPs) (15) because of their unique morphology: spoke-like structures radiating (thus the R type) from the electron-dense mature-appearing viral core to the periphery of the particles. Review of the published EMs of these IRPs (3, 4, 10) revealed that the morphology of the intracellular Env-deficient viral particles in Fig. 4D and E (2) closely resembled that of the endogenous virus of BHK-21 cells. They are similar in size; both have an electron-dense core, from which lines radiate to the outer border of the particles; and they occur in the lumen of the ER or within the nuclear envelope and sometimes appear to be budding into these spaces.

On recent reexamination of the EMs of our control cells, i.e., untransfected BHK-21-derived FAB cells, we recognized the presence of infrequent virus-like particles budding intracellularly. These mature-appearing, spikeless particles were morphologically indistinguishable from both the endogenous IRPs in the literature and the particles in panels D and E of Fig. 4 of reference 2. We believe that the particles in panels D and E of Fig. 4 (2) represent the endogenous virus of BHK-21 cells.

It is therefore appropriate to conclude, as did Fischer et al. and Pietschmann et al. (5, 14), that FV budding, both intracellularly and at the PM, requires the viral envelope glycoprotein.

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