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# Letter to the Editor

## Cytomegalovirus Primary Envelopment at Large Nuclear Membrane Infoldings: What's New?

Cytomegalovirus morphogenesis has been the object of ultrastructural studies by electron microscopy (EM) since the 1960s, and the nuclear egress of nucleocapsids has been accurately documented by several studies which, despite the use of the "oldest" fixation methods, observed and described the infoldings at the inner nuclear membrane and hypothesized their active role in the primary envelopment and the early step of the maturation of virions (2–7).

The paper by Buser et al. (1) reports ultrastructural studies on the nuclear morphogenesis of human and murine cytomegalovirus by transmission EM. The authors observe "large tubular infoldings of the inner nuclear membrane that were . . . active in primary envelopment," as reported in the abstract. They describe and photographically document these virus-induced domains and performed semiquantitative analysis of the enlarged areas and the locations of primary enveloped nucleocapsids. Finally, they claim that "this is a previously undescribed structural element relevant in cytomegalovirus morphogenesis."

The aim of this letter is to challenge their statements, because these domains have been reported in many works and by different groups, including our research team, and they were first reported many years ago. We will cite only some of those papers and show how they defined this enlarged area that is continuous with the perinuclear space. This area has been documented by excellent photographs and sometimes results of semiquantitative analyses and/or schemes of virion morphogenesis from the nucleus that are very similar to those shown in Fig. 5 in the article by Buser et al. (1).

Ruebner et al. (5) observed for the first time intranuclear inclusions induced by murine cytomegalovirus in hepatic parenchymal cells. They described, by using EM, a frequently tortuous and multiple nuclear envelope where viral particles seemed to acquire a second membrane. In particular, in this process the inner nuclear membrane appeared to be more active than the outer one.

Severi et al. (6) suggested that the main way of egress from the nucleus is via peculiar structures described as invaginations or enlarged cisternae and denoted as "pseudoinclusions" and that nucleocapsids undergo sequential envelopment and deen-

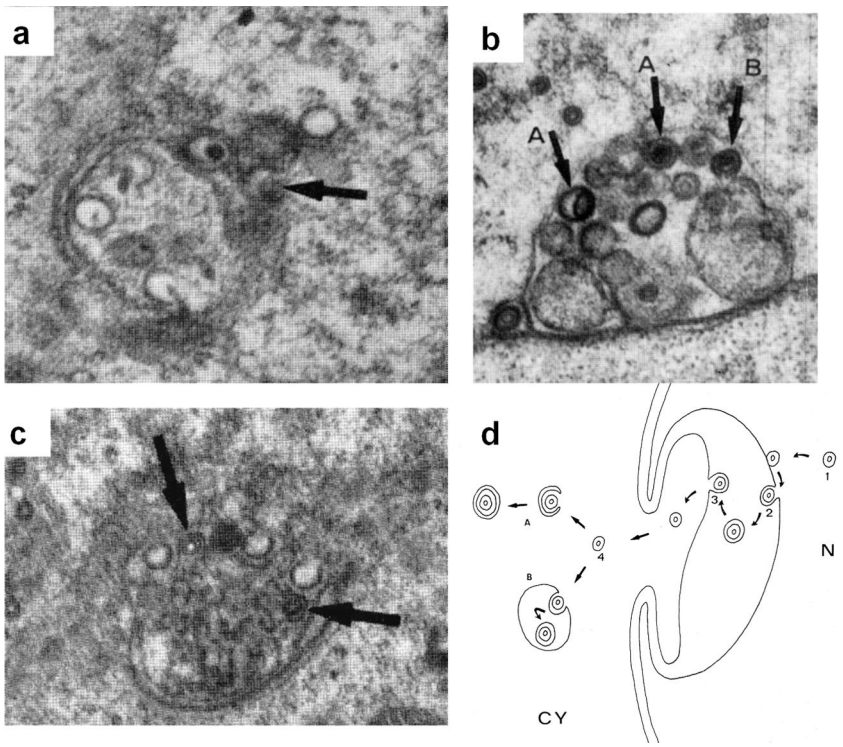


FIG. 1. Virus morphogenesis at nuclear pseudoinclusions. (a) Viral particle (arrow) budding into an enlargement of the perinuclear cisterna. (b) Primary enveloped viral particles in the enlarged perinuclear cisterna (arrows). (c) Two particles being released into the cytoplasm of the pseudoinclusion with the loss of their envelopes. (d) Model of cytomegalovirus nuclear egress. (Areas 1 and 2) Nucleocapsids budding from the nucleoplasm into the enlarged cisterna, acquiring a temporary envelope; (area 3) virion losing its envelope, entering the cytoplasmic portion of the pseudoinclusion; (area 4) free unenveloped viral particle in the cytoplasm, whose final envelopment occurs in the late cytoplasmic phases, as described in detail by Severi et al. (6). N, nucleus; CY, cytoplasm. (Reprinted from *Microbiologica* [5] with permission of the publisher.)

velopment when crossing the pseudoinclusion membranes (Fig. 1).

Papadimitriou et al. (4) investigated murine cytomegalovirus nuclear maturation and showed that nucleocapsids bud into the perinuclear cisternae and acquire an outer envelope from the inner nuclear membrane, lost by fusion with the outer nuclear membrane. Deep invaginations where virions most frequently bud from the nucleus were also observed, and perinuclear cisterna enlargement was photographically documented.

Severi et al. (7) confirmed by EM morphological changes in the nuclear structures of human cytomegalovirus-infected fibroblasts, describing irregularities of the nuclear outline, a widening of the perinuclear space, and the appearance of peculiar intranuclear saclike, membranous invaginations. Nucleocapsids were shown to acquire a temporary envelope budding from the inner nuclear membrane and to lose it by fusion with the outer membrane.

Gilloteaux and Nassiri (3) presented extensive EM ultrastructural observations on human bone marrow fibroblasts infected by cytomegalovirus. Many focal sites along the inner nuclear membrane displaying thickening and conspicuous invaginations are described in their report. Many viral particles were seen associated with this envelope, demonstrating what are known as pseudoinclusions or nuclear envelope proliferations as possible stages of exiting by the viral particles.

Dal Monte et al. (2) also described nuclear pseudoinclusions further characterized by the presence of the tegument protein pUL53, which is supposed to be acquired during the budding of virions. The authors cite only M53 and not human cytomegalovirus pUL53 in the context of virus maturation and egress from the nucleus.

Also, the intracytoplasmic events of virion maturation (envelopment by Golgi body-derived vesicles and egress from the cell membrane) are described by Buser et al. in exactly the same way that Severi et al. (7) described them.

The original but speculative datum is the lack of nuclear lamina in correspondence to inner nuclear membrane infoldings. Indeed, only immunolabeling using commercially available antibodies against lamins and the subsequent quantitative analysis may definitively confirm what Buser and colleagues claim.

None of the above-mentioned articles were cited as references by Buser et al. Thus, we include with this letter a panel of pictures (Fig. 1) from the report of Severi et al. (6), originally published in 1979, which show those nuclear structures and their suggested role in viral morphogenesis, and a list of essential references, as we are sure that the previous worthy work of other authors should be acknowledged by all readers of the *Journal of Virology*.

In conclusion, although we consider the extensive work from Buser et al., their semiquantitative analysis and their excellent photographic material, further clarifying the role of nuclear infoldings for viral morphogenesis, their study does not definitively elucidate the process. The study should have been done using other approaches (e.g., immunolabeling) and does not add any significantly new and original information, being largely a rerelease of previously published studies.

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## Authors' Reply

Thank you for your comments regarding our paper, "Cytomegalovirus Primary Envelopment Occurs at Large Infoldings of the Inner Nuclear Membrane" (1). We appreciate the fine work of the six publications that you bring to our attention, and we agree that it would have been plausible to cite these articles in our paper.

To answer your question, "What is new?" we will summarize the data and descriptions from the mentioned papers.

The papers mostly agree in that the nuclear egress takes place via an envelopment and deenvelopment process at the nuclear membranes and that primary enveloped virions are found in a sometimes enlarged perinuclear space.

The intranuclear "pseudoinclusions" described in these papers are cytoplasmic invaginations surrounded by both nuclear membranes. Severi et al. (6) state that "egress from the nucleus is via peculiar cytoplasmic pseudoinclusions" and is "interpreted as cytoplasmic pseudoinclusions for two main reasons: 1) they are surrounded by a double membrane which in the same areas forms a tetralamellar profile becoming tightly as-

sociated with the nuclear membranes; 2) they contain free ribosomes and material with the same electron density of the cytoplasmic matrix."

Ruebner et al. (5) wrote that the "nuclear membrane itself frequently became tortuous and sometimes multiple." The sentence cited in your letter, "The inner nuclear membrane appeared to be more active than the outer one," is not related to infoldings.

Papadimitriou et al. (4) also showed a cross-section of such a "cytoplasmic invagination," with virus particles within the perinuclear space (Fig. 4 in that article).

Severi et al. (7) showed a model (Fig. 10 in that article) where particles bud either into cytoplasmic invaginations or into the unmodified inner nuclear membrane, giving no information about the relative frequencies of these events.

Gilloteaux and Nassiri (3) showed infoldings with several membranes. The model (Fig. 11 in that article) shows invaginations of both nuclear membranes and a dilated perinuclear space (Fig. 11) by a widening of the outer nuclear membrane into the cytoplasm.

Dal Monte et al. (2) again described "pseudoinclusions" and referred to the articles by Severi et al. (6, 7). We did not observe such cytoplasmic infoldings, which differ fundamentally from the infoldings we reported.

Our new findings that were possible due to the improved structural preservation obtained with high-pressure freezing and freeze substitution are the following. We describe large tubular infoldings of only the inner nuclear membrane reaching deeply into the nucleus and present EM data that these infoldings appear free of lamina. It is very important to note that the outer nuclear membrane remains unaffected, which is crucial for our interpretation that the nuclear morphology is maintained during this phase of infection.

By statistical analysis, we found that about 86% of nucleocapsids bud at these infoldings, which constitute only about 4.8% of the total inner nuclear membrane surface.

This information cannot be found in the cited papers. We wish to emphasize that by no means do we want to debase the important contributions of the cited articles, but we are convinced that our paper clearly makes a new contribution to the process of cytomegalovirus nuclear egress.

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