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ORIGIN OF CYTOPLASMIC NUCLEOCAPSIDS IN MDBK-CELLS INFECTED WITH BOVINE HERPESVIRUS 1 (bHV-1)

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The nucleocapsid of herpesviruses is assembled within the nucleus and transported to the perinuclear space by budding through the inner nuclear membrane. The route from the perinuclear space to the plasma membrane for exocytotic release is assumed to involve loss of the acquired envelope by fusion with the outer nuclear membrane followed by wrapping of the nucleocapsid by Golgi membranes[1]. Alternatively, virions are thought to leave the perinuclear space via vacuoles originating from the outer nuclear membrane[1]. None of these processes has been shown so far. Instead, we showed intimate connection between Golgi complex and the perinuclear space implying immediate access of fully enveloped virions to Golgi cisterns for packaging[2]. These results, however do not explain the origin of naked nucleocapsids within the cytoplasm that are enveloped via wrapping by Golgi membranes. We thus examined the nuclear periphery of MDBK cells infected with BHV-1 by cryobased electron microscopy. Infected cells were high-pressure frozen at 4, 5, 6 and 7 hours of incubation, and freeze-substituted employing a protocol yielding high resolution of membranes[3].

Thin sections stained with uranyl acetate and lead citrate distinctly showed indications for perturbation of nuclear pores. The morphologic equivalent of the nuclear complex in thin sections of cryofixed specimens is a dense central layer filling entirely the nuclear pore of 100 to 110 nm, flanked by cloudy but broad layers at the nuclear and cytoplasmic side. Mild changes comprised widening of nuclear pores up to about 200 nm and loss of nuclear pore complex structures. The nuclear matrix made indentations into the cytoplasmic matrix through nuclear pores of 200 to 300 nm in diameter. Some of the nuclear matrix merged in the cytoplasm through nuclear pores enlarged up to 700 nm but formed delta-like protrusions at nuclear pores with a diameter between 700 and 1900 nm. Nucleocapsids were present within or close to enlarged nuclear pores. Changes of nuclear pores were rarely found in cells incubated for 4h. The number of impaired nuclear pores was drastically increased at 6 and 7 hours of incubation. The same was true for the number of naked nucleocapsids within the cytoplasm as reported elsewhere[2].

The findings strongly suggest that nucleocapsids are released from the nucleus via impaired nuclear pores. The cytoplasmic nucleocapsids are obviously transported to Golgi areas where they are wrapped by Golgi membranes[1,2]. Wrapping results in small sphere-like transport vacuoles containing a single enveloped virion that is transported to the plasma membrane for exocytotic release. Considering that one pathway of BHV-1 envelopment includes budding at the inner nuclear membrane, intracisternal transport followed by packaging within the Golgi-complex the pathway described here represents a second entirely different pathway that seems to take place predominantly late in infection.

References:
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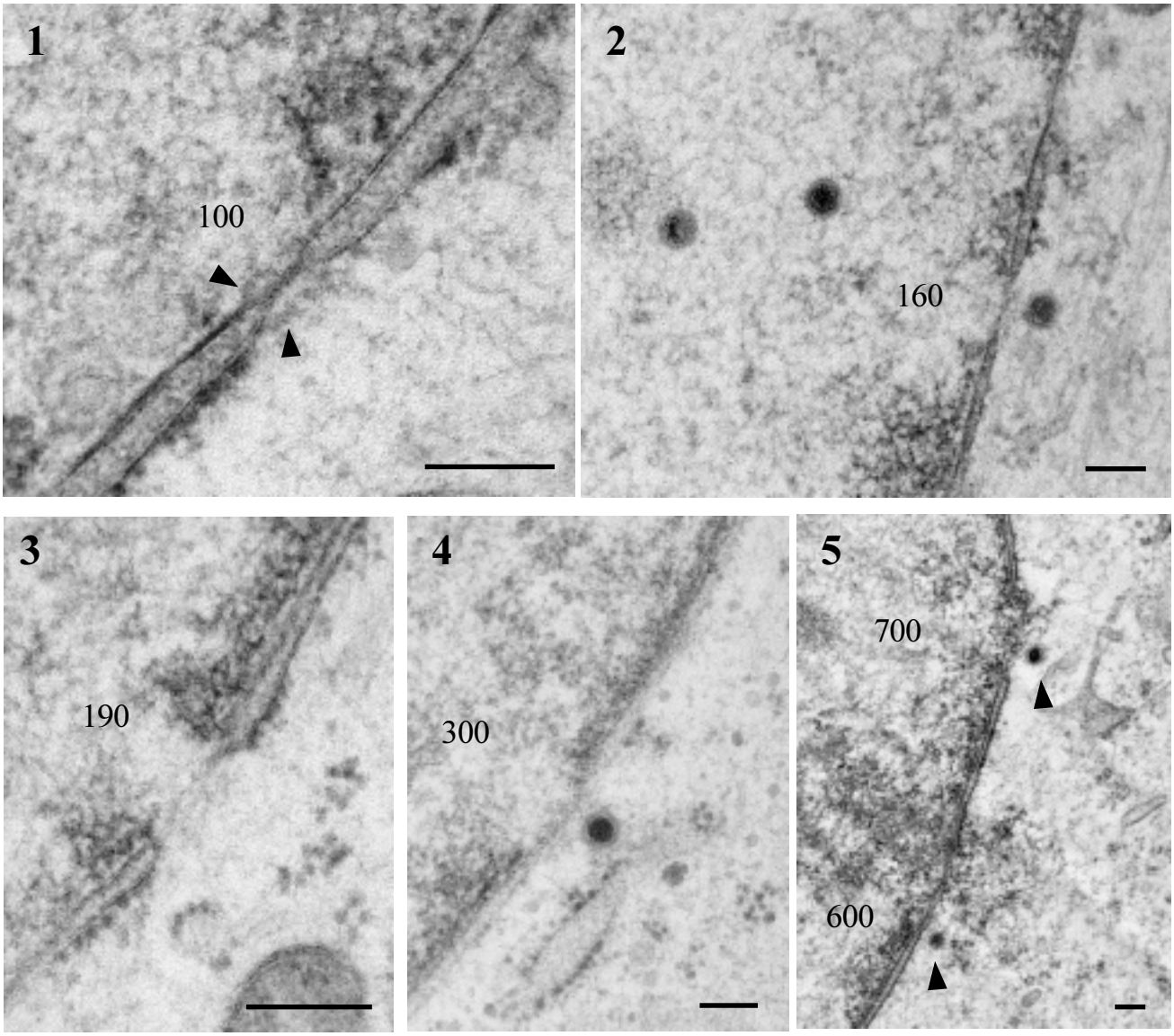

FIG. 1 Nuclear pore (100nm) with intact nuclear pore complex showing a distinct central layer and a cloudy layer (arrowheads) at the nuclear and the cytoplasmic site. Bars, 200nm

FIG. 2 Two naked nucleocapsids within the nucleus and one within the cytoplasm close to a wide (160 nm) nuclear pore. Bars, 200nm

FIG. 3 Nuclear pore of 190 nm in diameter missing distinct nuclear pore structures. Bars, 200nm

FIG. 4 Nuclear pore 300 nm in width. Nuclear matrix is slightly bent toward the cytoplasm which contains a naked nucleocapsid immediately adjacent to the nuclear pore. Bars, 200nm

FIG. 5 Two enlarged nuclear pores (600 and 700 nm) through which nuclear matrix merges into the cytoplasm that contains nucleocapsids (arrowheads) just outside of the impaired nuclear pores. Bars, 200nm

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