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SHORT COMMUNICATION

"Cell-in-cell" phenomena among the smallest cells

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Summary

The note briefly mentions facts reminiscent of the "cell-in-cell" phenomenon for some of the smallest cells in nature. Comparisons with higher eukaryotic cells and unicellular organisms (protists), among which the "cell-in-cell" structures are widely known, are presented. It is concluded that such biological phenomena may be universal for cells without a rigid cell wall.

Key words: cell-in-cell, eukaryotic cell, protists, bacteria, mycoplasmas, general biological significance

Can the "cell-in-cell" phenomenon be global in nature and persist in parallel in extremely evolutionary-distant biological objects? The "cell-in-cell" structures are widely known among eukaryotes (Fais and Overholtzer, 2018; Davies et al., 2020; Demin et al., 2022). It is characteristic not only of the cells functioning with some disorders (cancer), but also of the completely healthy cells of both multicellular and unicellular organisms, e.g. protists. It is worth noting from the very beginning that this term is applicable only to cells of the same species, contrary to many known cases of parasitism or symbiosis of some unicellular organisms inside the cells of the other species (Vishnyakov, 2021). As for protists, there the cases of "cell-in-cell" are largely attributed to the natural cannibalism shown for various unicellular organisms (Devi, 1964; Pierce et al., 1978). This phenomenon can lead to temporal multinuclearity of protists and facilitate the exchange of genetic information. Apart from that, the complete or partial fusion of vegetative cells – somatogamy – is common in the protistan life cycles (Demin et al., 2022).

As for bacteria, usually a cell wall prevents cells from fusing with each other. However, some objects resembling the "cell-in-cell" structures have been described for the wall-less prokaryotes – mycoplasmas (Vishnyakov, 2022). Mycoplasmas (class Mollicutes) do not have a cell wall but possess only the bilayer cytoplasmic membrane (reviewed by Borchsenius et al., 2016). Earlier, the ability of mycoplasma cells to fuse (but not of one cell to penetrate into another) has been shown only through artificial induction from the outside (Tarshis et al., 1991). In the recent work (Vishnyakov, 2022) it was established that rather different mycoplasmas – a phytopathogen *Acholeplasma laidlawii* (Chernov et al., 2014) and a human pathogen *Ureaplasma parvum* (Sweeney et al., 2016) – both could form unusual "cell-in-cell"-like structures (see the schematic representation of the structures in Fig. 1).

The probable absorption by the *A. laidlawii* cell of some material of low electron density but granular consistency (Fig. 1, A, 1), or structures similar to extracellular vesicles (Fig. 1, A, 2), as well as the relatively small vegetative cells (about 200–300 nm

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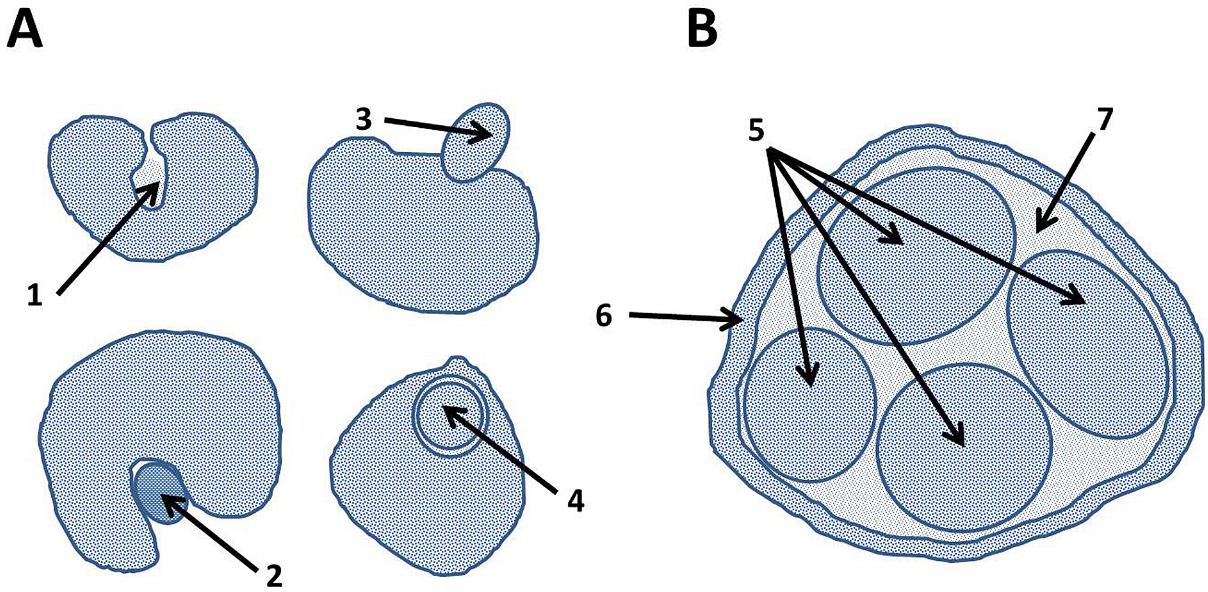

Fig. 1. Schematic representation of *Acholeplasma laidlawii* (A) and *Ureaplasma parvum* (B) cells in the light of the "cell-in-cell" phenomenon (according to the electron microscopic data presented in Vishnyakov, 2022). 1 – The probable membrane invagination and absorption of some material of low electron density but granular consistency; 2 – the probable membrane invagination and absorption of a structure resembling one of the extracellular vesicles of *A. laidlawii*; 3 – an initial moment of the probable membrane invagination and absorption of a structure resembling one of the mini-bodies (mini-cells) of *A. laidlawii*; 4 – the probable cell-in-cell structure of *A. laidlawii*; 5 – smaller cells of *U. parvum* inside the larger one (6); 7 – electron-dense material between the inner cytoplasmic membrane of the host *U. parvum* cell and the encapsulated *U. parvum* cells of smaller size.

in diameter) (Fig. 1, A, 3, 4) was observed earlier (Vishnyakov, 2022). Even under optimal growth conditions, this phenomenon was extremely rare. The presence of an electron-dense fibrillar material between the internalized (donor) and the host (recipient) cells (Fig. 1, A, 4) excludes an accidental entry of a smaller cell into a large one. In general, this may indicate the natural character of the observed phenomenon.

Also very rarely, *U. parvum* cells of a smaller size (300-500 nm in diameter) (Fig. 1, B, 5) can be enclosed inside a larger cell (with size ~1 μm or more in diameter) (Fig. 1, B, 6) (Vishnyakov, 2022).

The cells inside the larger one can keep a classic two-layer cytoplasmic membrane, at least for some time. At the same time, the presence of certain strands of electron-dense material in the cavity between the host cell and the smaller ones was also well observed (Fig. 1, B, 7). This speaks in favor of the natural character of the observed phenomenon rather than of possible artifacts.

Presumably, the ability to fuse and penetrate into other cells is one of the fundamental features of all living biological objects without a rigid cell wall at the cellular level of organization, which provides them with the mainstream strategy of survival and biodiversity maintenance. However, what are the causes and "purposes" driving forces of such phenomena? For example, a much larger exchange of genetic material can take place when cells merge and infiltrate into other cells of the same species, and this can play a role at the evolutionary-large scale. Specifically, the presence of ICEs (integrative conjugative elements) could facilitate an intensive exchange of large regions of mycoplasmal nucleoids, which are enclosed in a common space, with each other (García-Galán et al., 2021). Due to ICEs, the time scale of the mutational pathway leading to high-level of antimicrobial resistance of mycoplasmas can be readily compressed into a single conjugative step (Faucher et al., 2019). A global genetic exchange (ICEs + "cell-in-cell") in theory may prevent their genomes from stasis and contribute to adaptation to new hosts.

The probable "cell-in-cell" facts of mycoplasma cells presented in this paper do not resemble cell-

cell fusions but rather recall the process of material absorption like phagocytosis with the subsequent loss of the lipid membrane in the case of internalized cells (cannibalism?). It likely reminds entosis, when cell-cell invasion and retarded lysis of internalized cell occurs (Mlynarczuk-Bialy et al., 2020), or emperipolesis, when the engulfed cell can move inside the host cell for some time and even exit it (Rastogi et al., 2014). Moreover, cases of not only destruction of internalized cells, but also their proliferation within cancer cells of higher eukaryotes have been described (Durgan and Florey, 2018). This is very similar to what we see in the case of *U. parvum* (Fig. 1, B).

Despite the rare occurrence in mycoplasmal cultures, the fusion of microbial cells with each other and their potential penetration one into another with the probable genetic exchange may be of potential interest. If, in addition to the "classic" horizontal gene transfer and "non-classic" conjugation, "cell--in-cell" phenomena in wall-less bacteria are possible, then the mechanisms of such fusion can potentially affect the stability and plasticity of the genome of pathogenic mycoplasmas. The provisional mechanism, in theory, can also help these bacteria and the relative ones (phytoplasmas) to escape from the protective systems of the host organisms or medications.

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References

Borchsenius S.N., Chernova O.A., Chernov V.M. and Vishnyakov I.E. 2016. Mycoplasmas in Biology and Medicine of the Early 21st Century. Nauka, St. Petersburg (in Russian). https://www.researchgate.net/publication/329324534_Mikoplazmy_v_biologii_i_medicine_nacala_XXI_veka

Chernov V.M., Mouzykantov A.A., Baranova N.B., Medvedeva E.S., et al. 2014. Extracellular membrane vesicles secreted by mycoplasma *Acholeplasma laidlawii* PG8 are enriched in virulence proteins. J. Proteomics. 110: 117–128. https://doi.org/10.1016/j.jprot.2014.07.020

Davies S.P., Terry L.V., Wilkinson A.L. and Stamataki Z. 2020. Cell-in-cell structures in the liver: a tale of four E's. Front. Immunol. 11: 650. https://doi.org/10.3389/fimmu.2020.00650

Demin S., Berdieva M. and Goodkov A. 2022. Cell-cell fusions and cell-in-cell phenomena in healthy cells and cancer: Lessons from protists and invertebrates. Semin. Cancer Biol. 81: 96–105. https://doi.org/10.1016/j.semcancer.2021.03.005

Devi R.V. 1964. Cannibalism in *Frontonia leucas* Her. J. Protozool. 11: 304–307. https://doi.org/10.1111/j.1550-7408.1964.tb01758.x

Durgan J. and Florey O. 2018. Cancer cell cannibalism: Multiple triggers emerge for entosis. Biochim. Biophys. Acta Mol. Cell. Res. 1865 (6): 831–841. https://doi.org/10.1016/j.bbamcr.2018.03.004

Fais S. and Overholtzer M. 2018. Cell-in-cell phenomena in cancer. Nat. Rev. Cancer. 18 (12): 758–766. https://doi.org/10.1038/s41568-018-0073-9

Faucher M., Nouvel L.X., Dordet-Frisoni E., Sagné E., et al. 2019. Mycoplasmas under experimental antimicrobial selection: The unpredicted contribution of horizontal chromosomal transfer. PLoS Genet. 15 (1): e1007910. https://doi.org/10.1371/journal.pgen.1007910

García-Galán A., Baranowski E., Hygonenq M.C., Walch M., et al. 2021. Genome mosaicism in field strains of *Mycoplasma bovis* as footprints of in-host horizontal chromosomal transfer. Appl. Environ. Microbiol. 20: AEM0166121. https://doi.org/10.1128/AEM.01661-21

Mlynarczuk-Bialy I., Dziuba I., Sarnecka A., Platos E., et al. 2020. Entosis: from cell diology to clinical cancer pathology. Cancers (Basel). 12 (9): 2481. https://doi.org/10.3390/cancers12092481

Pierce E., Isquith I. and Repak A.J. 1978. Quantitative study of cannibal-giantism in *Blepharisma*. Acta Protozool. 17 (4): 493–501.

Rastogi V., Sharma R., Misra S.R., Yadav L. and Sharma V. 2014. Emperipolesis – a review. J. Clin. Diagn. Res. 8 (12): ZM01–ZM02. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4316366

Sweeney E.L., Dando S.J., Kallapur S.G. and Knox C.L. 2016. The human *Ureaplasma* species as causative agents of chorioamnionitis. Clin. Microbiol. Rev. 30 (1): 349–379. https://doi.org/10.1128/cmr.00091-16

Tarshis M., Salman M. and Rottem S. 1991. Fusion of mycoplasmas: the formation of cell hybrids.

FEMS Microbiol. Lett. 66 (1): 67–71. https://doi.org/10.1111/j.1574-6968.1991.tb04841.x

Vishnyakov I.E. 2021. Symphony of minimalism: peculiar endosymbiosis of mycoplasmas and protists. Protistology. 15 (1): 24–33. https://www.zin.ru/journals/protistology/num15_1/vishnyakov_protistology_15-1.pdf

Vishnyakov I.E. 2022. Cell-in-Cell phenomena in wall-less bacteria: Is it possible? Int. J. Mol. Sci. 23 (8): 4345. https://doi.org/10.3390/ijms23084345