HRVATSKO MIKROSKOPIJSKO DRUŠTVO

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Hrvatskog mikroskopijskog društva, koji će se održati u prostorijama
Instituta „Ruđer Bošković“, Bijenička cesta 54, predavaonica **I. krila**
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u
utorak, 26. srpnja 2022. u 16:00 sati
u organizaciji Vide Strasser

uz sljedeći

D n e v n i r e d:

1. **Predavanje stipendista 4. hrvatskog mikroskopijskog kongresa:**
   - **Kruno Vukušić:** Small GTPases interacting with IqgC on *Dictyostelium macropinosomes*
   - **Bruno Komazec:** Effect of silver nanoparticles and ions on oxidative stress formation and antioxidative machinery of *Chlorella vulgaris*

2. **Razno**

Tajnica:
Vida Strasser

Predsjednica:
Suzana Šegota

Peripheral chromosomes positioned behind the spindle poles are prone to chronic unalignment in tumour cell lines

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Mitosis encompasses series of ordered and controlled events that result in a precise separation of sister chromatids into two daughter cells. At a core of mitotic fidelity is the process of congression, movement of chromosomes towards the equatorial plane of the mitotic spindle. At metaphase in a healthy cell, most chromosomes have successfully aligned at the spindle equator. However, by large-scale and long-term live-cell imaging of several human cell lines from prophase to telophase, we observed that congression occasionally fails, resulting in chronically unaligned chromosomes, which remain close to the pole and induce substantial mitotic delays. We observed that the frequencies of unaligned chromosomes range from 42-22 % in tumour U2OS and HeLa cells, to 1.9 % in non-transformed RPE-1 cells. Furthermore, in 4 % of tumour cells, the unaligned chromosome remained at the pole through mitosis, resulting in aneuploidy. However, it is unknown whether there is any intrinsic spindle-based bias that would predispose certain chromosomes to unalignment and consequentially missegregation. Here we show, by tracking the movements of chronically unaligned chromosomes in U2OS cells, that they originated mainly from the nuclear periphery during prophase, contrary to pairs that rapidly aligned. Furthermore, unaligned chromosomes were preferentially positioned behind their respective spindle poles at the beginning of mitosis, indicating that passage across the polar region can drastically impede the speed of chromosome congression in tumour cells. Interestingly, during metaphase, unaligned chromosomes were characterized by persistent positions behind and near their respective poles, indicating that incapacity to congress is often combined with a high extent of minus-end directed movements during metaphase. Furthermore, unaligned chromosomes have severely reduced stability within the metaphase plate after the attempt of congression, compared to pairs that aligned during prometaphase. Even after successful alignment, unaligned chromosomes are repeatedly followed by persistent laggards, whereas chromatin bridges are not related to this phenomenon, indicating a possible connection between late alignment and a higher incidence of merotelic attachments. Lastly, by using stimulated emission depletion (STED) super-resolution microscopy, we showed that most laggards during anaphase in U2OS cells are indeed characterized by unresolved complex merotelic attachments. In conclusion, peripheral chromosomes located behind the spindle poles at the beginning of mitosis are particularly prone to chronic unalignment, which is harmful for mitotic fidelity.

Keywords: aneuploidy, chromosome missegregation bias, mitosis, live-cell imaging, super-resolution microscopy

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Effect of silver nanoparticles and ions on oxidative stress formation and antioxidative machinery of *Chlorella vulgaris*

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Silver nanoparticles (AgNPs) are used in a variety of industries and, due to their tendency to agglomerate in various media, they are commonly stabilised with various surface coatings such as carboxylic acids (citrate) or surfactant cetyltrimethylammonium bromide (CTAB), among others [1]. As the use of AgNPs increases, so does the potential for their release into the aquatic environment. Since *Chlorella vulgaris* is one of the most ubiquitous algae inhabiting aquatic ecosystems, it is widely used as a model organism for assessing the impact of materials of anthropogenic origin, e.g. AgNPs, on aquatic habitats [2]. To evaluate the impact of AgNP on *C. vulgaris*, algal cultures were grown in a liquid BBM nutrient medium for four days, after which they were exposed to AgNPs coated with citrate or CTAB and to $AgNO_3$, which was used as ionic Ag control. Concentration endpoints were obtained by growth inhibition test (72 h) and the estimated 25 % inhibition of growth rate (EC25) values for the AgNP-citrate, AgNP-CTAB and $AgNO_3$ were 0.188 mg L^{-1}, 0.895 mg L^{-1} and 0.130 mg L^{-1}, respectively. After 72 h treatment, the quantity of newly synthesised oxygen, damage to membrane lipids as measured through malondialdehyde (MDA) content, levels of reactive oxygen species (ROS) and activity of antioxidant enzymes (superoxide dismutase-SOD, catalase-CAT, ascorbate and pyrogallol peroxidase-APX and PPX) were analysed. To understand the ROS formation, algal suspensions were incubated with fluorogenic dyes through which hydroxyl and peroxyl activity within the cells (H_2DCFDA) or superoxide ($O_2^{\cdot -}$) generation (DHE) were determined, with both dyes providing a measure of other ROS activity through non-specific oxidation. Moreover, to further examine the interaction between AgNPs and algal cells, the treated organisms were observed directly by transmission electron microscopy (TEM). Obtained results showed a detrimental effect of all treatments on the photosystem of *C. vulgaris* since a significant decrease in oxygen synthesis was reported. Similarly, all treatments have increased ROS formation and MDA content, which is indicative of membrane lipids damage. Interestingly, only increases in PPX and CAT activity were observed after treatment with AgNP-citrate, while for APX and for other treatments a trend of decreasing activity of antioxidant enzymes was observed. TEM analysis of algae cells showed multiple NPs in extracellular polymeric substances (EPS) of algae cells which could aid in decreasing toxic effect of AgNPs on algal cells (Fig. 1). In conclusion, all silver nanoparticles (AgNP-citrate and AgNP-CTAB) and ionic silver showed damaging effects on the photosynthetic apparatus of *C. vulgaris* algae. The parallel increase in ROS formation and decrease in the activity of the antioxidant machinery after 72 h treatment can be explained by an overproduction of ROS (Fig. 1), which consequently results in a change of enzyme structure and activity [3].

Keywords: *Chlorella vulgaris*, oxidative stress, silver nanoparticles

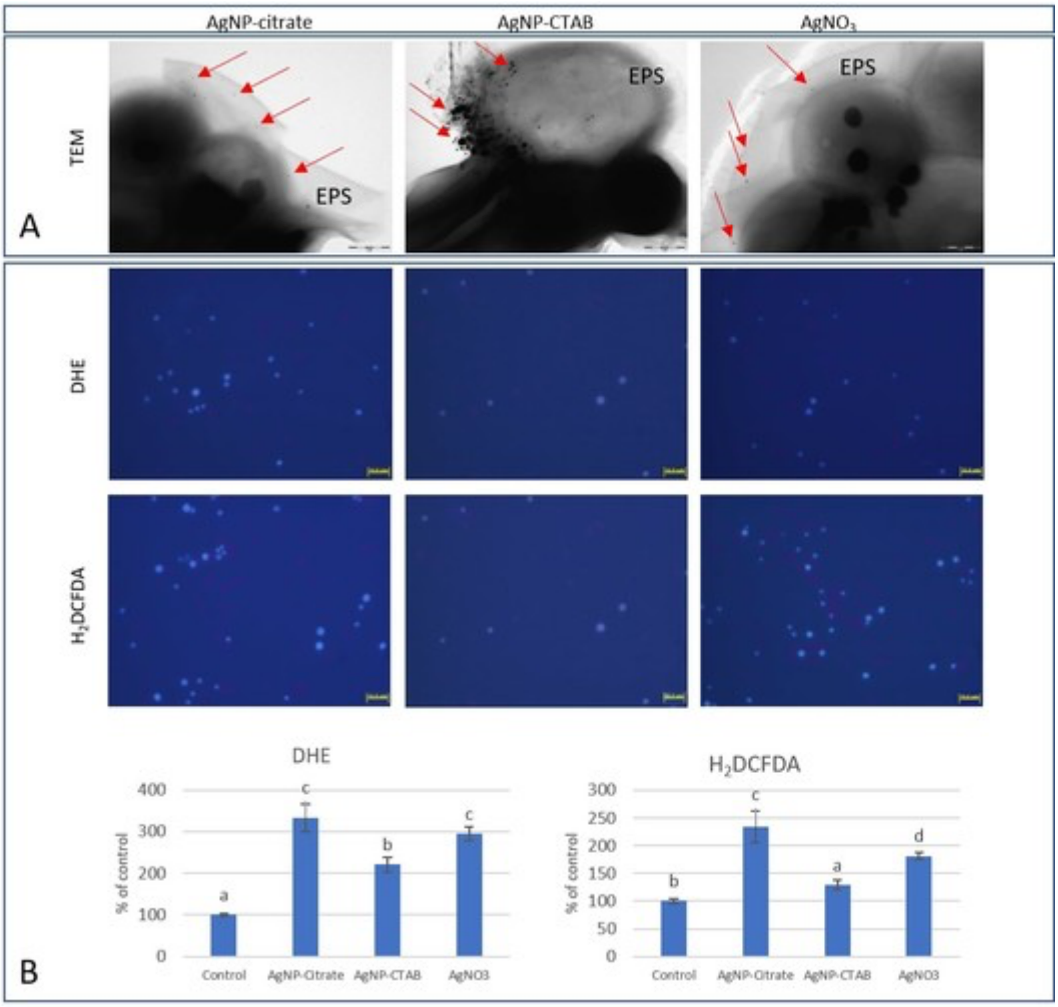

Figure 1. *C. vulgaris* cells after AgNP-citrate, AgNP-CTAB or $AgNO_3$ treatment obtained by (A) TEM, showing NPs (arrows) in the EPS (scale = 1 μm); (B) fluorescence microscopy showing ROS increase via fluorescent probes DHE or H_2DCFDA (scale = 10.8 μm).

References:
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2. S. Wu et al., Environ. Eng. Sci. 31 (2014) 9–17.
3. R. Van der Oost et al., Environ. Toxicol. Pharmacol. 13 (2003) 57–149.