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DISCOORDINATION OF *HOILUNGIA HONGKONGENSIS* (PLACOZOA) MOVEMENTS IN THE PRESENCE OF Zn^{2+} IONS

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Placozoa are the simplest multicellular organisms with a dynamic body plan. Calcium ions play a significant role in maintaining the integrity of these animals. We studied the effect of zinc ions on the interaction of *Hoilungia hongkongensis* cells. The coordination of amoeboid movement was disrupted by the addition of 20–25 μM Zn^{2+} ions, which led to the formation of "branched" forms of animals. Locomotor ciliated cells moved without coordination and independently of each other. Experimental research showed that the contact interaction of *H. hongkongensis* cells is important for coordinated movements of the organism, whereas zinc ions can compete with calcium ions, disrupting the regulation and destroying the connection between cells.

Keywords: Placozoa, locomotion, calcium and zinc ions

Trace elements may have played a key role as catalysts for the emergence of life on Earth. The change in trace element composition in the oceans over geological time suggests how their availability in the marine environment affected the early stages of biological evolution [Dupont et al., 2006]. Especially in the Archean Eon the excess of Fe, Co, and Cu ensured their widespread utilization by prokaryotes. In the Paleoproterozoic–Mesoproterozoic, there was a rise in Mo content in the ocean and a notable drop in Fe, Co, and Cu, which contributed to the appearance of alternative metabolic pathways and the birth of eukaryotes. In the Neoproterozoic and Cambrian, the general gain in Mo, Zn, Ni, and Cu concentration was favorable for the complexity and diversification of ancient biota [Robbins et al., 2016]. It has been suggested that the emergence of the first Placozoa organisms was associated with the ratio of Mg^{2+} and Ca^{2+} ions concentrations in the aragonite ocean during the Ediacaran or Cryogenian [Erwin, 2015; Mayorova et al., 2018].

Hoilungia hongkongensis Eitel, Schierwater & Wörheide, 2018 (haplotype H13), the same as *Trichoplax adhaerens* Schulze, 1883 (haplotype H1), is the simplest multicellular organism up to 1–2 mm in size, which consists of about 50,000 cells that form a three-layer plate [Smith et al., 2014]. The movement of this animal is performed due to the beating of the cilia of the ventral epithelium and the contraction of the plate [Armon et al., 2018]. As believed, the organism's integrity is maintained by Ca^{2+} bridges since trichoplax is destroyed by chelating agents that bind calcium ions [Ruthmann, Terwelp, 1979].

It is well known that metal ions compete for binding sites with proteins, according to the Irving–Williams order of stability of bivalent transition metal complexes as $Mg^{2+}/Ca^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} \sim Zn^{2+}$ [Rosenzweig, 2002], while Zn^{2+} ions replace Ca^{2+} ions during adsorption [Zachara et al., 1988]. A previous study [Kuznetsov et al., 2021] showed that Zn^{2+} ions, the same as Ca^{2+} ions, are located in specific binding sites of cadherin cell adhesion molecules and, thus, destroy calcium bridges that leads to the dissociation of the trichoplax body into individual cells. In this paper, the emphasis is on the analysis of a violation of the interaction between *H. hongkongensis* cells under the effect of zinc ions.

The aim of this work is to study morphological changes in *Hoilungia hongkongensis* when Zn^{2+} ions are added.

MATERIAL AND METHODS

H. hongkongensis was cultivated in 90-mm glass Petri dishes on mats of the unicellular green microalga *Tetraselmis marina* (Cienkowski) R. E. Norris, Hori & Chihara, 1980. The dishes were kept in a thermostat at +25 °C. Animals were transferred to a fresh mat every three weeks, and artificial seawater (ASW) with a salinity of 35 ‰ was changed once a week [Kuznetsov et al., 2021, 2022]. At least 20 *H. hongkongensis* organisms were picked up for each experiment. More than 500 animals were used in a series of 3 experiments. For adaptation, *H. hongkongensis* were transferred into plastic Petri dishes with ASW without algae 30–45 min before the beginning of the experiment. $ZnCl_2$ solution was added to the individuals that were kept in 50 mL of ASW, so that Zn^{2+} ions final concentration was raised by 10–25 μM, and the animals were then investigated for different time intervals: 15 and 30 min, as well as 1, 2, 4, and 24 h. The studies were carried out at magnification from ×40 to ×400 under light microscopes Zeiss Stemi 305 (Germany) and Nikon Eclipse Ts2R (Japan) equipped with digital cameras.

RESULTS AND DISCUSSION

H. hongkongensis incubation for 2 h after the addition of 15–25 μM Zn^{2+} ions caused an alteration in the structure and shape of the body plate, which manifested itself in its thickening and the appearance of multiple “branched” structures resembling tentacles in some animals. These “tentacles” did not move, while the organism body moved forward. The animals retained the vortex motions of cell groups in the center of the plate and were able to move forward.

Further exposure of *H. hongkongensis* for 24 h with additional 15 μM Zn^{2+} ions in ASW caused an elevation in the share of organisms with the “branched” structure (68.1%) or partially decomposed animals (2.8%). Interestingly, the parts of the disintegrated individuals continued to move without coordination and independently of each other due to the beating of the cilia of the ventral epithelium cells (Fig. 1b, d). The addition of 25 μM zinc ions resulted in a gradual increase in the proportion of “branched” and destroyed animals to 77.1 and 16.3%, respectively (Fig. 2).

There are such mechanisms of zinc toxicity as copper displacement from intracellular proteins [Plum et al., 2010], superoxide generation [Ninsontia et al., 2016], and binding to thiol groups [Gazaryan et al., 2002]. Zn^{2+} ions can interact with Ca^{2+} binding sites on cadherin and calmodulin [Kuznetsov et al., 2021]. It is worth noting that with a gradual rising in the concentration of Zn^{2+} ions capable of replacing Ca^{2+} ions, the share of “branched” and decaying animals increased proportionally. Apparently, the described morphological alterations are due to initial disruption of signaling between individual cells [Jékely, 2021; Senatore et al., 2017; Varoqueaux et al., 2018] and the consequent destruction of direct physical contacts between them [Ruthmann, Terwelp, 1979], since Ca^{2+} ions are involved in the transmission of signals into the cell in addition to cell adhesion [Berridge et al., 2003].

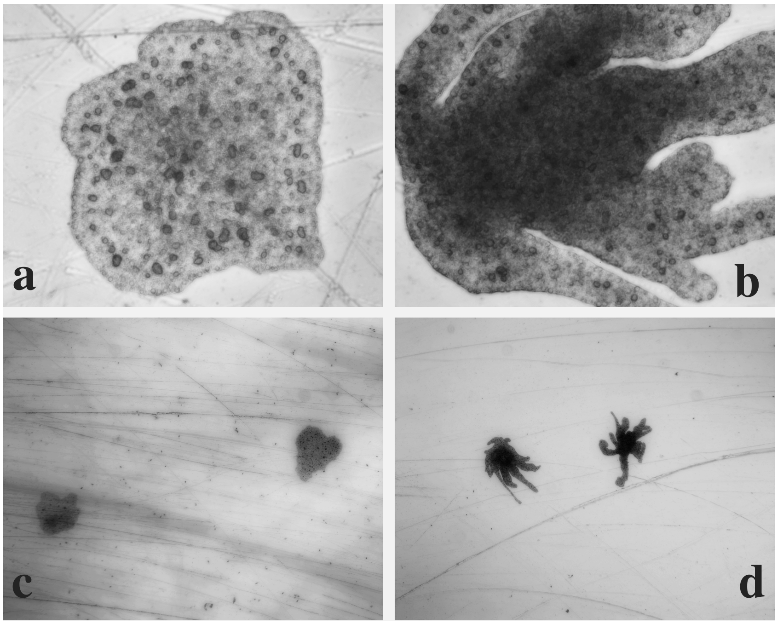

Fig. 1. *Hoilungia hongkongensis* morphological changes following a day of incubation with zinc ions: a, c, control without reagents; b, d, 20 μM Zn^{2+}. Magnification: a, b, 400 times; c, d, 40 times

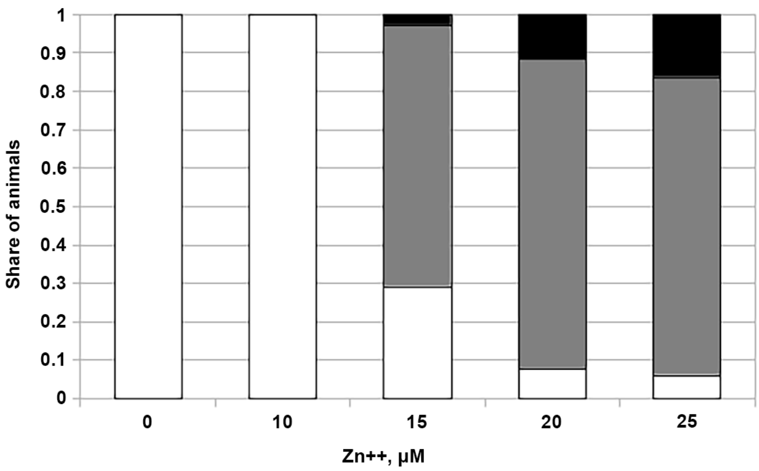

Fig. 2. Quantitative examination of abnormalities that occurred when *Hoilungia hongkongensis* was incubated with zinc ions at various doses for a day. Intact individuals are shown in white color; "branched" individuals are shown in gray; motionless, damaged, or completely destroyed individuals are shown in black. There were 245 animals used in the experiment

Let us pay attention to zinc toxicity for the freshwater sponge *Ephydatia fluviatilis* (Linnaeus, 1759) at a concentration exceeding 0.1 μM [John, Harrison, 1988]. This is comparable to data from our experiments on Placozoa organisms, as well as to data for two *Hydra* species, *Hydra vulgaris* Pallas, 1766 and *Hydra viridissima* Pallas, 1766, for which 96-h LD_{50} values are about 35 and 14 mM, respectively [Holdway et al., 2001]. As shown, the starlet sea anemone *Nematostella vectensis* Stephenson, 1935 activates the genes for early transcription factors Egr1, AP1, and NF-κB already an hour after exposure to Hg, Cu, Cd, and Zn ions. This is followed by the expression of stress response genes, including *Hsp*, *ABC*, and *CYP* [Elran et al., 2014], which can compensate for the toxic effect of heavy metal ions. It is known that *Trichoplax* sp. H2, whose genome is well annotated [Kamm et al., 2018], contains orthologous proteins, such as Egr1 (RDD41957.1), NF-κB (RDD44621.1), and Hsp (RDD46759.1). Perhaps, a similar defense systems exist in *H. hongkongensis*.

Thus, experimental exposure to Zn^{2+} ions leads to a discoordination of amoeboid movement in *H. hongkongensis*, which is observed in the emergence of uncontrolled "tentacles." This is consistent with the results of experiments on *Trichoplax* sp. H2 [Kuznetsov et al., 2021]. Some areas of *H. hongkongensis* plate begin to move without coordination and independently of each other. Later, the animal body dissociates into separate cells, which may be the result of Zn^{2+} ions interference in the regulation of two fundamental processes by Ca^{2+} ions: 1) coordinating the joint cell functioning and 2) maintaining the integrity of the animal body structure.

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НАРУШЕНИЕ КООРДИНАЦИИ ДВИЖЕНИЙ *HOILUNGIA HONGKONGENSIS* (PLACOZOA) В ПРИСУТСТВИИ ИОНОВ Zn^{2+}

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Hoilungia hongkongensis принадлежит к типу пластинчатые (Placozoa) — простейшим многоклеточным организмам с динамическим планом строения тела. В поддержании целостности этих животных важную роль играют ионы кальция. В настоящей работе экспериментально изучено влияние ионов цинка на взаимодействие клеток *H. hongkongensis*. При увеличении концентрации ионов Zn^{2+} на 20–25 мкМ нарушается согласованность амёбоидного движения, что приводит к образованию «ветвистых» форм животного. Локомоторные реснитчатые клетки двигаются хаотично и независимо друг от друга. Эксперименты показали, что контактное взаимодействие клеток *H. hongkongensis* важно для скоординированных движений организма, в то время как ионы цинка могут конкурировать с ионами кальция, нарушая регуляцию и разрушая связь между клетками.

Ключевые слова: пластинчатые, локомоция, ионы кальция и цинка