Continuous monitoring of cell division in Dictyostelium and its response to interference with sterol biosynthesis

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Introduction

Cells of the model system Dictyostelium discoideum can be grown under various conditions. The food source can be bacteria, which resemble the natural situation of these unicellular amoebae, or an axenic medium, consisting of sugar, protein hydrolysate and yeast extract for providing nutrients, vitamins and trace elements. Both food sources can be offered on agar or plastic surfaces, respectively, as well as in Erlenmeyer flasks under continuous orbital shaking to ensure aeration (Maniak and Nellen, 2014). Growth in axenic medium with shaking increases the number of cells with more than one nucleus, indicating that cytokinesis is impaired, while the other three combinations of growth conditions yield predominantly mononucleate cells. Using the zenCELL owl, our goals were to observe division of multinucleate cells and to determine exact cell numbers over time, when sterol biosynthesis was perturbed chemically.

Materials and Methods

A 24-well cell tissue culture plate from Sarstedt (Standard F), was preloaded with 0.5 ml of axenic medium without further additives (untreated), with ethanol (solvent control), with terbinafine (5µM final conc.) and three concentrations of cholesterol dissolved in ethanol (final conc. 5, 10, or 25 µM) and mixed thoroughly. Appropriate dilutions ensured that the amount of solvent was 2 µl per well under all test conditions. Dictyostelium cells of the AX2 strain from axenic shaking condition were diluted to 20,000 cells/ml and 0.5 ml were dispensed into each well. Imaging was started soon after setting the illumination intensity, exposure time and focal plane in the zenCELL owl at ambient conditions and a controlled temperature of 21oC for three days. Because the zenCELL owl software does not routinely recognize the unusually small Dictyostelium cells (average diameter ca. 10 µm), a so called “AI prediction model” was trained with appropriate images of about 10,000 cells at neuralab (www.neuralab.de) to increase the efficiency of cell recognition from initially 10% to finally routinely over 95%.

Results and Discussion

Mere visual inspection of videos generated from three days of continuous observation of untreated cells cell already shows numerous cell division events including a cytokinesis of a (presumably multinucleated) large cell synchronously splitting into 6 daughters (Figure 1A) as well as more frequently normal divisions resulting in two daughter cells (Figure 1B).

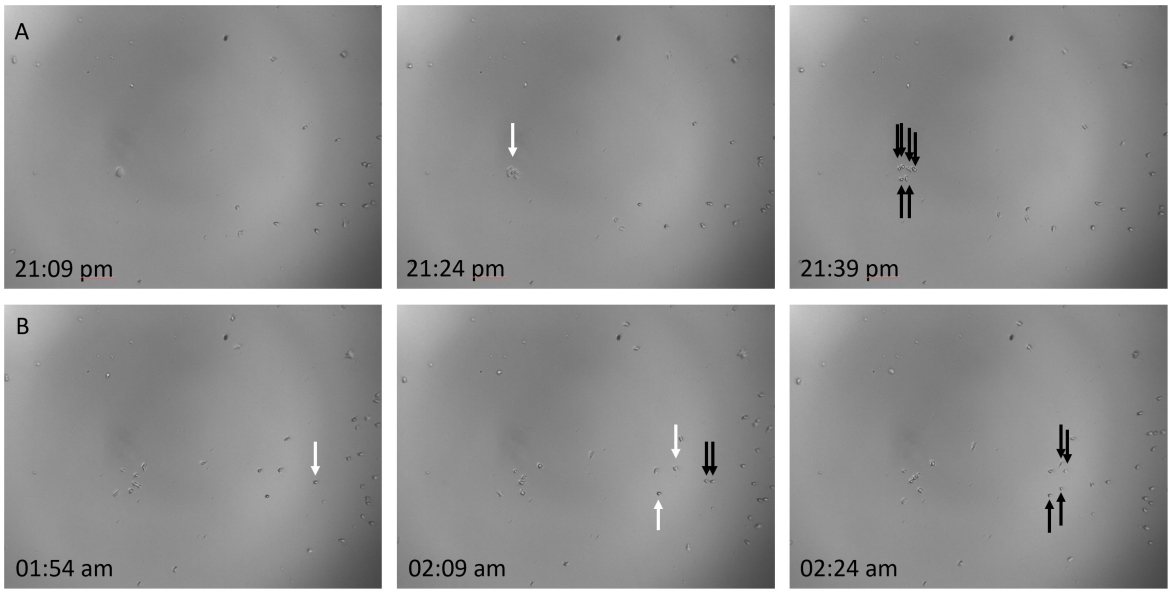

Figure 1. Cytokinesis of large and small cells. Time series of consecutive frames taken by the zenCELL owl in a single well 15 min apart with real times given in the lower left corner. Mother cells are marked by a white arrow in the frame before division and daughter cells are indicated by black arrows at the following timepoint. In Panel A, one single cell has split into 6 daughter cells, whereas panel B shows a high frequency of events generating two daughter cells each.

We also investigate the general role of lipids in various aspects of Dictyostelium cell physiology. As all kingdoms of life, like animals, plants and fungi produce their own specific species of sterols which act as essential membrane components, it was of interest to see how the growth of a protist, like Dictyostelium, would respond to sterol perturbation. Treating Dictyostelium with terbinafine, an inhibitor of fungal sterol synthesis, immediately stops cell division and addition of cholesterol, the major sterol of animal cells cannot overcome this block (Figure 2) regardless of the concentration. This observation is intriguing, because we had previously shown that Dictyostelium can use animal cholesterol to synthesize steryl-esters and store them in lipid droplets (Du et al., 2013). In contrast, cholesterol apparently cannot replace endogenous sterols in Dictyostelium membranes.

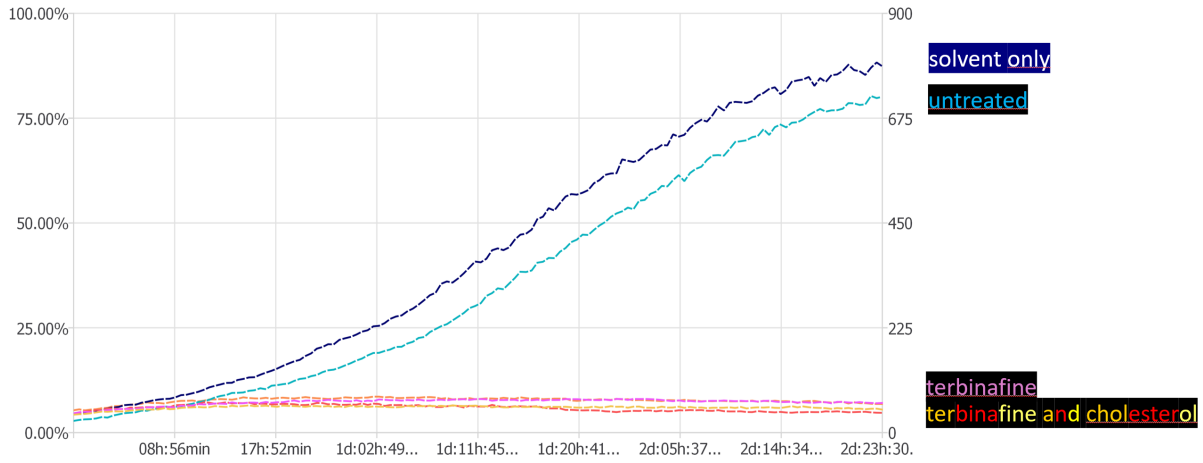

Figure 2. No cell growth occurs when sterol synthesis is impaired. Cells were treated with cholesterol and/or terbinafine (yellow, orange, red and pink lines), received the solvent only, or were left untreated (light blue and navy lines). Four wells for each of the six conditions were grouped in the zenCELL owl software and the average cell counts (right axis) over time (in days, hours and minutes) are displayed as coloured lines in the above diagram, which is an unmodified output of the zenCELL owl software.

Conclusions and Outlook

The zenCELL owl device is an easy to use live cell imaging system for monitoring up to 24 small-scale cultures in parallel. It was apparently developed with mammalian cells in mind, but if the software is appropriately trained, it can also cope with much smaller cells, like Dictyostelium amoebae. As Dictyostelium cells move much faster than most cultured mammalian cells, a shorter interval between the images would be attractive, which we have not achieved yet. For generating reliable curves of cell growth, a 10-minute interval is fully sufficient. Cytokinesis (the act of cell fission) occurs, however, faster than that. The instrument shows a good stability of focus over 3 days, our maximum period of use, representing 4-5 successive Dictyostelium generations. We envision a future application, requiring another round of training of the “AI prediction model” to also quantify the process of Dictyostelium spore germination, i.e. the process when an amoeba hatches from a cell-wall encased spore.

References

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