2D-Protein Gels of the Circadian Rhythm in the algae E. gracilis

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Abstract

To study the moleculer origine of the circadian rhythm a simple model system is advantages. We have choosen the unicellular algae Euglena gracilis which displays a clear circadian rhythm with a maximum activity during the time of ligth at day. The algea were cultured at laboratory condition with an alternating twelfe hour day (ligth) and night (dark) time at room temperature. From the synchronized culture (of ~25 l) aliquots (of ~100 ml) were taken at ~1h intervals and analysed by 2-D-IEF-SDS-electrphoresis gels. The polypeptide spots were studies for variation in intensity of their coomassie stain. There are spots that show a quite constant intensity in all aliquots and a few others vary in time. The polypeptide in the spots have not yet been identified.

Take Home & Conclusion

1: In Euglena gracilis, the circadian clock changes the cell shape between a spheriod immobile night form and an elongated mobile day form.

2: The clock process seems to effect only some of the cellular proteins.

Introduction

The algae Euglena gracilis is one of the solitary cell eucaryotic organisms which show a definitive circadian rhythm. The shape of the cell is a superficial property which reflects the day-night rhythm. In the dark, the cells take on an immobile spherical form, whereas in day-light the algae actively swim by means of its flagellae and show an elongated form.
The intracellular variation of many proteins with time is determined by 2D gel electrophoresis.

Results

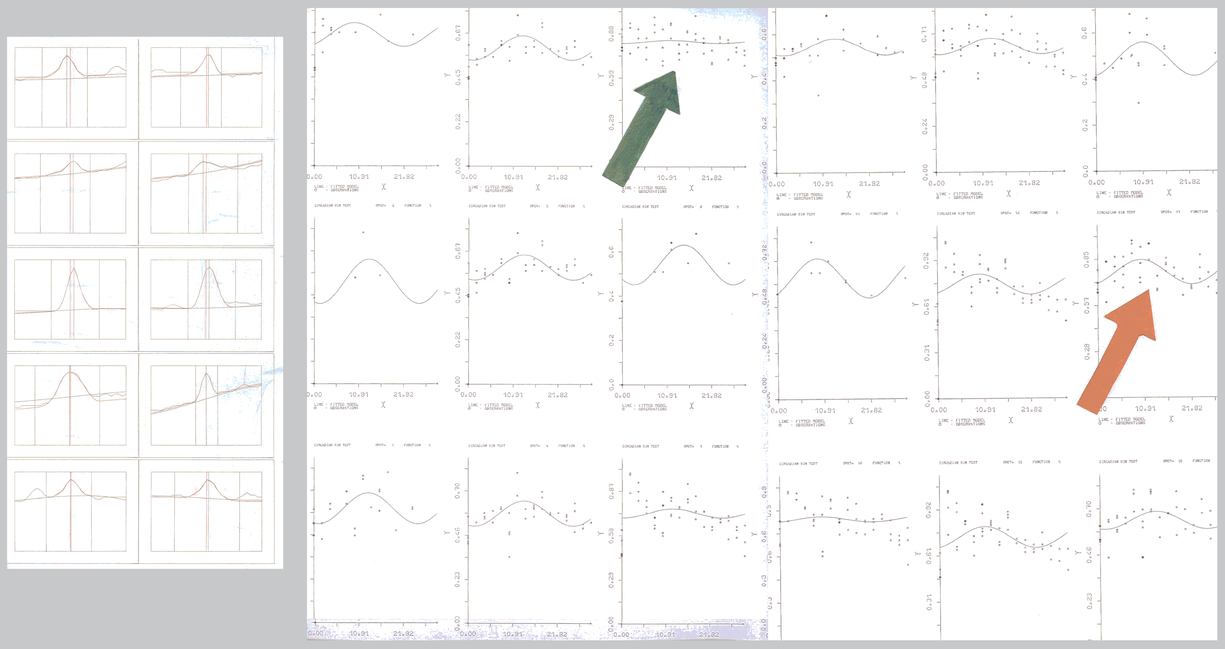
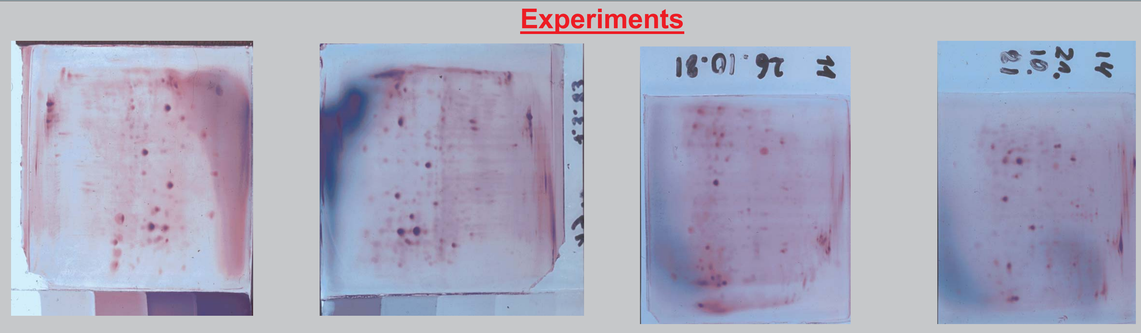


Fig. 2 Time series of the spot intensities of specific proteins.
The red arrow marks a protein which varies with circadian time. The green arrow indicates a protein which intensity remains constant.

Experiments



Some dels of the data base on the circadian rhythm of E. gracilis.
The database of about 300 gels is soon available at http://BusseLab.uni-kiel.de/rhythm
For future we are looking for a host to maintain the database system. Please leave your contact information.

Experimental Conditions

A 3 ltr. batch culture of Euglena gracilis was grown to the stationary phase under steady light. Then, the culture was synchronized by a 12 hours light and a 12 hours dark phase. The light was switched on at 7 a.m. After ten day-night-cycles constant dark was kept apart from a 15 minutes pulse of light every 2 hours (i.e. switch to the free running state in the dark). After 18 hours in the free running state (one and a half day-night-cycle), samples of 50 ml were withdrawn every two hours during the pulse of light.

The samples were analysed:
(1) by counting of the different forms of the cells in the light microscope (see Fig. 1),
(2) by assaying chemical and biochemical intra- and extracellular compounds (cAMP, Calmodulin and Ca++ -- not shown here),
(3) by separating the proteins on 2-D-IEF/SDS gels and by quantifying the spot intensity versus time (see Fig 2)

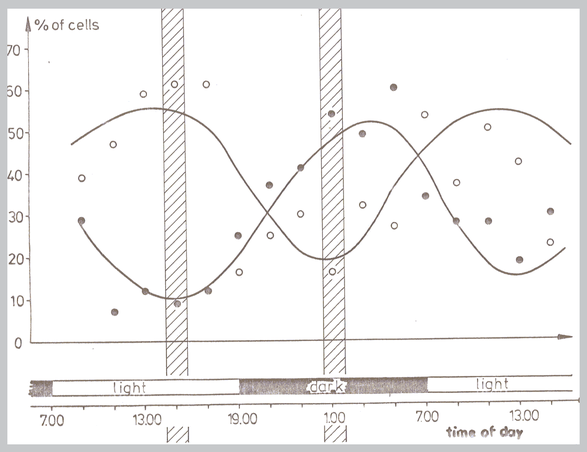
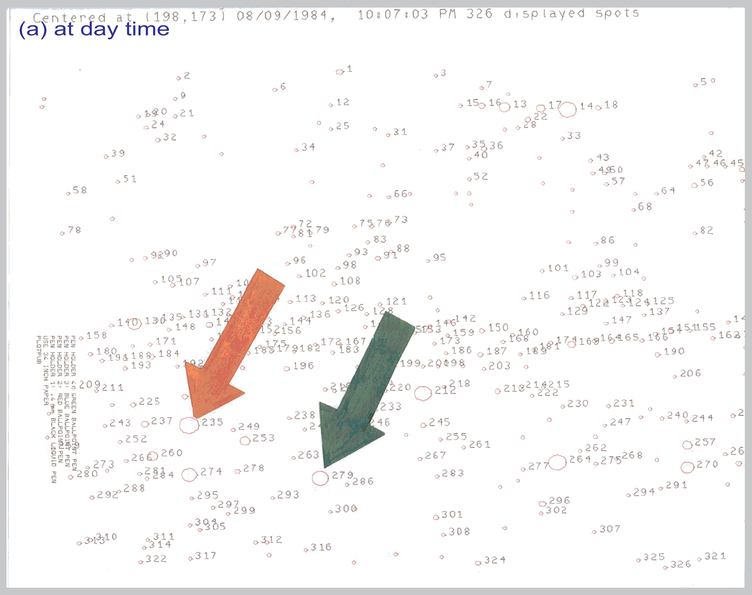


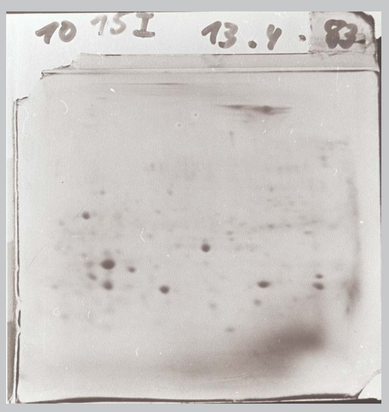
Fig. 1 Under constant night conditions:
The number of cells in the spheroid form (Fig. 3 lower right pannel) varies in the free running circadian oscillation with a maximum at night. The elongated mobile form (Fig. 3 upper right pannel) is found during the day phase.

Analysis of Experimental Data

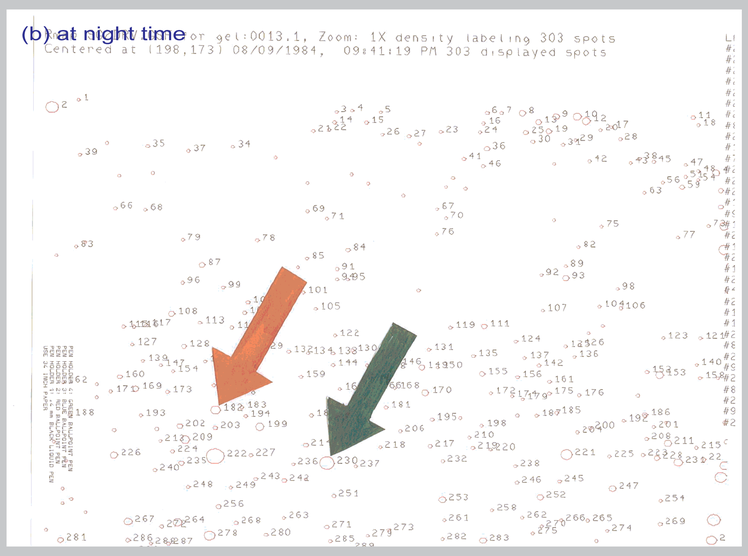
Fig. 3. Data for one night (upper figures) and one day (lower figures) time.

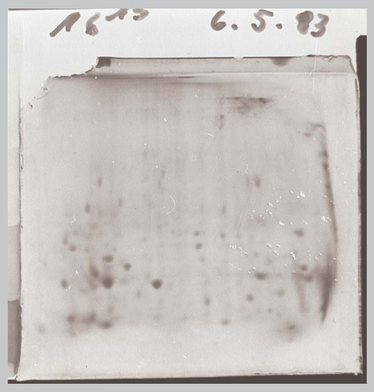
Right figure shows the microscopic view of the cells (i.e. at night cells are mostly spheroid - at day elongated)
Middle figure 2-dimensional IEF/SDS gel of the protein homogenate of the state in the left figure.
Left figure is the computer output of a scan of the gel shown in the middle figure (the arrows point to specific spots - the red to one that varies with circadian time and the green to one spot that stays constant in intensity over time) see also figure 2 for coresponding red and green arrows.





(a) at day time





(b) at night time