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Circumnutation in the growth of *Chara* rhizoids

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Abstract

Chara rhizoids, cylindrical cells growing in their apex, having the capability of positive orthogravitropic response, while growing without any change of position are not ideally straight, but they are characterised by slight and repeating cyclic bendings. The mean distance between successive bendings is 150 μm. The bendings appear more or less every 1.5 hour at rhizoids' growth rate of about 100 μm h^{-1}. After the displacement of the rhizoids from the vertical to the horizontal position, during the gravireaction, the curvature proceeds in stages, in which, alternatingly, there appear periods of higher or lower curvature. The curvature of successive higher bendings decreases when the rhizoid reaches the vertical direction. It seems that gravireaction is based on the increase of the maximal curvature of cyclic bendings and directing the bendings into a vertical plane. Other tip growing cells, generally straight, such as root hairs or hyphae of *Mucor* and *Phycomyces*, also show the repeating bendings.

Key words: Chara rhizoids, circumnutation, gravitropism, spiral growth

INTRODUCTION

Chara rhizoids are cylinder-shaped cells. They grow in the apex. The maximum growth rate is in the tip (Hejnowicz and Sievers 1971, Hejnowicz et al. 1977). Also other cells are characterised by the tip growth, e.g. fungi hyphae, root hairs or pollen tubes of higher plants (Castle 1942, 1958, Grove et al. 1970, Howard 1981, Sievers and Schnepf 1981, Emons 1986, 1987, Franke et al. 1972, Reiss and Herth 1978, 1979a, b, 1980). The rhizoids differ from them by the capability of gravitropic reaction (Sievers 1965, 1967a, b, Sievers and Schröter 1971, Friedrich and Hertel 1973, Sievers et al. 1979). In the apical zone of rhizoids (15-20 μm in the basal direction from the tip) a group of statoliths is located. Statoliths are small vesicles filled with dense crystallites of $BaSO_4$ (Schröter et al. 1973, 1975, Sievers and Schmitz 1982). They are assocciated with the perception of gravity stimulus. During gravireaction a displacement of statoliths occurs and there appears an asymmetry of distribution of Golgi vesicles at the rhizoid tip. This causes a differentiation of a cell wall growth rate and changes in a shape of a newly formed part of the rhizoid (downward bending) (Sievers and Volkman 1979, Sievers 1984). A hypothesis has been put forward that microfilaments are involved in controlling the position of statoliths (Hejnowicz and Sievers 1981). The preliminary examinations of rhizoids growing in a vertical position as well as during the gravireaction have pointed at an occurrence of slight and repeating bendings. More detailed study on this phenomenon is presented in this paper.

MATERIAL AND METHODS

The culture of the *Chara* sp. was established in laboratory conditions in an aquarium of a volume of 200 dm^3 (a four-year culture). Periodically some segments of thalli were collected for the needs of experiments. Each segment contained an internode and two intact nodes. In a lower node (with respect to) the direction of growth) "leaves" were cut off. A few fragments of thalli prepared this way were placed in a vertical position in a transparent chamber made of plexiglass ($14 \times 14 \times 3$ cm). This chamber could be turned so as to obtain a horizontal position of the rhizoids. After about 7 days rhizoids appeared at the bottom of some lower nodes. The mean growth rate for 20 rhizoids was 104 ± 17 $\mu m\ h^{-1}$. Two variants of experiments were carried out: 1) the rhizoids grew vertically without any change in a chamber position, 2) the rhizoids grew vertically at first and then the 90° turn of the chamber introduced a gravireaction resulting in their curvature. The segments of thalli with the rhizoids were then carefully placed on a slide and photographed with use of Docuval microscope. Each rhizoid was photographed in segments, magnified about 800 times. These images were used to analyse the rhizoid shape. The analysis was carried out as follows: on one side of the cell wall, equally distant points A_1, A_2, ... A_n were marked (Fig. 1) and normals were drawn through them. The normals cut the opposite side of the wall in points B_1, B_2, ... B_n. Thus, two sequences of segments: Δx_1, Δx_2, ... Δx_n between points A_i and Δy_1, Δy_2, ... Δy_n between points B_i were obtained. For successive i, $\Delta x_i / \Delta y_i = \delta_i$ was calculated, and changes of δ along the rhizoid were studied.

If a rhizoid was straight, then $\delta = 1$, if there was a bending towards side x, then $\delta < 1$, if there was a bending towards side y, then $\delta > 1$. The more different δ from 1 the higher the curvature of a given segment.

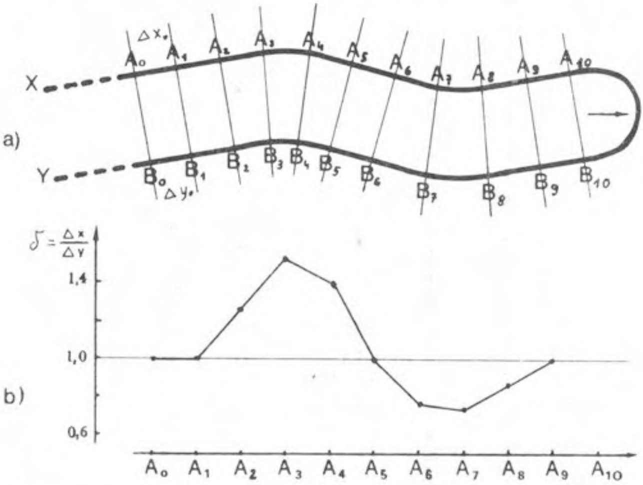

Fig. 1. Scheme of the assignation of curvature changes along the rhizoid

RESULTS

THE GROWTH OF RHIZOIDS WITHOUT ANY CHANGE OF CHAMBER POSITION

Rhizoids growing vertically without any change in their position are not ideally straight. Seen magnified about 100 times, they exhibt slight and repeating bendings (Fig. 2). The mean distance between successive bendings is 141 ± 24 μm. The mean maximal angle of bendings is 8° ± 3°. The successive repeating bendings are directed into the opposite side and, together, they create a zig-zag. Looking at the rhizoid from one side, one may notice that the zig-zag is visible at times better and at times worse. It is because the plane in which the bendings are being created turns slowly with respect to the plane of slight. The rhizoids do not represent a flat zig-zag but rather the one which rotates along its axis. The length of the rhizoid that falls into one cycle of rotation is about 2.4 mm. Since the mean distance between the successive bendings is 141 μm, then during one cycle of rotation the rhizoid makes about 17 bendings. Hence, the planes of successive bendings are one to another at the angle of 21°. Taking into account the mean growth rate of the rhizoids, the rotation cycle of the zig-zag bendings equals about 24 hours. It is difficult to say whether it is constant enough to call it circadian because only a few rhizoids were studied.

Beside the rhizoids characterized by the zig-zag wound along their axes,

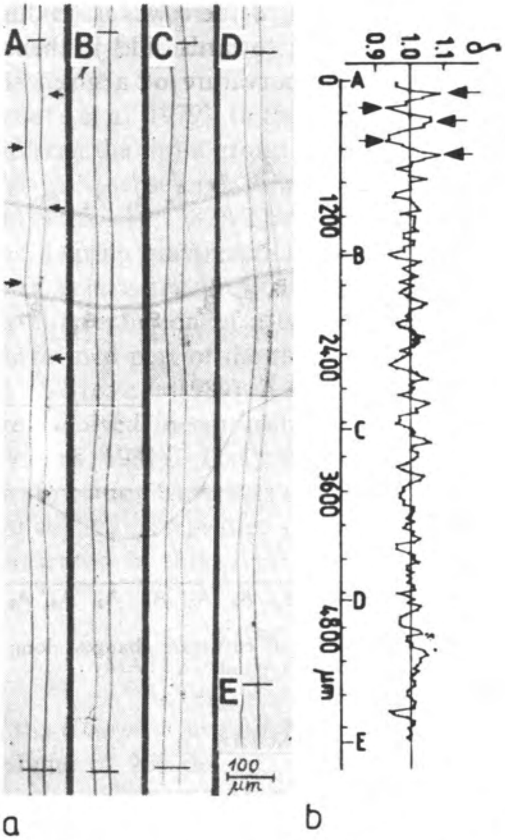

Fig. 2. a – Successive segments of a typical rhizoid growing steadily in a vertical direction. b – Graph of the curvature changes referring to segment AE of this rhizoid. Some slight and alternating bendings are marked with arrows

relatively seldom there are rhizoids wound spirally (Fig. 3a). In this case, the length of the rhizoid, which falls into one stroke of the spiral is also about 2.4 mm. On the spiral arcs (Fig. 3b), higher bendings connected with the zig-zag are visible. Bendings towards the axis of the spiral, dominate. The bendings towards the opposite side are minimal or reduced to zero. The factor causing an asymmetry of bendings is unknown. The mean distance between the successive, alternating bendings is 155 ± 30 µm.

THE GROWTH OF RHIZOIDS AFTER THE CHANGE IN CHAMBER POSITION

Thirty rhizoids which at first grew in a vertical position making a weak zig-zag were turned through 90° and left in this position. Within about 4 hours

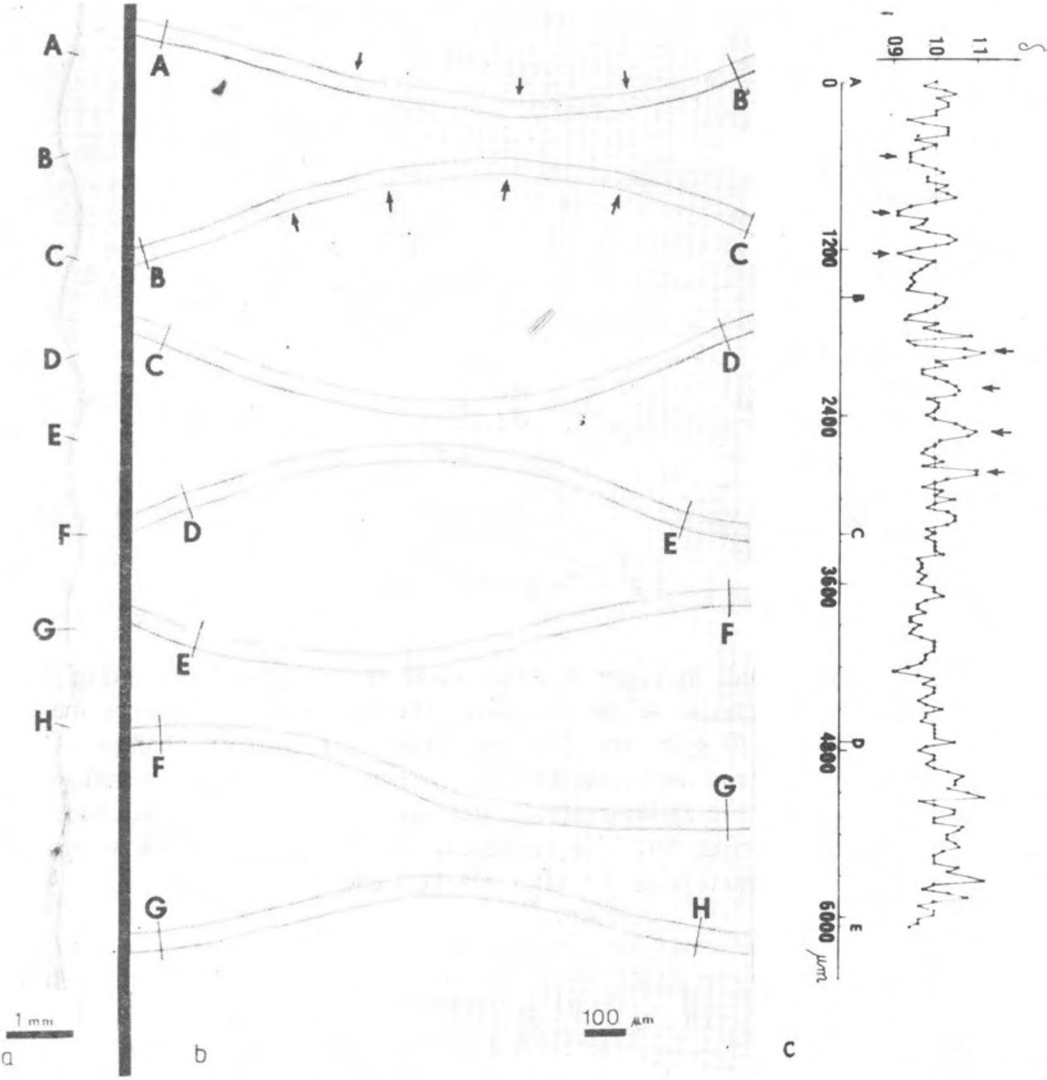

Fig. 3. a — Rhizoid growing spirally in a vertical direction shown after being pressed against the slide which causes flattening of the spiral. b — Successive segments of this rhizoid. Some bendings towards spiral axis are marked with arrows. c — Graph of the curvature changes referring to segment AE of the rhizoid

they ended a graviresponse, directing their tip to the vertical position (Fig. 4). Figure 5 shows the graphs of curvature changes for two typical rhizoids in which the gravireaction occurred. The exact moment, in which the rhizoids were turned, is unknown. However, it is certain that the moment precedes a violent curvature increase. Along the rhizoids, including the gravireaction arcs, zig-zag oscillatory changes of curvature are clearly visible. The gravireac-

Fig. 4. The gravitropic arc

tion (Fig. 6a) proceeds in stages in which there are periods of alternatingly higher and lower increases of the curvature. The mean distance between the higher bendings is 91 ± 25 μm. The amplitude of oscillatory changes of curvature 1) is greater at the beginning of gravireaction then before the change in rhizoid position (Fig 5), 2) gradually decreases with the rhizoid reaching vertical orientation (Fig. 6b). The maximum of δ changes from 1.4 at the beginning of gravireaction to 1.1 after reaction ended.

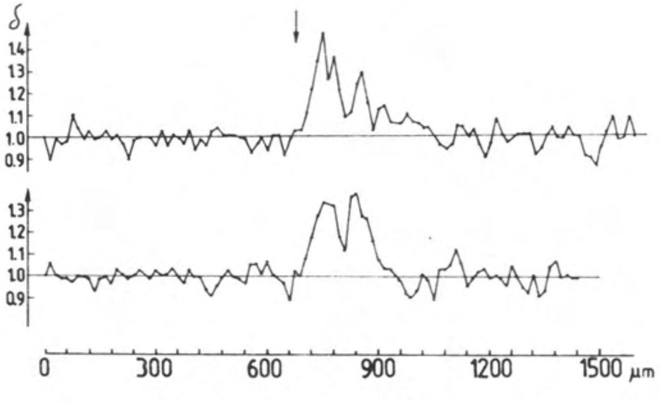

Fig. 5. Graphs of the curvature changes for two rhizoids in which graviresponse occurred after changing their position from the vertical to the horizontal one. The beginning of gravireaction is marked with an arrow

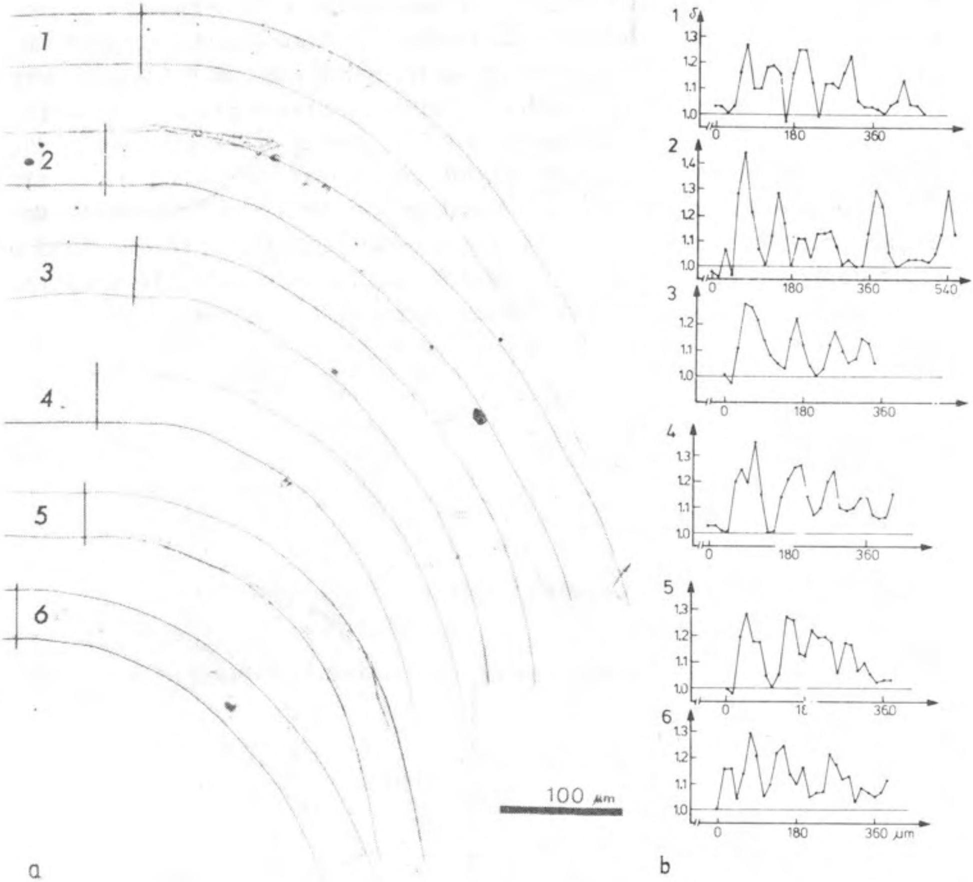

Fig. 6. a – The gravitropic arcs of 6 rhizoids. The vertical lines refer to the beginning of the graphs. b – Graphs of curvature changes for the arcs. On the arcs, the locations of stronger curvature are visible

DISCUSSION

Along the rhizoids, including the gravireaction arcs, their curvature changes periodically. This possesses a question, concerning the relation between the zig-zag of vertically growing rhizoids and the gravitropic curve of the rhizoids whose position has been changed from the vertical to the horizontal one. First, let us compare the rhizoids representing a zig-zag wound along its axis with the spiral rhizoids. In the vertically growing zig-zag rhizoids, the plane in which slight and alternating bendings occur (marked with "+" and "–" in Fig. 7a), turns slowly around the zig-zag rhizoid axis. In the spirally growing rhizoids one category of bendings (marked with "+" in Fig. 7b) dominates distinctly. The opposite bendings (−) are smaller or reduced to zero. A hypothesis can be put forward that the spiral rhizoids are a particular case of the zig-zag rhizoids in which occurs an asymmetry of alternating bendings. The hypothesis has been tested on a model of a wire. First a flat zig-zag, i.e. such in which equally distant, alternating bendings were in one plane, was constructed. Next, each bending was rotated in respect to the previous one by a small angle. This way a model of a zig-zag rhizoid wound along its axis was obtained. Then, by straightening every second bending, i.e. the bendings of the same orientation, a spiral was produced.

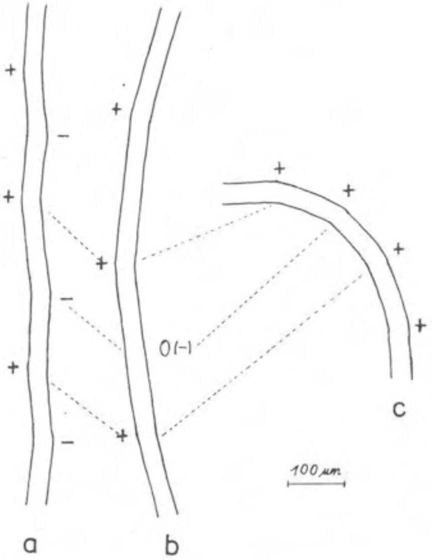

Fig. 7. Graph showing: a — zig-zag rhizoid growing in a normal position, b — the arc of a spiral rhizoid having the length of about 1/2 of the spiral stroke shown after the spiral rhizoid has been flattened by pressing against the slide, c — the gravitropic arc. Slight and alternating bendings of the zig-zag rhizoid (a) marked with "+" and "−". In the spirally growing rhizoids (b) the bendings marked with "−" are minimal or reduced to zero. During gravireaction (c) the bendings are in one plane and they have the same orientation "+"

If it is possible to obtain a spiral from a zig-zag, then perhaps it is also possible to obtain a gravireaction arc from tiny bendings, namely, in such a way that all the bendings are put to a vertical plane and all of them have the same orientation. To obtain the spiral rhizoid, bendings of one orientation must be eliminated. To obtain a gravireaction arc, all the bendings must be of the same orientation but they also have to be in one plane! Indeed, on the gravireaction arcs there appear from three to five higher downward bendings

("+" in Fig. 7c). If a graviresponse depended only on the fact that all the bendings occurred downward in one plane and they had the same orientation, the rhizoid would not yet reach the vertical direction through three or five bendings (keeping their angles). Apart from directing properly the bendings, their curvature would have to be amplified. Indeed, after the displacement of rhizoids from the vertical to horizontal position, the curvature of bendings increases distinctly. At the beginning of gravireaction, the maximal curvature of bendings is greater then the one before gravireaction and it decreases gradually toward the end of it as the rhizoid reaches the vertical position. The

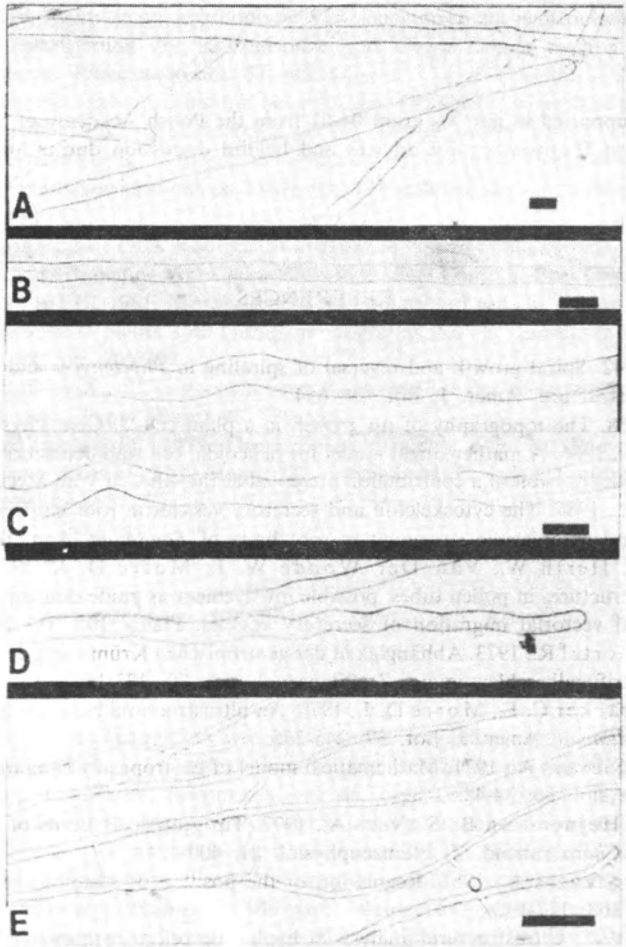

Fig. 8. Tip growing cells of root hairs (a – *Avena sativa* L., b – *Brasica oleracea* var. *gongledos*, c – *Raphanus sativus* var. *sativa*, d – *Tradescantia vulgaris* L.) and hyphae of *Mucor* sp. (e). Periodical bendings are visible. Magnification bar represents 20 μm

distance between stronger bendings on the gravireaction arcs is more or less 30% shorter than before the position change of the rhizoids, however it is difficult to say whether it is due to the shortening the period of cyclic curvature changes or to the decreasing growth rate of rhizoids during gravireaction. Periodical changes in the shape of the rhizoids indicate periodical changes in the system controlling the growth rate of the cell wall. It has been proved that other tip growing cells, generally straight, such as root hairs or hyphae of *Mucor* show oscillatory "zig-zag" bendings (Fig. 8). It is possible that the periodical changes in the shape of the cells are connected with the maintenance of a defined growth direction similarly as in graviresponsive cells of the *Chara* rhizoids.

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Cirkumnutacje we wzroście szczytowo rosnących komórek na przykładzie ryzoidów Chara

Ryzoidy *Chara* – grawitropicznie wrażliwe komórki o cylindrycznym kształcie, rosnące tylko w obrębie czaszy apikalnej – rosnąc bez zmiany położenia nie są idealnie proste, lecz charakteryzują się niewielkimi, powtarzającymi się, czyli oscylacyjnymi, wygięciami. Średnia odległość między kolejnymi wygięciami wynosi 150 µm. Przy szybkości wzrostu ryzoidów ok. 100 µm na godzinę wygięcia te pojawiają się mniej więcej co 1,5 godziny. Po zmianie położenia ryzoidów z pionowego na poziomie (w czasie grawireakcji) zakrzywianie postępuje etapami w których na przemian występują okresy silniejszej i słabszej krzywizny. Średnia odległość między miejscami silniejszej zmiany krzywizny wynosi 91 µm. Krzywizna kolejnych silniejszych wygięć maleje w miarę jak ryzoid dochodzi do kierunku pionowego. Nasuwa się przypuszczenie, że reakcja grawitropiczna polega na zwiększeniu maksymalnej krzywizny oscylacyjnych wygięć i ukierunkowaniu tych wygięć. Inne komórki rosnące szczytowo, ogólnie proste, takie jak włośniki czy strzępki niektórych grzybów wykazują również "zygzakowate" wygięcia.