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Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*

I. Action spectrum

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Several action spectra active in the photo-displacements of chloroplasts have been reported for different objects (Zurzycki 1962 for *Lemna trisulca*, Haupt and Schönfeld 1962, Fischer-Arnold 1963 for *Vaucheria sessilis*, Mayer 1964 for *Selaginella martensii*) in the recent years. In the all above mentioned investigations two maxima of activity of radiation were found: in the blue region and in near UV. Suggestion was made that riboflavin, the substance of similar absorption properties, may play the role of a photoreceptor in these cases (Haupt and Schönfeld 1962; Haupt 1964). On the other hand some differences in the position of blue maximum among different objects were noticed. It seems therefore worthwhile to collect more data about the activity of this spectral region in other plants.

Most of the measurements of the activity of radiation were made in visible and near UV regions. This is only Mayer (1964) who found that far UV (at wave length 254 nm) also exerts a strong influence on the displacements of chloroplasts in *Selaginella*. Taking into consideration the high absorption of riboflavin in the far UV the extension of measurements over this region may be important.

The aim of the present study was to estimate the action spectrum for displacements of chloroplasts in *Funaria hygrometrica* in the spectral regions including far UV. The absorption properties of some cell components (i.e. call walls) may influence the energy of radiation reaching the photoreceptor. Therefore optical properties of the cells were also measured in order to obtain a background for interpretation of the action spectrum of radiation.

METHOD

Fully grown leaves of *Funaria hygrometrica* were used for experiments.Plants were obtained from spores sown on humus soil in small glass containers. Cultures were kept in daylight. In order to obtain a dark position of chloroplasts, which was the starting position in most experiments, leaves were cut out from the stem with a sharp razor blade, mounted in water on the microscopic slide and kept in darknes in a humid chamber for 18—24 hours. Before irradiations were begun leaves were

transferred to another drop of water on another slide. When the last step of procedure is omitted the displacement of chloroplasts is sluggish and irregular probably because of exhaustion of oxygen from the medium surrounding the objects while pretreating in darkness. The cover glass of the final preparations was sealed with hot vaseline to prevent water evaporation and shifting of the cover glass when irradiation of slides takes place in a vertical position.

Objects were irradiated on the microscopic table. For the illumination in visible and near UV regions the previously described equipment (Zurzycki 1961)

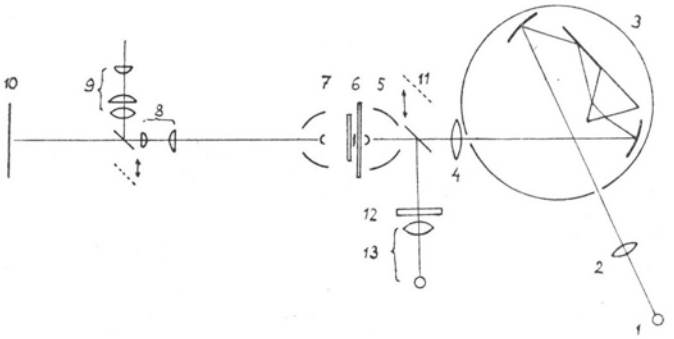

Fig. 1. Schematic diagram of the apparatus used for UV irradiation. *1* — Hg lamp, *2, 4* — quarz lenses, *3* — mirror monochromator, *5*, 7 — Mirror condensor and objective, 6 — objects between quarz plates, 8 — quarz oculare, 9 — side ocular 10 — camera, *11* — mirror, *12* — red filter, *13* — microscopic lamp.

was used. The light system of the microscope was based on the Kohler's principle, using an incadescent lamp 750 W 110 V supplied through a variable auto — transformer. A 5 cm thick layer of $CuSO_4$ solution (40 g/l) was used as heat filter. Only for red light water was applied insted of $CuSO_4$. Required spectral ranges were selected by means of following interference filters (half widths in breckets): 362 (20), 382 (14), 400 (11,1), 429 (8,9) 442 (8,4), 454 (6,0), 464 (7,4), 470 (10,2), 480 (5,6), 497 (6,3) 506 (6,7), 523 (5,3), 530 (7,1), 543 (10,1) and 668 (11,0). The diameter of the light spot in the plane of objects was 2 mm.

For irradiation in UV an equipment schematically presented in fig. 1. was used. Radiation from mercury lamp (Q 700 — Hanau) concentrated with a quarz lens was passed through a Zeiss mirror monochromator with a quarz prism. Selected lines of radiation concentrated by another quarz lens placed near the exit slit of the monochromator were projected on the condensor of the microscope. The microscope had a horisontal position and was equipped with a mirror condensor (NA 0,4), mirror objective (20×NA 0,45) and quarz ocular (Zeiss). The objects were mounted between quarz plates. The irradiated area of the objects was 0,83 mm. For a periodical control of the position of chloroplasts objects were illuminated with weak red light comming from the microscopic lamp and reflected from the mirror which was shifted into the optical axis for the time of observations.

Light intensity was measured with microthermoelement (Zurzycki 1961) sup-

plied with a quarz window. Intensities of radiation at 280 and 334 nm only w er too low to be measured with the applied method. They were calculated on th base of the emission spectrum of the lamp (according to Hanau leaflet) in comparision with the measured intensity of two neighbouring lines. Radiation intensity was regulated by changing slits of the monochromator.

Observations were made in the middle part of the leaf. Measurements of the chloroplasts position, based on determination of the number of chloroplasts in flat position on the upper and lower cell walls was described in the previous publication (Zurzycki 1962).

Absorption properties of the cells were registered by means of photographic method in the mercury lines isolated by monochromator. Photographs were made on negative material ORWO NP 18 with the equipment shown in fig 1. Densities of negatives were subsequently determined with densitometer, in the field corresponding to the area of 4μ in diameter of the microscopic preparation. Calculation of light transmission was based on the density gradation curves estimated for each wave length separately.

RESULTS

Placing the leaf, with the chloroplasts in a flat position (low light intensity arrangement), in darkness causes a rather slow disapearance of chloroplasts from the upper and lower cell walls and their displacement to the side walls. The percent of chloroplasts in a flat position (% E) initially about 100 reaches about 30% after 2 hours and after 18—24 hours a typical for *Funaria* dark position is reached in which all or nearly all chloroplasts are gathered on the side walls of the cell (Fig. 2a). The %E decreases then to 0—10%. This dark position of chloroplasts was used as starting position for the study of the action spectrum.

The same speed of displacements as in darkness was registered by red light irradiation (Fig. 3) even in strong intensity of this light. It may be concluded that the photokinetic effect of long wave length of the spectrum found in *Lemna* (Zurzycki 1962) is not distinguishable in *Funaria* cells.

The exposure of the leaves pretreated in darkness (see — method) with low intensity of radiation causes displacements to a flat position much faster than in the above described reaction in darkness. If the typical dark arrangement is used as starting position a great majority of chloroplasts displace themselves to the cell wall facing the light source (Fig. 2*b*, *c*). This pattern is characteristic of the whole region of spectrum which is able to cause the „phototactic" arrangements. Fig. 4 illustrates typical time curves of displacements. As shown by the graph the rearrangements last 1—1,5 hours and after that time the %E reached a stable value depending on the radiation intensity and wave length and subsequently did not change significantly. Basing on these results 1,5 hour was chosen as the time of irradiation to obtain the relation between radiation intensity and %E.

In figure 5 the dependence of chloroplasts arrangement on the radiation intensity

for different wave lengths is plotted. Taking as the criterion of activity of radiation the intensity which causes the value 50% E and expressing it in number of quanta/cm² sec the action spectrum of low light intensity displacements was calculated. The results are listed in Tabl 1. and presented graphically in fig. 6. The action spectrum has three peaks at 266 (main), between 360 and 380, and by 454 nm. The last one is not very sharp the longer wave lengths till about 480 nm have comparable activities. The relative activities of radiations at the maxima is: 3,8:0,92: 1 for 266, 366 and 454 respectively.

Table 1

Radiation intensities inducing the half displacements in the darkness → low light intensity rearrangements and the quantum activity of the reaction

Wave length nm.	Radiation intensity erg/cm² sec.	Quantum activity 1/quanta 10^{13}
254	17	4.6
266	7	10.65
280	21	3.36
302	61	1.07
313	43	1.47
334	34	1.75
362	25.5	2.15
366	21	2.57
382	27.5	1.89
400	61	0.817
405	54	0.904
429	33	1.405
436	31	1.465
442	21	2.15
454	15.5	2.80
464	19	2.246
470	19	2.218
480	17.7	2.336
497	51	0.784
506	99	0.397
523	380	0.0993
530	810	0.0463
543	2900	0.0126

Absorption properties of the cell were measured in two modifications. In the first transmission of the whole cell was recorded. In cells which chloroplasts are in a dark position the transmission of radiation passing through the centre of the cell (omitting chloroplasts) was measured. In the second case the leaves were homogenized in phosphate buffer pH 7.0 and washed several times by centrifugation. In the obtained preparation the 2-layers fragments of cell walls were chosen and their transmission was recorded. In this way the transmission of the walls was

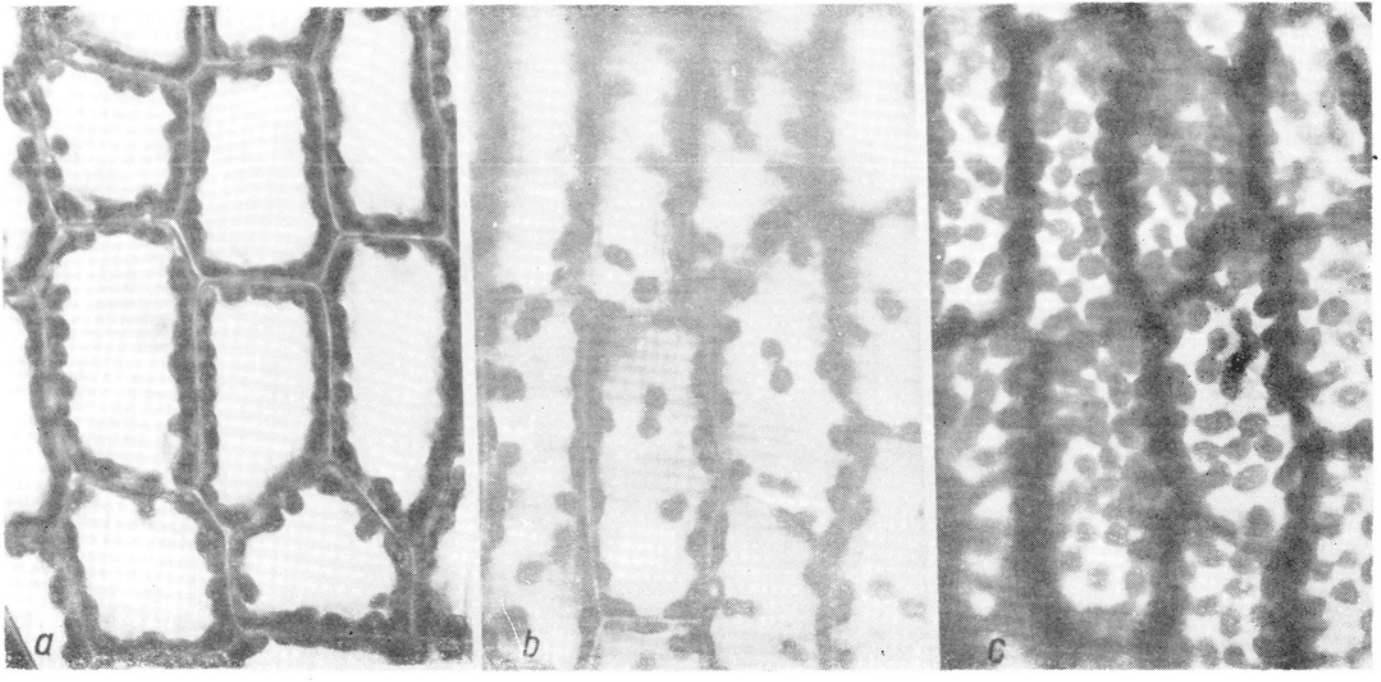

Fig. 2. *a* — dark arrangement of chloroplasts, *b*, *c* — arrangement which took place after irradiation with low light intensity *b* — microscope focussed on the upper (facing away from), *c* — on the lower (facing light source) cell walls.

obtained, because proteins of protoplasm were probably in a great part dissolved during washing.

The results of measurements show that absorption of the whole cell (excluding chloroplasts) is negligible in visible range and in UV till 330 nm. and increases distinctly in the shorter wave length of radiation (Fig. 7, 1). The absorption curve shows a shoulder by 280 nm. Few measurements made on the cut out cells (which lost their cell sap) show no significant differences in absorption in comparison to

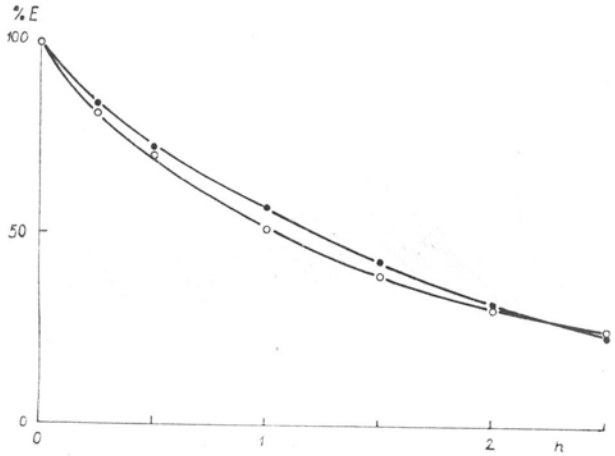

Fig. 3. The course of chloroplasts displacements in darkness (black points) and in red light: 668 nm, 55000 erg/cm² sec (white circles). Starting position — low light arrangement.

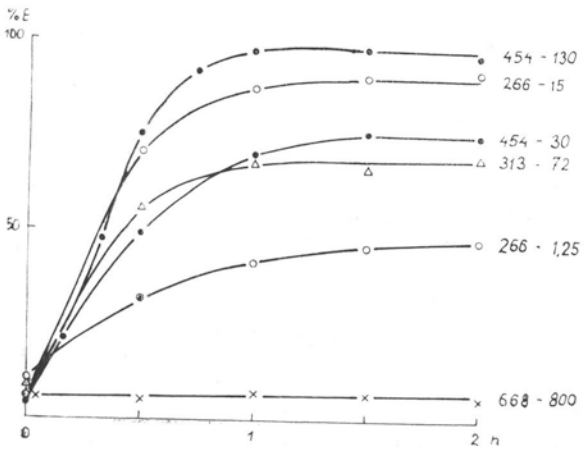

Fig. 4. Time course of the displacements of chloroplasts in UV, blue and red light. Starting position — dark arrangement. Figures by the curves denote the wave lengh and radiation intensity in erg/cm² sec.

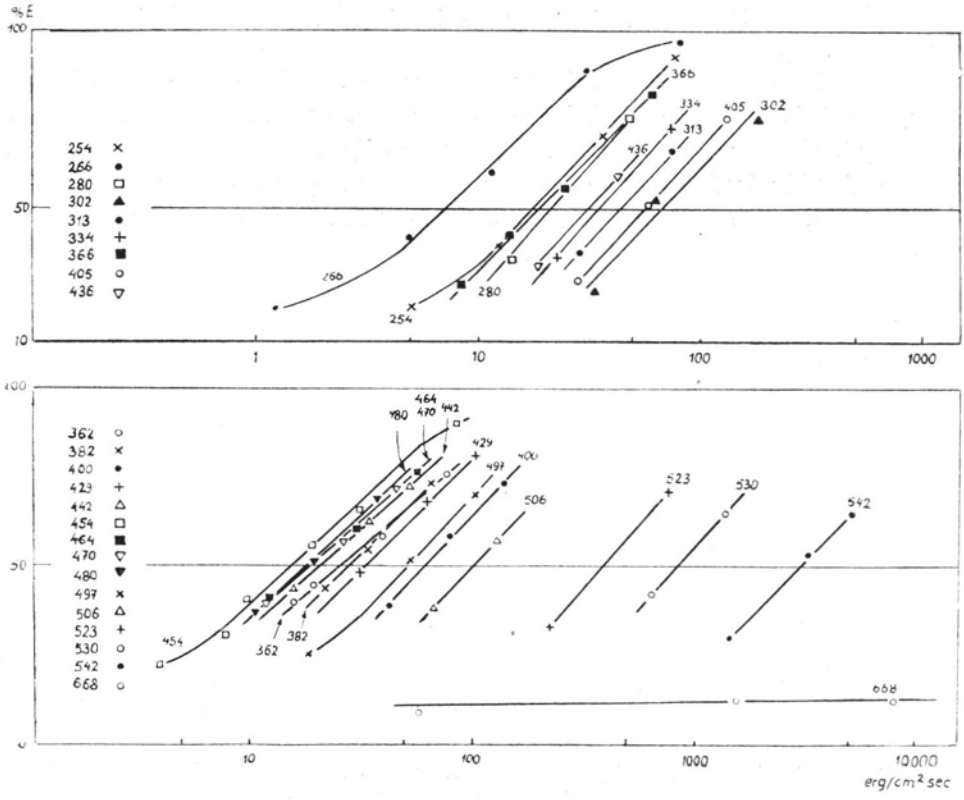

Fig. 5. Dependence of chloroplasts arrangement (Y axis — %E) on radiation intensity (X axis — erg/cm² sec). Starting position — dark arrangement, time of irradiation 90 min. Figures by the curves mark the wave length of radiation in nm.

whole cells. It may be supposed that for absorption in far UV cell walls and the protoplasm layer are responsible in the first place. On the other hand absorption in cell walls in the far UV is much weaker (Fig 7, *2*). Although in the region 250—280 nm the cell walls absorb significantly; the absorption of the main part of radiant energy in this region should be ascribed in the cell to proteins and nucleic acids in the protoplasm.

DISCUSSION

The obtained action spectrum for low light intensity displacements of chloroplasts in *Funaria hygrometrica* is in the visible and near UV range comparable to similar spectra recorded for *Lemna*, *Vaucheria* and *Selaginella*. Extension of the experiments till 245 nm shows a disinct third maximum of activity by 266 nm. Riboflavin has its main peak of absorption exactly in this region. These facts support

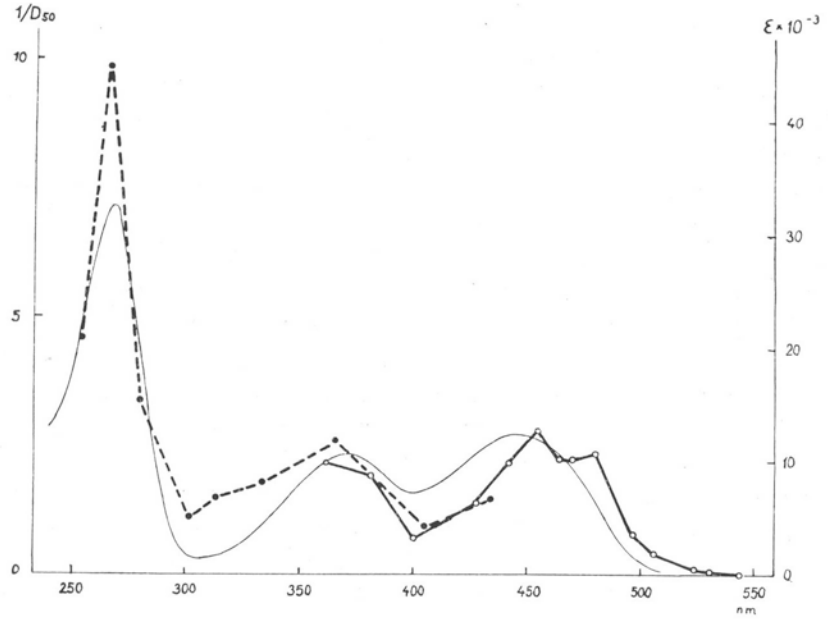

Fig. 6. Action spectrum for the low light intensity displacements of chloroplasts. X axis — wave length, Y axis — quantum activity (inverse of the intensities of radiation expressed in quanta causing 50%E). White circles — data obtained with interference filters, black points — with mercury lines. Thin line — absorption of riboflavin according to Whitby (1953).

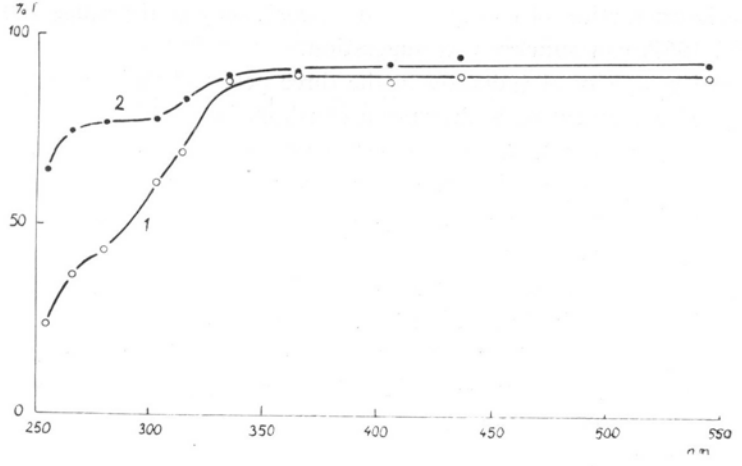

Fig. 7. Optical properties of the cell. X axis — wave length, Y axis — Transmission of radiation passing trough the whole cell (*1*) and trough prepared cell walls (*2*).

the hypothesis given by Haupt that riboflavin or its compound acts as a photoreceptor in the radiation induced displacements of chloroplasts.

Some disagreement between the absorption spectrum of riboflavin and action spectrum of the displacements is to be seen in the region 450—500 nm. The activity of radiation in this range is higher than it should be expected from the absorption curve of riboflavin. Similar disagreements are to be found in the action spectrum for *Lemna* where an accessory peak at 485 nm exists (Zurzycki 1962) or by *Selaginella* (Mayer 1964) where the greatest activity in blue region was found at 472 nm. Action spectra of photodinesis and related processes show also an accessory maximum or at least shoulder in the range 470—490 nm. (Seitz 1964, 1966). Mayer supposed that the disagreement between the action spectrum and riboflavin absorption can be explained by the shift of the maximum of absorption in the flavin-protein complex to longer wave lengths. It is striking however, that the relative activities in the range 450—490 nm are in every investigated object different. In the author's opinion this may have two other explanations:

1. If riboflavin is the only photoreceptor screening its molecule by another inactive pigment (i.e. carotenoids) may cause a shift of the most active radiation to the longer wave lengths where absorption in screening pigments is distinctly lower. The different ratio riboflavin: carotenoids this may be responsible for differences in spectral activity in the blue region.

2. Riboflavin is not the only photoreceptor and light absorbed by another blue pigment can also induce photo-displacements. According to Schönbohm (1963) the negative phototaxis of chloroplasts in *Maugeotia* is controlled by a pigment absorbing in blue between 400 and 510 nm with the maximum of absorption at 477 nm. It may be supposed that the same or similar blue absorbing pigment acts as an accessory photoreceptor in objects where photo-displacements are controlled by riboflavin. Different ratios between both pigments can induce differences in activity of the blue region of light. Finding out that polarized light induces characteristic orientation of chloroplasts in *Funaria* only in the range 400—530 nm (Zurzycki 1967) can support this suggestion.

The relative activity of radiation in the three peaks of the action spectrum is in rather good agreement with absorption of riboflavin. Taking into consideration the high absorption of UV in the proteins of protoplasm it may be suggested that the photoreceptor is localized in the outer layer of protoplasm. When localized in the deeper parts of the cytoplasm relative activity of 266 nm radiation should be considerably lower.

In contrast to the present study no full low light intensity arrangement of chloroplasts could be induced by irradiation with UV in *Lemna trisulca* (Zurzycki 1963). In the same ranges of intensities only a weak tendency to this arrangement appeared. The above discrepancies may be explained either by specific differences between both objects or, more probably, by the fact that not monochromatical light was used in *Lemna* studies.

The photokinetic effect of red light found in *Lemna* (Zurzycki 1962, 1965) is finally supported by the findings of Seitz (1966) that red light induces a lo-

wering of viscosity and acceleration of rotation of chloroplasts in *Elodea*. In the present study no specific effect of red light was found but the long wave length of spectrum was not intensively investigated.

SUMMARY

1. The action spectrum of low light intensity displacements of chloroplasts in *Funaria hygrometrica* was studied in the range from the visible till the far UV region. Three maxima of activity of radiation at 266, 360—380 and 454 nm were found. The position of the maxima and the relative activity of radiation speak in favour of the suggestion considering riboflavin a photoreceptor active in the photodisplacements.

2. The suggestion was developed of the existence of an accessory pigment with the maximum absorption about 480 nm cooperating with riboflavin in the photo-reception. The different ratio of both pigments can explain the differences in relative activity of radiation in the region 450—490 nm found in some objects.

3. Absorption of radiation in the cell walls and in the protoplasm was measured. The results compared with the action spectrum suggest that riboflavin is localized in the outer layer of protoplasm.

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Własności i lokalizacja fotoreceptora czynnego w przemieszczeniach chloroplastów u Funaria hygrometrica

I. Widmo działania

Streszczenie

1. Określono widmo działania promieniowania czynnego w przemieszczeniach chloroplastów *Funaria hygrometrica*. Stwierdzono maksima aktywności promieniowania leżące przy 266, 360—380 i 454 nm. Zarówno położenie maksimów, jak i względna aktywność poszczególnych zakresów spektralnych przemawiają za przyjęciem barwnika flawinowego (ryboflawiny) jako substancji pełniącej rolę fotoreceptora w zjawisku ruchów chloroplastów.

2. Wysunięto przypuszczenie o istnieniu drugiego barwnika żółtego z maksimum absorbcji przy około 480 nm który współdziała z ryboflawiną. Różny stosunek ilościowy obu barwników byłby odpowiedzialny za różnice aktywności właściwej promieniowania niebieskiego w zakresie 450—500 nm stwierdzone u badanych dotąd obiektów.

3. Określono absorbcję promieniowania w błonach komórkowych i protoplazmie komórki. Na podstawie uzyskanych wyników porównanych z widmem działania, wysunięto przypuszczenie, że drobiny fotoreceptora zlokalizowane są w zewnętrznej warstwie cytoplazmy.