Interferometric measurements of dry mass content in nuclei and cytoplasm in the life cycle of antheridial filaments cells of *Chara vulgaris* L. in their successive developmental stages

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Abstract

Interferometric measurements of the nucleus and cytoplasm dry mass during interphase in the successive stages of development of antheridial filaments of *Chara vulgaris* demonstrated that the dry mass and surface area of cell nuclei double in size in each of the successive generations of the filaments, whereas neither the surface nor the dry mass of the cytoplasm increase in such proportion in the same period. In the successive stages of development of the antheridial filaments the dry mass and surface area of the nuclei and cytoplasm gradually diminish.

INTRODUCTION

The compounds synthesized in the cell cycle significantly affect the dry mass content of nuclei and whole cells. Investigations on changes in dry mass content in the nuclei of meristematic tissues (Sandritter et al., 1960; Rodkiewicz, 1961; Kuran, Olszewska, 1975) demonstrated an at least twice increase of dry mass during interphase. With the progress of interphase, an increase of cell surface and volume was observed beside the increase in dry mass. In diploid tissues an increase in nuclear volume was noted in interphase, exceeding largely the dry mass increment (Sandritter et al., 1960). According to Woodard et al. (1961) the cell volume increases five fold in interphase. Thus, for tissues of meristematic type a considerable increase in volume, and consequently, of the cell surface in the interphase period is the rule. On the other hand, in the object of the present study — the antheridial filaments of *Chara vulgaris* — the cells reach during interphase at most 2/3 of the length of

pre-prophase cells of the preceding generation (Olszewska, Godlewski, 1972).

The antheridial filaments of *Chara vulgaris* develop within the antheridium where, owing to synchronous divisions 2, 4, 8, 16, 32 and 64 cells are formed. Interphase consists of only two phases, S and G₂ (Olszewska, Godlewski, 1972). In each generation the surface of the nuclei increases mainly in phase S, while the cytoplasm increment appears later, in phase G₂ (Olszewska, 1974b).

Autoradiographic investigations on nucleic acid and protein synthesis in the life cycles of the successive cells generations of antheridial filaments showed that with limitation of growth a gradual reduction of protein and RNA synthesis (Olszewska, Godlewski, 1972) as well as of the ability of ³H-actinomycin D binding occurs (Olszewska, 1974a) in the successive development stages (4-, 8-, 16- and 32-cell) of the *Chara vulgaris* filaments.

The aim of the present studies was to compare the changes in dry mass content in the nucleus and cytoplasm in 4-, 8-, 16-, and 32-cell filaments. The results indicate that the gradual limitation of cell growth and their protein and RNA-synthesizing activity causes a corresponding decrease of their dry mass content.

MATERIAL AND METHODS

The apical parts of the *Chara vulgaris* thallus were fixed in 2 per cent glutaraldehyde solution in 0.1 M cacodyl buffer, pH 7.2. This buffer was chosen in view of the results of previous investigations from which it resulted that this substance, as compared with phosphate or veronal buffer, does not cause decreasing of the dry mass of nuclei (Kuran, Olszewska, 1975). The fixed material was kept in 70 per cent alcohol. After placing in a water medium the antheridial filaments were pressed out of the antheridium. Squashes were prepared and the dry mass of the nuclei and cytoplasm was calculated at three phases of interphase (early S, early G₂ and late G₂) in the successive stages of development. As criterion for the particular interphase stages the cell length was adopted, according to the data of Olszewska (1975).

The dry mass content was calculated with the aid of an interference-polarization microscope MPI-5 (PZO) according to the formula

$$m = \frac{\delta}{100\sigma} A$$

where

δ — difference of optic path

A — surface

σ — specific refractive increment (Pluta, 1965).

The nuclear surface was treated as the surface of an ellipsoid and the cytoplasm surface as a rectangular surface minus the nuclear surface, and corresponding formulae were used for calculation.

RESULTS

Increase of nuclear surface area

It results from the calculation of surface area increase of the cell nuclei in the successive generations of antheridial filaments that the surface of the nucleus increases within the interphase period about twice in each generation, whereas it decreases, although slightly in the same interphase periods of the successive generations (Fig. 1). The increase in surface area of the nuclei occurs above all in the initial period of inter-

Fig. 1. Increase in surface area of nucleus during interphase in successive developmental stages of *Chara vulgaris* antheridial filaments

phase that is in S phase. The difference in the surface area increment in both the distinguished interphase periods indicates a twice larger increment during phase S as compared with that in stage G₂. It should be added that as starting point the nuclear surface in the period of early post-telophase was adopted, that is the period when full individualization of both the daughter cells had not yet occurred. In the successive phases of filament development, in the same interphase stages, the surface of the cell nuclei shows a slightly decreasing tendency. It is only in late stage G₂ that the nuclei do not reach the dimensions characteristic for the preceding generation, this decrease is most pronounced from the 4- to the 32-cell generation (Fig. 1).

Changes in dry mass of the nuclei

The dry mass of the nuclei was, like their surface, calculated for three successive stages of interphase (early S, early G₂ and late G₂) in 4-, 8-, 16- and 36-cell antheridial filaments. The results show that in early phase S the dry mass of the nuclei gradually diminishes in the cells of the successive antheridial filament generations. In all generations the

Fig. 2. Changes in dry mass content of nuclei during interphase in successive developmental stages of *Chara vulgaris* antheridial filaments

Table 1

Changes in dry mass content of nuclei during interphase

Stage of interphase	Generation			
	4-cell	8-cell	16-cell	32-cell
Early S	79.6	48.2	45.7	35.7
	± 4.1	± 3.3	± 3.5	± 0.3
Early G ₂	128.4	96.3	83.7	67.1
	± 2.9	± 6.3	± 2.2	± 2.7
Late G ₂	164.8	111.9	96.6	76.8
	± 6.2	± 3.4	± 3.0	± 2.5

highest increase in nuclear dry mass, similarly as that of surface area is noted in the first stage of interphase, since the differences in the weight of the nuclei are widest between early phase S and early phase G₂ (Fig. 2). These differences for the successive generations amount to 49, 48,

38 and 31 pg. In the second stage of interphase, during G_2 , the increment of dry mass of the nuclei is much smaller, the increase is distinctly limited beginning with the 4-cell and ending with the 32-cell generation (from 36.4 to 9.7 pg). In the course of the entire interphase, in all the generations examined, nuclear dry mass is at least doubled (Table 1). On the other hand, in the corresponding interphase periods the cell nuclei of the successive generations of antheridial filaments contain less and less dry mass, the widest differences in weight being found in the 4- and 8-cell generations (Fig. 2).

Increase of cytoplasm surface area

The cytoplasm surface area is not doubled in the interphase period in any of the antheridial filaments generations (Fig. 3). It only increases by about 75 per cent. The lowest surface increment of the cytoplasm was noted in 32-cell filaments. In early interphase the increase of the

Fig. 3. Increase in cytoplasm surface area during interphase in successive developmental stages of *Chara vulgaris* antheridial filaments

cytoplasm surface is slight or does not occur at all. It is only in stage G_2 that a distinct increase is noted. If we examine, however, the successive generations of antheridial filaments in the same interphase stages, a gradual reduction of the cytoplasm surface is seen, the larger the more advanced is the stage of development of the antheridial filaments.

Changes of the cytoplasm dry mass

The cytoplasm dry mass was calculated similarly as that of nuclei for the same three stages of interphase (Fig. 4). When the successive antheridial filaments generations are considered, a certain dry mass increase is

found for the entire interphase period. This increase occurs mainly between early and late G_2 stage, although this actually is true only for the 4-cell generation, and even in this case the dry mass content is not doubled. In further generations the dry mass increase is slight, moreover in the first stage of interphase, between early stage S and early stage G_2

Fig. 4. Changes in cytoplasm dry mass content during interphase in successive developmental stages of *Chara vulgaris* antheridial filaments

Table 2

Changes in dry mass content of cytoplasm during interphase

Stage of interphase	Generation			
	4-cell	8-cell	16-cell	32-cell
Early S	175.5 ± 7.8	139.4 ± 9.3	85.9 ± 5.4	62.4 ± 2.2
Early G_2	168.0 ± 9.9	101.5 ± 9.3	68.4 ± 3.5	59.7 ± 5.6
Late G_2	272.8 ± 13.2	135.2 ± 13.8	88.2 ± 10.3	65.8 ± 7.9

a certain decrease occurs in dry mass, most pronounced in 8- and 16-cell generations (Fig. 4). In the corresponding interphase stages the dry mass of cytoplasm decreases from the 4-cell to the 32-cell generation of antheridial filaments (Fig. 4, Table 2).

DISCUSSION

One of the indispensable parameters necessary for dry mass determination is the surface area of the given object. In each generation of antheridial filaments of *Chara vulgaris* the cells grow in the interphase period. This is manifested in a gradual increase of the nucleus and cytoplasm surface area. The surface increment of the nucleus has, however, a different course than that of the cytoplasm, both as regards quantity and the stage of interphase at which it occurs. The results indicating a greater surface area increment of the nucleus than of the cytoplasm over the entire interphase agree with the results of earlier measurements on the same object, and also with the fact that the increase in surface area of the nucleus takes place in phase S, while the cytoplasm surface enlarges most in stage G₂ (Olszewska, 1974b). Moreover, in the corresponding interphase periods in the successive generations of antheridial filaments the cells gradually become smaller. The same is true of the cell nuclei, to a smaller extent, however, so that the plasma-nucleus ratio changes in the successive generations in favour of the nucleus (Olszewska, Godlewski, 1972). The length of the cells shorter than that characteristic for the given interphase period in the preceding filament generation is connected in *Chara* with a shortened life cycle. This shortening is gradual and occurs at the cost of stage G₂, since the duration of phase S is the same in all generations, whereas stage G₂ is longer in filament cells during earlier developmental stages (Godlewski, Olszewska, 1973). This fact is probably connected with the limitation of cytoplasm increase in the successive filaments generations, since this increase takes place above all in the second part of interphase.

The changes in dry mass content strictly connected with the changes of surface also show that nuclear and cytoplasmic components are synthesized at different stages of interphase.

The results of nuclear dry mass determination show a doubling of its content in the interphase in all generations. Thus, in spite of the shortening of the cell cycle, the components of the nucleus are fully restored, ensuring a division which leads to the formation of the next cell generation. Dry mass measurement results, obtained in investigations of *Physarum polycephalum* with a similar type of life cycle, also show a doubling of dry mass content in the nuclei (Bevey, Ruch, 1972). The most characteristic feature of the growth and differentiation process of antheridial filaments cell differentiation in *Chara* is the diminishing dry mass of the nuclei at the beginning of interphase, that is in early stage S (see Table 1). If we analyse the data listed in this table, it is seen that the dry mass of both daughter nuclei in stage S of the particular generations of antheridial filaments is markedly smaller than that of nuclei

of the preceding generation in late G₂ stage. The reason for this may be, that beside the equivalent division of the chromosome material during mitosis, the rest of the nuclear dry mass (ca. 75 per cent) is mixed in prophase with cytoplasm and its full amount does not return to the daughter nuclei (Mitchison, 1971). This is confirmed to some extent by analysis of the data in table 2. It results from them that the cytoplasm dry mass value in stage S of the particular generation of antheridial filaments amounts to more than one half of the cytoplasm dry mass in the period of late G₂ stage in the preceding generations. The gradual increase of nuclear dry mass in the period between early S stage and early G₂ stage may occur owing to the transport of protein substances from the cytoplasm to the nucleus through the nuclear membrane (Mitchison, 1971). This suggestion will be checked on the studied material in the course of further investigations.

Sandritter et al. (1958, cf. Wied, 1966) report that 50 per cent of the nuclear dry mass consists of DNA. Other authors mention, for different objects, a lower per cent, giving priority to protein substances (Lyndon, 1968). In the antheridial filaments of *Chara vulgaris* the first period of interphase that is phase S is characterized by an increase of at least 50 per cent in nuclear dry mass in all the generations examined. No doubt, beside the synthesis of DNA in this time, there also takes place RNA synthesis since they occur simultaneously, and radioactivity intensity converted to 100 μm^2 is similar in all generations (Olszewska, Godlewski, 1972). Intensive ¹⁴C-adenine incorporation as RNA synthesis index occurs in early S stage when part of this precursor is incorporated into replicating DNA as well as in G₂ stage. In the second part of interphase, that is in late G₂ stage the intensity of RNA synthesis diminishes (Olszewska, Godlewski, 1972). Investigations on the interphase period in which synthesis of nuclear proteins occurs in *Chara vulgaris*, did not demonstrate unequivocally on the basis of ³H-phenylalanin incorporation a more intensive activity in the first interphase stage (Olszewska, Godlewski, 1972). On the other hand, at least part of the radioactive amino acids indicative of histone protein synthesis is incorporated into the nuclei above all in stage S (Olszewska, 1974c). In stage G₂ almost the whole nuclear dry mass increment would be attributable to nonhistone proteins. The latter proteins in the nucleus are, according to Roels (1963), the component contributing most to the increase of dry mass content.

The dry mass of the cytoplasm is not doubled in any developmental stage of *Chara vulgaris* antheridial filaments. The increase in surface area is only weakly correlated with the dry mass increment, the latter being out of proportion lower than the change in surface area. This may be connected with the state of the hydration of the cytoplasm. It is known from the studies of Sandritter et al. (1960) that in early interphase

the increase in cell volume is caused, on the one hand, by the synthesized chemical compounds, while, on the other hand, it results from water uptake. Water is released only in the second period of interphase, and the further increase in volume is due only to the increasing dry mass content.

In *Chara vulgaris* the cytoplasm surface increases in interphase by about 75 per cent in each generation. In stage S this increment is smaller than in G₂ stage, but dry mass does not increase at all in this time, it even decreases somewhat. It seems reasonable to refer this phenomenon to cytoplasm hydration, since the variations in cytoplasm dry mass content in both stages of interphase are the smallest in the 32-cell generation in which the cytoplasm volume is most reduced.

The decrease in dry mass content of the cytoplasm in the corresponding interphase period in the successive phases of development of antheridial filaments in *Chara* is certainly due to a reduction of RNA and protein synthesis. Labelled adenine incorporation, estimated by the number of traces per cell, decreases in the successive generations. The intensity of incorporation of this precursor into the nucleus diminishes in stage G₂, but the radioactivity of the cytoplasm remains at the same level (Olszewska, Godlewski, 1972). Protein synthesis in the cytoplasm, estimated on the basis of labelled phenylalanine, agrinine and tryptophane incorporation decreases similarly in the successive generations of antheridial filaments, although in the course of interphase it persists at the same level in each generation (Olszewska, Godlewski, 1972; Olszewska, 1974b, Olszewska, 1974c). The decrease in intensity of protein and RNA synthesis is connected here with the limitation of cell growth, thus, with the diminution of the surface area of the cytoplasm in the successive development phases of antheridial filaments.

To sum up it may be stated that the gradual decrease in intensity of synthesis of nuclear and cytoplasmic components with development of antheridial filaments, observed in earlier investigations seems to be confirmed by the quantitative results concerning changes in dry mass content in the nucleus and cytoplasm.

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*Interferometryczne pomiary zawartości suchej masy jąder i cytoplazmy
w cyklu życiowym komórek nici spermatogenicznych Chara vulgaris L.
w kolejnych stadiach rozwoju*

Streszczenie

Metodą mikroskopii interferencyjnej wyznaczono suchą masę jąder i cytoplazmy w trzech okresach interfazy (wczesna S, wczesna G₂, późna G₂) w 4, 8, 16 i 32 komórkowych niciach spermatogenicznych *Chara vulgaris*. Stwierdzono, że sucha masa i powierzchnia jąder ulegają podwojeniu podczas interfazy w każdym z kolejnych pokoleń nici spermatogenicznych, natomiast ani powierzchnia, ani sucha masa cytoplazmy nie podwajają się w tym czasie. Przyrost powierzchni i suchej masy jądra ma miejsce we wczesnym okresie cyklu życiowego, tj. w fazie S, zaś przyrost powierzchni i suchej masy cytoplazmy następuje w późniejszym okresie cyklu, tj. w fazie G₂. W kolejnych pokoleniach nici spermatogenicznych zarówno sucha masa jak i powierzchnia tak jąder, jak i cytoplazmy ulegają wyraźnemu i stopniowemu zmniejszeniu.