Specific Nuclear Elimination in Polyploid Plasmodia of the Slime Mold Physarum polycephalum

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In growing plasmodia of the myxomycete Physarum polycephalum (G2-phase), three distinct classes of nuclei with a relative DNA content of 1x, 2x, and 4x are observed in the presumed haploid strain CL. The 2x and 4x species comprise up to 35% and 5% of the nuclei. Quantitative cytofluorometric studies of nuclei isolated in either G2- or S-phase or after FUDR treatment (G1 arrest) show that the three nuclear populations undergo a synchronous mitotic cycle and that the relative DNA content of the nuclear fractions in G2-phase reflects the 2c, 4c, and 8c state.

The heterogeneity of the nuclear population does, however, seem to be restricted to the growth phase. During a starvation period of 4 days that always precedes sporulation (and also meiosis), the 4c nuclear population is reduced to 7%, 8c nuclei are no longer detected. These results suggest that a mechanism exists in Physarum for the selective detection and elimination of polyploid nuclei.

Key terms: Macroplasmodia, myxomycete, haploid stain, starvation, cell growth

Plasmodia of the myxomycete Physarum polycephalum are unicellular, multinucleate organisms. The plasmodal cell cycle is characterized by synchronous nuclear divisions (6,9,15) and the lack of a G1-phase (21). After nutrient deprivation for 3 to 5 days, plasmodia are induced to sporulate by light (4). The formation of spores is accompanied by meiotic divisions (11). Germinating spores release single uninucleate amoebae, which, upon fusion with amoebae of different mating type, reform plasmodia.

The colonia (CL) isolate differs from heterothallic Physarum strains in that plasmodium formation occurs without amoebal fusion. CL plasmodia are therefore haploid in contrast to the generally diploid heterothallic strains (1,13). A low degree of diploidization, however, appears to be essential for the formation of viable spores in the CL strain (12). It has recently been reported that haploid strains, which were kept for extended periods in submerse culture, revealed a high degree of mixoploidy (10).

A specific elimination of nuclei has been observed in Physarum heterokaryons. In a genetic analysis, Dee and Anderson (5) reported the elimination of diploid nuclei from haploid/diploid heterokaryons during growth phase; a similar effect was observed under starvation conditions (14).

In this report we show that polyploidization in the strain CL may occur at a high level in plasmodia during growth and that the polyploid nuclei are gradually reduced to a minute amount during a prolonged starvation period. Thus we extend earlier findings of nuclear degradation in Physarum heterokaryons to a naturally polyploidized strain, suggesting that a selective mechanism exists to include polyploid nuclei from meiosis.

MATERIALS AND METHODS

Plasmodial Culture

Plasmodia of the colonia strain (CL) were generated by plating spores on agar made from dilute (33%) semidefined growth medium (3). Surface plasmodia were scraped from the agar, transferred to shake flasks containing growth medium, and cultured as macroplasmodia at 24°C at 240 rev/min. Macroplasmodia were obtained by coalescence of 2-h-old microplasmodia. Growth and starvation conditions were kept exactly as described (17). Sporulation was induced at the fourth day of starvation by illumination with fluorescent white light (17). For 5-fluoro-2'-deoxy-uridine (FUDR) inhibitor

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studies, macroplasmodia were transferred with the paper support to 10 ml growth medium containing appropriate concentrations of the drug. Determination of mitosis was performed by evaluating plasmodia smears under the light microscope. Nuclei were isolated after the protocol of Nothacker and Hildebrandt (16). No nuclear aggregates were detectable by microscopic observation. Throughout this manuscript, lc is defined as the nonreplicated haploid nuclear DNA content and 2c, 4c, and 8c as multiple values of lc.

Flow Cytometry

The DNA of Physarum nuclei was stained for 10 min at 22°C by diluting 5 μl nuclear preparation with 250 μl of a buffer solution pH 7.35 containing 0.15 M NaCl, 100 μg/ml mithramycin (Serva, Heidelberg) (2), 15 mM Mg2+, and 5 mM HEPES. Five milliliters of porous latex particles (5 μm diameter), stained with fluorescein-isothiocyanate, monosized, and containing free amino group, were also added to serve as an internal standard for volume and fluorescence.

The electrical volume and the fluorescence of the mithramycin-stained nuclei were measured simultaneously in a Fluvo-Metricell flow cytometer (8) (HEKA Elektronik, Lambrecht/Pfalz, FRG), using a hydrodynamically focused cylindrical orifice of 50 μm diameter and 60 μm length at an electrical current of 0.23 mA. The buffer, without Mg2+ and mithramycin, served as sheath fluid. Fluorescence was excited with an HBO-100 high-pressure mercury arc lamp between 400 and 500 nm. Fluorescence emission was measured between 510 and 700 nm. The volume and the fluorescence pulses of all nuclei were logarithmically amplified. The maximum amplitude of each signal was digitized by 128-step analog-digital converters and stored on magnetic tape in list mode. The data were subsequently classified, displayed and quantitatively evaluated by software developed earlier (19,20). The DNA and volume pulses were logarithmically amplified in order to visualize haploid, diploid, tetraploid, and octoploid nuclei, nuclear aggregates, and calibration particles in the same graph, which was not possible with linear amplification.

RESULTS

Nuclear Heterogeneity in Growing Plasmodia

In cytofluorimetric analysis of nuclear preparations from Physarum macroplasmodia (strain CL), three major distinct classes of nuclei were detected (Fig. 1 and 2A, peaks α, β, and γ). The fractions had a relative DNA content of 1:2:4 and occurred with a relative abundance of 60.2, 35.0, and 5.2%, respectively (see Table 1). To characterize these nuclear populations with respect to mitotic activity and cell cycle stage, we compared the flow cytometric patterns obtained from nuclear preparations in G1-, G2-, and S-phase. Since the cell cycle of

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**Fig. 1.** Volume versus DNA of mithramycin-stained Physarum polycephalum nuclei. The 2c (α), 4c (β), 8c (γ), and 1c (δ) nuclei, as well as the calibration particles (part) and the cell debris, are visible. The graph is standardized to the maximum logarithmic channel content as 100% and contour lines are plotted at 10% intervals downwards. A total of 36,112 nuclei, particles, and cell debris, with a maximum of 806 nuclei/channel were measured. The lowest contour line corresponds to a channel content of 2 nuclei, i.e., the position of the majority (95%) of the nuclei is labeled in the graph. One logarithmic volume class corresponds to a volume of 0.33 μm³.

**Fig. 2.** DNA distribution curves of nuclei 30 min before mitosis (A) and in the presence of 25 μg/ml FUDR (B). Peak identification as in the experiment of Figure 1. The nonlabeled peaks in B are assumed to represent unreplicated material from peak β and γ in A (see text). The curves contain 4,174 (A) and 9,997 (B) nuclei and are standardized to the respective maximum channel frequencies of 931 (A), 3,195 (B) nuclei as 100%.
Physarum lacks a G1-phase, the unreplicated nuclear state was induced by treatment of the cells with FUDR, an inhibitor of DNA replication (18). Nuclear isolates from plasmodia grown for 24 h in the presence of FUDR (25 μg/ml) contain an additional peak (δ, Fig. 2A), with half the DNA content of peak α in the experiment of Figure 2A. This peak very likely represents the 1c state of peak α, trapped before the beginning of DNA replication by FUDR treatment. The changes in peak positions and intensities in the experiment of Figure 2B relative to the profile in Figure 2A suggest that peaks α, β, and γ represent the 2c, 4c, and 8c of nuclei (see also Materials and Methods) and that all ploidy classes participate in the synchronous mitotic cycle. This interpretation was further confirmed by the comparison of nuclei isolated in prophase and early S-phase of the cell cycle (Fig. 3). In this experiment, a shift of the major peak α to a position with approximately half the DNA content is observed. The position of this early S-phase-specific peak is found at a slightly higher DNA content, as compared with FUDR-induced peak δ in the experiment of Figure 2B. This shift in peak position indicates that 5 min after mitosis, newly synthesized DNA has already accumulated to some extent. This finding correlates with the observations of other authors (9). Taken together, these results show that growing plasmodia of the CL strain contain, besides haploid nuclei, a considerable amount of 4c and 8c nuclei that also are mitotically active and undergo a synchronous cell cycle.

The relative abundance of the individual nuclear fractions in growing plasmodia varied considerably (20–40% for 4c) in preparations from different plasmodial batches, as indicated by the different nuclear profiles in the experiments of Figures 2A and 3A. This effect clearly was not a function of total culture time after plasmodium formation and had no obvious effect on specific biological functions such as growth rate and differentiation. Thus we did not observe any change in sporulation and germination frequency.

**Fate of Polyploid Nuclei in the Life Cycle**

Since haploid nuclei cannot undergo a regular meiosis, the formation of euploid spores in the strain CL is a rare event. The low but significant frequency of spore germination observed has been attributed to the presence of a small fraction of diploid nuclei, as reported by Lafler and Dove (12). The same authors also reported a correlation of germination frequency and the amount of diploidization in the CL-strain. In contrast with these findings, the frequency of spore germination in the CL variety used in our experiments was very low (< 1%, data not shown), in spite of the high percentage of 4c nuclei in growing plasmodia. We therefore analyzed the fate of the 4c and 8c nuclei throughout the plasmodial stage of the life cycle, i.e., during the acquisition of sporulation competence and the differentiation into sporangiophores and spores. Nuclei were isolated from plasmodia after 2 and 4 d of starvation and from sporulating cells (14 h after photostimulation on the fourth day of starvation). The results depicted in Figure 4 indicate that 4c and 8c nuclei gradually disappear during the starvation period. After 4 d of starvation, the 4c nuclear fraction is reduced to 7%, and 8c nuclei are no longer detected (Fig. 4 and Table 1). This value remains unchanged throughout sporangiogenesis (Fig. 4D). The reduction of the relative amount of polyploid nuclei to a low level was observed in all plasmodial batches anal-

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**Table 1**

<table>
<thead>
<tr>
<th>Peak</th>
<th>δ</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Mean volume of all nuclei (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Growth (MII - 30 min)</td>
<td>4.2</td>
<td>60.2</td>
<td>35.0</td>
<td>5.2</td>
<td>4.23</td>
</tr>
<tr>
<td>2-d starvation</td>
<td>0.5</td>
<td>74.5</td>
<td>25.0</td>
<td>7.6</td>
<td>2.57</td>
</tr>
<tr>
<td>4-d starvation</td>
<td>5.7</td>
<td>86.9</td>
<td>7.4</td>
<td>1.5</td>
<td>3.65</td>
</tr>
<tr>
<td>4-d starvation</td>
<td>6.0</td>
<td>87.0</td>
<td>7.0</td>
<td>1.0</td>
<td>3.73</td>
</tr>
<tr>
<td>14-h light</td>
<td>38.2</td>
<td>45.4</td>
<td>26.4</td>
<td>5.0</td>
<td>2.57</td>
</tr>
<tr>
<td>B MIL - 10 min</td>
<td>6.7</td>
<td>77.1</td>
<td>13.8</td>
<td>2.4</td>
<td>3.85</td>
</tr>
<tr>
<td>MIL + 5 min</td>
<td>81.5</td>
<td>14.4</td>
<td>4.1</td>
<td>1.0</td>
<td>2.41</td>
</tr>
</tbody>
</table>

All data were obtained by quantitative evaluation of the experiments in Figures 1–4. Peak γ possibly contains insignificant amounts of nuclear aggregates.
FIG. 4. DNA distribution curves of nuclei 10 min before mitosis (A) and after starvation for 2 d (B) and 4 d (C) and 14-h white light illumination on day 4 (D). A total of 4,280, 20,990, 21,637, and 13,880 nuclei with peak values of 931, 7,640, 7,289, and 5,546 were measured for the curves.

lyzed, independently of the initial nuclear distribution profile.

DISCUSSION

Using flow cytometry, we have presented evidence that Physarum plasmodia of the presumed haploid strain CL contain nuclei with 1, 2, and 4 replicated chromosome complements during growth, and that the 4c and 8c species are eliminated prior to differentiation and meiosis. We assume that the 4c and 8c nuclei arise by polyploidization rather than by polytenization, even though the quantitative methods used in this study do not discriminate between these alternatives.

The characterization of the nuclear populations was based on two lines of evidence:

1. Treatment with FUDR, a drug well known to inhibit DNA replication in Physarum, reduced the DNA content of all the nuclear fractions by 50%, indicating that the nuclei had been arrested in a lc state after a mitotic division before the onset of replication.

2. Accumulation of nuclei in the lc state was also observed in isolates from S-phase plasmodia. We conclude from these results that all nuclear species are mitotically active and follow a synchronous cell cycle, as has also been reported for nuclei with differing DNA content in a mixoploid plasmodial strain (9).

We have not observed any correlation between the relative amount of 4c and 8c nuclei and biological parameters such as growth rate or sporulation competence. Since an increased amount of 4c nuclei in the haploid strain CL raises the germination frequency of the spores considerably (12), it is interesting to note that 4c and 8c nuclei disappear during a starvation period, i.e., at the stage preceding spore formation and meiosis. This observation seems to rule out the assumption that the observed polyploidisation is a strain-specific phenomenon that compensates for the failure of haploid nuclei to give rise to cuploid spores.

The polyploid nuclei are selectively lost from starving plasmodia, which are characterized by a massive reduction in protein, DNA, and RNA content (7). Since the protein:DNA ratio remains constant throughout growth and differentiation (unpublished observation), we infer a degradation of nuclei during starvation. Whether the polyploid nuclei are specifically detected and preferentially degraded or rather induced to undergo a mitosis cannot be derived from our data. Reports on a selective loss of diploid nuclei from fusion-induced heterokaryons of haploid and diploid plasmodia (5), however, support the assumption that mechanisms for the detection and elimination of specific nuclear fractions exist, even though direct experimental evidence for such a process has not been obtained so far.

LITERATURE CITED


