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The Relationship Between Enzyme Activity, Cell Geometry, and Fitness in *Saccharomyces cerevisiae**

(evolution of diploidy/gene dosage/regulation/population genetics)

RICHARD L. WEISS†, J. R. KUKORA, AND JULIAN ADAMS‡

Department of Botany, University of Michigan, Ann Arbor, Mich. 48104

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ABSTRACT **The relationship between enzyme activity, cell geometry, and the ploidy level has been investigated in *Saccharomyces cerevisiae*. Diploid cells have 1.57 times the volume of haploid cells under nonlimiting growth conditions (minimal medium). However, when diploid cells are grown under conditions of carbon limitation, they have the same volume as haploid cells. Thus, by altering the environmental conditions, cell size can be varied independently of the degree of ploidy. The results indicate that the basic biochemical parameters of the cell are primarily determined by cell geometry rather than ploidy level. RNA content, protein content, and ornithine transcarbamylase (carbamoylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3), tryptophan synthetase [L-serine hydro-lyase (adding indole), EC 4.2.1.20], and invertase (α-D-glucoside glucohydrolase, EC 3.2.1.20) activity are related to cell volume, whereas acid phosphatase (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.2) activity, a cell surface enzyme, is related to the surface area of the cells. Fitness is ultimately determined by the activity and concentration of the cellular components. Thus, when fitness is determined by the activity of certain cell surface enzymes, such as acid phosphatase, diploids would be expected to have a lower fitness than haploids because of the lower surface area/volume ratio. However, when fitness is determined by the activity of an internal enzyme, diploids would be expected to have the same fitness as haploids. Results from competition experiments between haploids and diploids are consistent with these predictions. The significance of these results to the evolution of diploidy as the predominant phase of the life cycle of higher plants and animals is discussed.**

Although diploid cells of *Saccharomyces cerevisiae* possess some functions that are unique, such as the production of ascospores, they can be considered, in many respects, to consist simply to two haploid genomes enclosed in one cell membrane. During exponential growth in complex medium, diploid cells have twice the volume of haploid cells (1, 2) and contain twice the amount of DNA and protein (3–5), and twice the total activities of a variety of internal enzymes (4, 6–8). However, the increase in certain amino-acid transport systems has been shown to be proportional to the increase in surface area (and not volume) enjoyed by diploid cells (9).

These strict proportionalities led Adams and Hansche (10) to predict and demonstrate experimentally that: (*i*) diploid cells would have a lower fitness than haploids in environments where fitness was determined by transport of a limiting substrate across a cell membrane and (*ii*) diploid cells would have the same fitness as haploids where fitness was determined by the internal metabolism of a limiting substrate. These results could be explained by the reduced surface area/volume ratio of diploids.

An unexpected feature of these results was that the geometric proportionalities between haploid and diploid cells were not preserved when the cells were grown under nonideal conditions. In particular, when diploid cells are grown under conditions of dextrose limitation, the sizes of diploid cells are virtually identical to those of haploid cells (10). This finding raises the question: Is the reduction in volume caused by reduction in vacuole size or by a reduction in the amount of all or certain cellular proteins and enzymes? A reduction in size caused by a general decrease in gene expression would further substantiate the idea that diploids are not intrinsically any more fit than haploid cells under conditions of suboptimal growth rates.

The experiments described below were designed to test the hypothesis that basic biochemical cell parameters, and thus ultimately fitness, are determined primarily by the geometry of the cell rather than the DNA content or ploidy level. In addition to total protein and RNA content, the activities of four enzymes, two internal and two located on the surface of the cell, were studied in detail. Both internal enzymes act in amino-acid biosynthetic pathways: tryptophan synthetase [L-serine hydro-lyase (adding indole), EC 4.2.1.20] and ornithine transcarbamylase (carbamoylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3). We chose these enzymes specifically because a gene dosage–activity relationship had previously been reported for both (4, 8), and we wished to include one repressible (ornithine transcarbamylase) and one nonrepressible enzyme (tryptophan synthetase). The two cell surface enzymes chosen were acid phosphatase (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.2) and invertase (α-D-glucoside glucohydrolase, EC 3.2.1.20) (11).

MATERIALS AND METHODS

Yeast Strains. Haploid strains used in these experiments were XC500A and XC500B obtained from R. K. Mortimer. These strains, derived from S288C, are assumed to be isogenic for all loci except for the mating type locus (10). Diploid strains used in these experiments were SCD3-1, SCD3-2, and SCD3-3, all derived from single zygotes isolated by micromanipulation after conjugation between XC500A and XC-

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† Present address: Department of Chemistry, University of California, Los Angeles, Calif. 90024.

‡ Author to whom reprint requests should be sent.

500B. These three diploid strains are assumed to be isogenic to each other and also to the haploid strains used. In all the experiments described below there was no significant variation among the three diploid strains, or among the two haploid strains.

Media. All experiments were conducted in defined media as previously described (10). Minimal medium with 10 g/liter of sucrose replacing dextrose as the carbon source was used for experiments to determine invertase activity.

Growth and Sampling. Unlimited growth cultures on minimal medium were grown at 30° in a gyratory shaker at 150–200 gyrations per min. Cultures were harvested by filtration through 0.45 μm pore cellulose nitrate filters when cell density was between 8.0×10^6–1.2×10^7 cells per ml, washed three times with 0.1 M phosphate buffer at pH 7.2 or pH 8.0, and either used immediately or stored at −20°. Chemostat cultures were maintained at 30° in chemostats of various sizes from 150–200 ml. All dextrose-limited chemostats were run at a dilution rate of 0.14 hr^{-1} or 55% of the maximum specific reproductive rate (i.e., reproductive rate when dextrose is nonlimiting) of the cells. This growth rate gave an equilibrium density in the chemostat of 1×10^7 cells per ml. At this growth rate the equilibrium concentration of dextrose in the chemostats is barely detectable with the anthrone test. All organic-phosphate-limited chemostats were run at a dilution rate of 0.17 hr^{-1} or 60% of the maximum specific reproductive rate of the cells. This growth rate gave an equilibrium density in the chemostats of 5×10^6 cells per ml.

Cells were collected from the chemostats after numerical equilibrium had been reached, either by emptying the culture vessel or by collecting cells from the overflow tube in a flask immersed in an ice-bath over a period of several hours. There were no significant differences between samples obtained using the different techniques. Cells were harvested by filtration as for the unlimited growth cultures, and stored at −20° or used immediately.

Cell Number and Size Estimation. Cell number was estimated with an electronic particle counter (Celloscope 111: Particle Data, Inc. Elmhurst, Ill.) equipped with a 60 μm orifice. In all experiments described below, buds were not counted as separate cells but assumed to be part of the parent cell. Cell size was estimated by direct measurement. The major and minor axes of the parent cells and buds were measured on at least 100 cells for each strain in each environment. Cell volumes and surface areas of the parents and buds were calculated on the assumption that the shapes approximated a prolate spheroid. Volumes and surface areas were then calculated for the cells by adding the values for the parents and buds. This method of representing cell size accounts for the differences between the figures presented in this paper and those published elsewhere (1, 2, 10).

Cell-Free Extracts. Cells were lyophilized and resuspended in ice-cold 0.1 M potassium phosphate buffer at pH 7.2 or pH 8.0. The suspension was cooled in an ice bath and sonicated (80% maximum power) in a Bronwill ultrasonic disintegrator for 3–5 min in 15–30-sec pulses to avoid excess heating. Following sonication, the suspension was centrifuged at $5000 \times g$ for 10 min in a refrigerated centrifuge. The clear supernatant was used within a few minutes for the determination of tryptophan synthetase activity. A portion of the supernatant was dialyzed for 18 hr at 4° against three changes of 0.05 M Tris·HCl, pH 8.0, 100 volumes each. This dialyzed extract was used to determine ornithine transcarbamylase activity.

Nucleic Acid and Protein Assays. DNA was extracted from a determined number of cells (1 to 5×10^9) with trichloroacetic acid as previously described (4). DNA was determined in the trichloroacetic acid extract by a modified diphenylamine procedure (12, 13). Standard curves were prepared with salmon sperm DNA (Mann). RNA was extracted and assayed as described by Ogur and Rosen (14) with yeast RNA (Sigma) as a standard. Total protein was determined (15) after extraction of a determined number of cells with 1 N NaOH for 24 hr, with bovine serum albumin as a standard.

Ornithine Transcarbamylase Activity. The assay procedure of Davis (16) was used except that we utilized 0.1 M Tris·HCl, pH 8.0.

Tryptophan Synthetase Activity. The enzyme was assayed as described by Manney (17) following indole disappearance (18).

Acid Phosphatase Activity. Enzyme activity was estimated using whole cells at pH 6 according to Francis and Hansche (19). The nonoptimal pH was used in order to make comparisons with previously published results (10, 19, 20).

Invertase Activity. Invertase activity was measured in whole cells by the method of Arnold (21). The cell suspension was buffered at pH 5.6 with 0.2 M sodium acetate buffer, the previously determined optimum for this enzyme in these strains, and pretreated with 30 μg/ml of Nystatin (Sigma) for 30 min at 30° (22).

Arginine Pool. A determined number of cells was suspended in water and heated at 100° for 10 min. After cooling, the suspension was centrifuged for 10 min at $10{,}000 \times g$. Arginine was purified by ion-exchange chromatography and assayed as previously described (23).

Tryptophan Pool. A determined number of lyophilized cells was treated with trichloroacetic acid (final concentration 5%) and the precipitate was removed by centrifugation and reextracted with an equal aliquot of 5% trichloroacetic acid. Tryptophan was then assayed in the combined supernatants using the procedure of Messineo and Musarra (24). Indole also reacts in this procedure. Enzymatic determination of tryptophan (25) demonstrated, however, that indole was undetectable in the samples.

RESULTS

Macromolecular Composition. The macromolecular composition of haploid and diploid cells was examined during both unlimited and carbon-limited growth. Diploid cells under unlimited (all components of the medium present in excess) growth conditions have 1.57 the volume of haploid cells. However, when diploid cells are grown under conditions of carbon-limitation, they possess exactly the same volume as haploid cells (Table 1). Thus, by varying the conditions of the environment we can vary cell size independent of the level of ploidy. The DNA contents of such cells are shown in Table 1. During unlimited growth, DNA content is directly related to ploidy. These results are in good agreement with those reported elsewhere (4, 5). During carbon-limited growth, DNA

TABLE 1. *Nucleic acid and protein content of S. cerevisiae, and cell volume*

Ploidy	Unlimited growth				Carbon-limited growth			
	DNA ($mg/10^{11}$ cells ± SEM)	RNA ($mg/10^{11}$ cells ± SEM)	Total protein ($mg/10^{11}$ cells ± SEM)	Mean cell volume (μm^3)	DNA ($mg/10^{11}$ cells ± SEM)	RNA ($mg/10^{11}$ cells ± SEM)	Total protein ($mg/10^{11}$ cells ± SEM)	Mean cell volume (μm^3)
Haploid	2.70 ± 0.13	118 ± 3	573 ± 17	66.9 ± 1.8	2.14 ± 0.05	54 ± 3	316 ± 17	43.96 ± 2.44*
Diploid	4.90 ± 0.11	184 ± 18	805 ± 14	105.0 ± 2.4	3.73 ± 0.14	51 ± 2	312 ± 27	41.56 ± 2.53†
Diploid/haploid	1.81	1.56	1.40	1.57	1.74	0.95	0.99	0.95

* XC500B only.
† SCD3-1 only.

content remains proportional to ploidy, despite the fact that both haploids and diploids are smaller than unlimited growth cells and have the same cell volume. The deviations of the diploid/haploid DNA content ratio from the theoretically expected value of two may reflect a difference in the average degree of DNA replication in the cell populations. In this respect it is interesting to note that the diploid/haploid DNA content ratio for stationary phase cells, after correction for buds, is 1.97. The difference in DNA content between unlimited growth cells and cells under carbon-limitation is probably a reflection of differences in the frequencies of unbudded cells in the two environments. Frequency of unbudded cells is higher ($(\bar{x} = 0.54)$ under carbon-limitation than under unlimited growth ($\bar{x} = 0.33$). When this factor is taken into account, the significant differences in DNA content between haploid cells and between diploid cells in the two environments disappear.

The proportional increase in RNA content in diploids compared to haploids in unlimited growth is almost identical to the proportional increase in volume (Table 1). A similar correlation is seen in cells grown in a carbon-limited environment, in which haploid and diploid cells have the same RNA content and the same cell volume despite significant differences in DNA content. This result suggests that diploid and haploid cells in a carbon-limited environment may differ only in DNA content.

Further support of this conjecture is gained from a consideration of the total protein contents of haploids and diploids in the different environments. The results (Table 1) show that total protein content is correlated with cell volume and is independent of ploidy (DNA content). The soluble protein fraction, extracted by sonication, is a constant proportion of the total protein content in both haploid and diploid cells. Thus, the soluble protein content of haploid and diploid cells is also closely correlated with cell volume and apparently independent of ploidy.

Although the macromolecular composition of haploid and diploid cells (Table 1) suggests that cellular parameters are related to cell geometry rather than ploidy, it is conceivable that individual macromolecular components, as well as small-molecular-weight compounds, may not show such a relationship. Accordingly, the activities of certain enzymes and amino-acid pool sizes were examined in haploid and diploid cells in different environmental conditions.

Amino-Acid Pools. The amino acids arginine and tryptophan were chosen for study as examples of small-molecular-weight compounds with high (arginine) and low (tryptophan) intracellular concentrations. Details of the pool sizes in haploid and diploid cells under the two environmental conditions are shown in Table 2. As was the case with RNA and protein, the sizes of the amino-acid pools are related to the cell volume rather than the ploidy level or DNA content. However, the results show that carbon-limitation can affect the levels of specific intracellular components quite drastically. Although cell volume is only reduced 34% in haploids, the intracellular arginine concentration is reduced almost 15-fold when a limitation for carbon is imposed.

Internal Enzyme Activities. The observed change in tryptophan and arginine concentrations under conditions of carbon limitation raised the possibility that enzymes in the tryptophan and arginine metabolic pathways may be derepressed under these conditions. Activities of two enzymes in these pathways, tryptophan synthetase and ornithine transcarbamylase, were therefore determined in haploid and diploid cells under the two environmental conditions. The results are shown in Table 3. The specific activity of both the enzymes is constant under all conditions. We are confident that these results are not an artifact of differential extraction, since sonication solubilizes a constant proportion of the total protein in every case. The apparent constancy of enzyme specific activities suggests that the reduction in total protein content

TABLE 2. *Amino-acid pools in S. cerevisiae*

Ploidy	Unlimited growth			Carbon-limited growth		
	Tryptophan ($mmol/10^8$ cells, ± SEM)	Arginine ($mmol/10^8$ cells, ± SEM)	Mean cell volume (μm^3)	Tryptophan ($mmol/10^8$ cells, ± SEM)	Arginine ($mmol/10^8$ cells, ± SEM)	Mean cell volume (μm^3)
Haploid	1.138 ± 0.046	182 ± 6	66.9 ± 1.8	0.994 ± 0.037	12.44 ± 0.54	43.96 ± 2.44*
Diploid	1.594 ± 0.077	258 ± 12	105.0 ± 2.4	0.961 ± 0.046	12.45 ± 1.20	41.56 ± 2.53†
Diploid/haploid	1.40	1.43	1.57	0.97	1.00	0.95

* XC500B only.
† SCD3-1 only.

TABLE 3. *Specific activities of tryptophan synthetase and ornithine transcarbamylase in S. cerevisiae* (mmol/min per mg of protein, ± SEM)

	Unlimited growth		Carbon-limited growth	
Ploidy	Tryptophan synthetase	Ornithine transcarbamylase	Tryptophan synthetase	Ornithine transcarbamylase
Haploid	6.02 ± 0.43	374 ± 25	5.50 ± 0.45	269 ± 2
Diploid	6.31 ± 0.33	366 ± 13	5.95 ± 0.25	282 ± 11
Diploid/haploid	1.05	0.98	1.08	1.05

during carbon-limited growth reflects a decrease in general gene expression. The results also substantiate the concept that haploids and diploids differ *only* in their DNA content during carbon-limited growth, and that intracellular parameters are related to cell volume rather than ploidy level.

Activity of Cell Surface Enzymes. The above results show that the volume of the cell rather than the DNA content determines the amount and activity of the intracellular components. This correlation of intracellular components with cell volume might suggest that the concentration of components located on the cell surface may be related to surface area rather than volume or DNA content. Accordingly, the activities of two enzymes, acid phosphatase and invertase, located on the surface of the cell, were assayed in haploids and diploids. The cells were grown in environments that promoted the derepression of these enzymes. For estimation of acid phosphatase activity cells were grown in chemostats, limiting growth with an organic source of phosphate (β-glycerol phosphate). In order to estimate invertase activity cells were grown in flask culture, in minimal medium, with sucrose as the sole carbon source. The results are shown in Table 4, together with estimates of cell surface areas and volumes. The activities of the two enzymes in haploids and diploids are in sharp contrast. Whereas the activity of acid phosphatase correlates well with cell surface area and not with cell volume, the reverse is true for invertase. Certainly, in neither case is the activity of these enzymes correlated with DNA content. A similar set of results was obtained by Hennaut *et al.* (9) for amino-acid transport systems.

DISCUSSION

Relationship Between Cell Geometry and Cell Physiology. The results presented in this paper indicate not only that the macromolecular components of the yeast cell vary substantially with the environment of the cells, but that this variation is: (*a*) independent of the number of gene copies present, and (*b*) closely related to the geometry of the cell. Whereas the DNA content per cell is strictly related to the ploidy level, irrespective of the environment in which the cells are grown, the RNA and protein content per cell are closely related to the cell volume rather than the ploidy level. Furthermore, the activities of the sample group of internal enzymes (ornithine transcarbamylase and tryptophan synthetase), a cell surface enzyme (invertase), and the pool sizes of arginine and tryptophan reflect the relationship between protein content and cell volume. The activity of acid phosphatase, an enzyme located on the cell surface, is, in contrast, related to the surface area of the cell, rather than cell volume or ploidy level. Thus, the geometry of the cell may be considered diagnostic of the activities and concentrations of the cell constituents, whether the constituents involved are located internally or on the surface of the cell. These results have implications regarding the adaptive significance of the diploid state of the life cycle, and it is worthwhile to examine them here in detail.

TABLE 4. *Cell surface enzyme activity in S. cerevisiae*

Ploidy	Enzyme activity	Mean cell surface area (μm^2)	Mean cell volume (μm^3)
Acid phosphatase (μg of PO_4/min per 10^7 cells ± SEM)			
Haploid*	5.21 ± 0.12	84.7 ± 2.7	61.1 ± 2.2
Diploid†	7.50 ± 0.37	117.4 ± 3.2	99.2 ± 2.8
Diploid/haploid	1.44	1.39	1.62
Invertase (μg of dextrose/min per 10^7 cells ± SEM)			
Haploid	5.69 ± 0.38	89.1 ± 1.6	61.5 ± 1.3
Diploid	8.94 ± 0.82	122.6 ± 2.0	97.3 ± 2.0
Diploid/haploid	1.57	1.38	1.58

* XC500B only.
† SCD3-1 only.

Adaptive Significance of Diploidy; Physiological Considerations. The results of Adams and Hansche (10) showed that under a variety of environmental conditions diploid cells have the same or lower reproductive rate compared to haploid cells. Their results could be explained if the fitness of these cells were related to, or determined by, the geometry of the cells. Now, the reproductive rate (and hence fitness) of cells in non-limiting conditions or in chemostat culture is ultimately determined by the activity of a single enzyme catalyzing the rate-limiting step. For some environments this enzyme may be easy to identify. For example, the fitness of cells grown in a medium buffered at pH 6, containing organic phosphate in limiting quantities as the sole phosphate source, is determined by the activity of the enzyme acid phosphatase (10). Our results show that acid phosphatase activity is closely related to the surface area of the cells, and so, for this environment at least, the fitness of the cells can be related to surface area. However, for other environments the enzyme limiting or determining the specific reproductive rate may be more difficult to identify. Nevertheless, the results of this paper indicate that the amounts/cell of macromolecular and small molecular components of the cell are commonly related to cell volume or surface area. Thus, the fitness of the cell can be related to its geometry whether it be cell surface area or volume. It follows therefore that diploid cells will have the same reproductive rate as haploids where fitness is determined by the activity of an intracellular enzyme or certain cell surface enzymes (such as invertase), because the same enzyme activity to unit volume ratio prevails in haploid and diploid cells. Diploid cells, however, will have a lower reproductive rate than haploids where fitness is determined by the activity of certain surface-located enzymes (such as acid phosphatase), because the ratio of enzyme activity/unit volume is lower in

diploids. In this respect it is interesting to note that diploid cells are more elongated than haploid cells. For example, in complex medium the eccentricity of diploid cells (0.696) is about 35% higher than that of haploid cells (0.513). The more elongated the cell, the higher the ratio of surface area to volume. Consequently, the shape of the diploid cell may represent an adaptation that increases this ratio, and thereby increases the fitness of diploid cells in the particular environments. Competition experiments between isogenic haploids and diploids (10) support these predictions. Diploid cells have the same or lower reproductive rate than haploids, depending on the specific enzyme determining fitness.

The results presented in this paper are inconsistent with the hypothesis that the evolution of the diploid phase as a major part of the life cycle of most organisms was a result of the direct physiological advantage of the diploid. However, this conclusion must be qualified by two considerations. (*1*) The experiments described above and also by Adams and Hansche (10) have only examined the relationships between haploid and diploid cells under quite restricted, constant environmental conditions. It may be that in other environments diploids do in fact have a higher reproductive rate than haploids. Complex medium may be such an environment (26). (*2*) Constant growth conditions rarely exist in nature and it may be that the extra genetic endowment of diploids may enable them to adapt more rapidly to changes in the environment than haploids. Although diploids show no advantage in constant environments, they may be selectively favored in varying environments.

Adaptive Significance of Diploidy; Genetic Considerations. Although it may be difficult to explain the predominance of the diploid state from a consideration of any physiological advantage of diploids, the possession of more than one genome per cell may be advantageous from three genetic standpoints. (*1*) One of the theories advanced for the predominance of the diploid state (27) is the protection it affords against somatic mutation. Recessive or partially recessive deleterious somatic mutations will be masked in the diploid, whereas they would be expressed in haploids. Although mutation rates for individual genes are low (about 1×10^{-6}), the mutation rate/genome may be significant. However, without an estimate of the number of genes in the yeast genome, the importance of this effect cannot be assessed. (*2*) The presence of two copies of an allele in diploids may allow the development of favorable allelic interactions or overdominance. Thus, diploids may be selectively favored due to the development of overdominant type interactions not available to haploids. (*3*) Although it may be difficult to explain the evolution of diploidy by the selective advantage of the diploid, the lack of reversion to the haploid state is easy to understand. It is now recognized that most diploid species have accumulated large amounts of variability and that as many as 30% of the loci in a genome may be polymorphic. Undoubtedly a proportion of this variability is deleterious, being comprised of recessive lethal or semi-lethal alleles. Consequently, a reversion to haploidy would result in inviable individuals due to the expression of these deleterious genes. Therefore, it may be that, even if there is no selective advantage to the diploid stage of the life cycle, reversion to haploidy is impossible.

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