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review

Development of a New Flow Cytometric Procedure for the Determination of Single-cell Growth Properties in Asynchronous *Saccharomyces cerevisiae* Populations

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Summary

A novel flow cytometric procedure has been developed with the aim of obtaining growth properties of individual Saccharomyces cerevisiae cells in asynchronous cultures. The method is based on labelling of the cell wall with FITC-conjugated to Concanavalin A and detection of the single cell fluorescence with flow cytometry after exposing the cells to growth conditions. Because the formation of new cell wall material in budded cells is restricted to the bud tip, exposure of the stained cells to growth conditions results in three cell types: (i) stained cells, (ii) partially stained cells, and (iii) unstained cells. This first staining has been coupled to the determination of the protein content of the individual cells, which gives a good estimation of the cell size. Analysis of the double staining patterns over time permits determination of cell age and specific growth rate of individual cell cohorts. The procedure has been tested with yeast cell populations growing at different growth rates. In this paper we present a short overview of the data obtained.

Keywords: flow cytometry, *S. cerevisiae*, cell cycle, population structure

Introduction

The major microbial biomass for human use that is produced commercially on a large scale is baker's yeast (*Sacch. cerevisiae*). *Sacch. cerevisiae* is also used in well established bioprocesses for the synthesis of products such as ethanol, vitamins, enzymes and heterologous proteins. The production of baker's yeast to be used specifically for bread-making started in the middle of the 19th century. The method used for the production of baker's yeast biomass (*i.e.*, fed-batch fermentation) directly reflects the physiology of this micro-organism. Optimisation and control of the bioprocess requires monitoring and manipulating of the cellular environment (*i.e.*, sugar and nutrients concentrations, oxygen availability, temperature and pH values). The dynamic reactions of the yeast population to changes and manipulations of its surrounding are determined by the distributions of the »single-cell growth rates« of the growing cells. The »single-cell growth rates« are defined by the rates at which cells accumulate proteins, nucleic acids, lipids, cell wall materials, secreted material and all the other cellular components *(1–2)*. In order to determine the »single-cell growth rates« of a growing population, it is necessary to obtain data on the cellular composition of the individual cells and how this composition is distributed within the whole growing cellular population.

Further optimisations of *Sacch. cerevisiae* as »biomass or cell factory« might depend on better understandings of the relations between the cellular environment and the distributions of »single-cell growth rates« in the growing population. Little information is available on these distributions since conventional sensors typically measure only population average quantities, and their direct determination is difficult.

Flow cytometry allows the measurement of physical and/or chemical bioparameters in single cells at rapid rate such that the distribution of these properties in the whole population can also be obtained in a very short period of time *(3)*. This analytical technique has

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been applied to growing yeast cells in several different applications. These range from the characterisation of the growth dynamics *(4–6)*, the analysis of the kinetics of heterologous protein production *(7)*, the selection of cells with specific properties *(8–9)*, to studies on the metabolic state of cell populations *(10)*, to give a few examples.

Sacch. cerevisiae divides with an asymmetrical process. The degree of asymmetry at division depends on the growth rate, with slow-growing cells dividing more asymmetrically. In an asynchronously growing *Sacch. cerevisiae* population, individual cells differ in their position within the cell division cycle, their genealogical age (*i.e.*, daughters, parents of first generation, parents of second generation) and their size, although cells which have the same size do not necessarily share the same age or the same cell cycle position. All these differences yield the cell size distribution (*i.e.* the cell protein content distribution) of the growing population. It has been shown that the protein content distribution of a given population in balanced exponential growth is stable and characteristic of each growth condition (for a review see ref. *6*).

Based on the work of May and Mitchison *(11)*, a novel flow cytometry procedure that permits determination of single-cell growth properties of *Sacch. cerevisiae* populations has been recently developed *(12–16)*. The aim of this paper is to provide a short overview of the latest results obtained.

A novel flow cytometric procedure

The procedure is based on labelling the yeast cell wall(s) with a lectin such as ConcanavalinA (ConA) conjugated to a fluorescent marker such as fluorescein isothiocyanate (FITC). The yeast cell wall is constructed almost entirely of two classes of polysaccharides: polymers of mannose covalently linked to peptides (mannoproteins) and polymers of glucose (glucans). A third sugar polymer of N-acetylglucosamine (chitin) is present only in small amounts *(17–18)*. Concanavalin A, the lectin obtained from Jack beans (*Canavalia ensiformis*) (19), specifically binds the mannoproteins of the yeast cell wall (20). Localisation of cell wall synthesis during growth of the bud has been demonstrated with fluorescent dyes and autoradiography *(20–21)*. The cell wall of the growing bud is not derived from the material of the mother cell wall, but it is synthesised *de novo (17–18,20–22)*.

The procedure requires the culturing of a yeast population, harvesting the cells, staining the cell wall(s) with ConA-FITC, exposing of the cells to growth conditions and detection of the single cell fluorescence with flow cytometry. Fig. 1 shows the cell cycle phases of a growing yeast cell. Since the synthesis of new cell wall material in the budded cells is restricted to the bud, the first effect of the new cell growth is the production of new-born daughter cells with a gradually decreasing amount of surface stain (*i.e.*, partially stained daughter cells). The partially stained cells are new-born daughter cells originated from cells stained during the budded phase (S + G2 + M + G1*) of the cell cycle *(12)*. Completely unstained daughter cells (Fig. 1) represent the new-born daughters originating from cells stained while they were in the unbudded phase (G1). The time of appearance of this last subpopulation of new daughter cells is function of the length of the S + G2 + M + G1* phase *(12)*. Both balanced and transient growth conditions can be analysed by resuspending the stained cells in the same or in a different medium. In order to use the surface staining as a permanent label for the observation of the growth properties of individual cells, fast staining conditions (the staining takes few minutes) have been developed so as to meet the requirements that the labelling procedure not perturb the growth behaviour and that the surface label be retained by the cells over the subsequent growth period *(12)*

This first staining has been coupled to the determination of the protein content of the individual cells, which gives a good estimation of the cell size. This double tag approach offers a sensible tool for the analysis of the single-cell growth properties of asynchronously growing yeast populations. In fact, on the one hand it allows the selection of individual yeast cells at the different set points of the cell cycle (see below), while on the other hand it yields the relative distribution of the protein content.

Experimental methods developed to obtain some of the above cited information are described below.

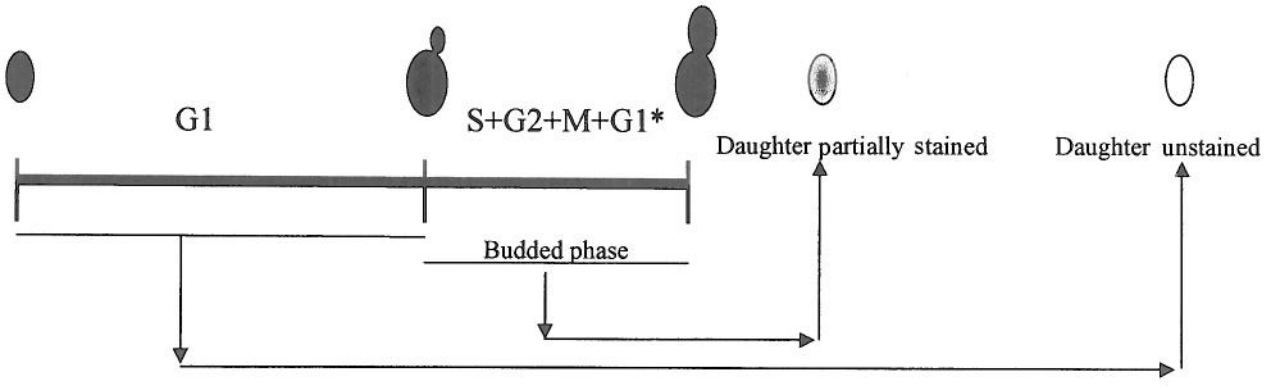

Fig. 1. Cell cycle phases of a growing yeast cell. The evolution of partially stained and unstained daughter cells after resuspension of stained cells is clearly deductible.

Identification of new-born daughter cells and quantification of the growth properties of single cells.

S288C yeast cells were cultured in a flask by shaking at 30 °C in the 6.7 g L^{-1} YNB-galactose medium to exponential phase, harvested, stained and resuspended in the same growth medium. Cells have been stained for 7 minutes by incubation in precooled fresh YNB-based medium, containing 120 μg mL^{-1} of conjugated ConA-FITC (approximately 3.6 moles of FITC per mole of lectin; SIGMA, St. Louis, MO, USA) at cell concentration of $2 \cdot 10^8$ cells/mL. At different times after resuspension of stained cells and new growth, yeast samples were collected by centrifugation, washed and fixed for 20 minutes in 70% volume fraction of ethanol. Fixed cells were centrifuged, washed once and total cell proteins stained for 30 minutes in 0.5 M sodium bicarbonate containing 50 μg/mL of tetramethylrhodamine isothiocyanate (TRITC; SIGMA, St. Louis, MO, USA). FITC and TRITC signals were finally acquired from a FACStar^{plus} (Becton & Dickinson) equipped with an argon-ion laser (excitation wavelength 488 nm, laser power 200 mW) *(13–15)*. Fig. 2 shows the dynamic of the double-tagged cell population after resuspension (t = 0). The specific growth rate of the overall population and the percentage of budded cells before and after resuspension were 0.22 ± 0.02 h^{-1} and 53.5 ± 0.5 %, respectively

The ConA-FITC signals shown in Fig. 2 were acquired with a logarithmic scale. On the one hand, the logarithmic scale allows a global evaluation of the experiment. However, it is very important to emphasize that all the data have been obtained analysing yeast samples with a linear scale which allows the recording of the smallest differences in the ConA-FITC fluorescence signal between each single cell. Further, after resuspension of stained cells, samples for subsequent analysis were prepared every 10–20 minutes of new growth *(12–16)*. In asynchronous yeast populations, cells occupy different cell cycle positions. At the time of staining with ConA-FITC the cell wall of each cell is completely stained (St. at t = 0; Fig. 2). Each individual cell of the yeast population is represented by a single dot. After the staining of the cells and the subsequent new growth, the newly synthesised cell wall is not stained while the older cell wall components retain the initial fluorescence (i.e., each cell retains the initial amount of fluorescence, the dye attached to the cells is only diluted on yeast surface by the new growth) *(12)*. The evolution of partially (P. st.) and unstained (Unst.) daughter cells is clearly visible. A long period of growth (*i.e.*, more than 1–2 generation times) clearly results in the accumulation of a large fraction of completely unstained cells. Such behaviour of the double staining pattern is clearly dependent upon the growth properties of the overall population as well as on the growth properties of the individual cell. For instance, the time of appearance of the unstained cells can be used to estimate the length of the budded phase of the growing population *(12)*. The simplest quantitative analysis of the staining pattern allows the determination of the cell size of the new-born daughter cells. Examples of the selection of new-born daughter cells are shown in Fig. 2; the gates r1 and r2 indicate the regions

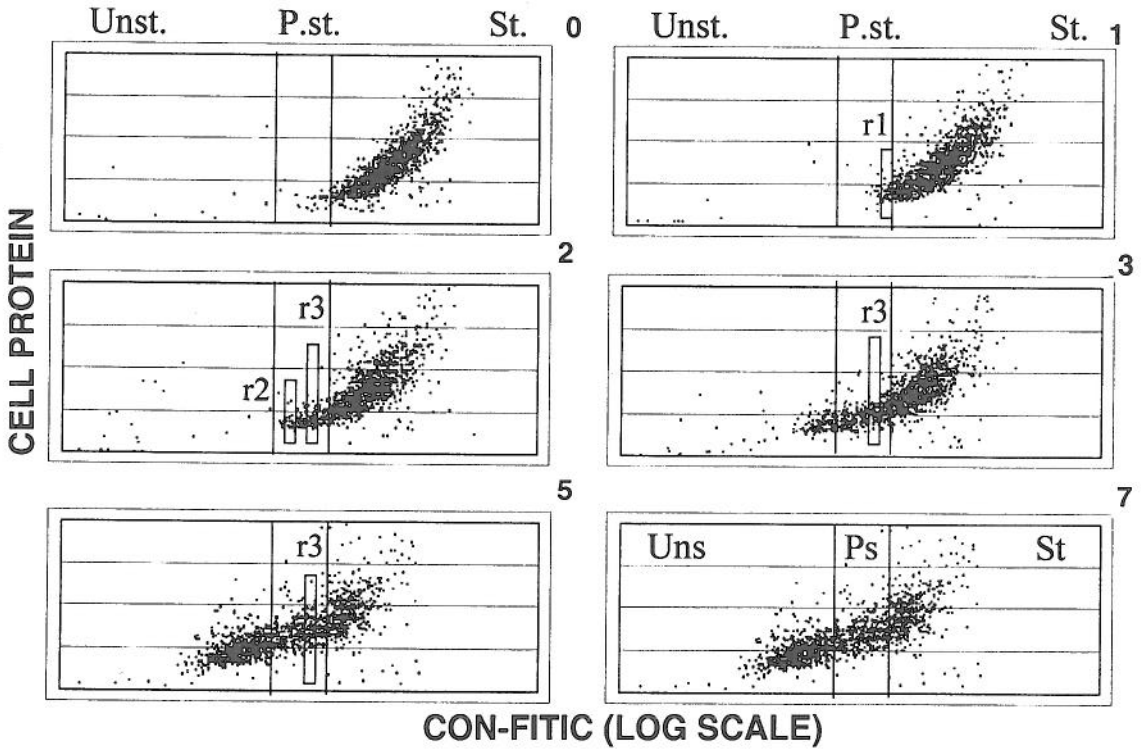

Fig. 2. Examples of dot-plots of cell-wall-tag (abscissa; ConA-FITC signals; Log scale) versus cell-protein-content-tag (ordinate; TRITC signals; Linear scale). Data shown have been acquired at time t = 0, 1, 2, 3, 5 and 7 h after resuspension of ConA-stained cells (all the data are expressed as channel number).

where the new-born partially stained daughter cells fall after resuspension.

The double-tag procedure also allows to directly determine the growth kinetic of the cell size of the daughter cells during cell cycle progression. Since the new-born partially ConA-stained cells retain the same initial amount of fluorescence (*i.e.* the dye on the single cells is only diluted by the new growth) *(12–16)*, it is possible to follow the growth dynamics of a cohort of selected cells born at the same time, and thus having the same age over time. In Fig. 2 an example of the selection of such cohort of daughter cells over time is also reported (gate r3). The kinetic of cell protein content increase is shown in Fig. 3. The cell protein content increase of the selected daughter cells has been followed for 6.5 h, a time which is much longer than the duplication time of the whole population (3.2 h). Data obtained during the first 4.5 h of growth after resuspension clearly fit an exponential increase of the cell size over time. In fact, the coefficient of correlation is higher than 0.99. Exactly after 4.4–4.5 h of growth (*i.e.*, 5 hours after resuspension, considering that the selected cells were born 0.5 h after the resuspension) an exponential rate law can no longer be used to fit the data. We proved earlier *(13–16)* that the time-deviation shown in Fig. 3 corresponds to the division of the daughter cells and therefore it can be used to determine the duplication time of the daughter subpopulation. A simple mathematical equation ($1 = e^{-\mu Td} + e^{-\mu Tp}$; where Td and Tp are the duplication times for the daughter and parent subpopulations, respectively) allows to extrapolate the duplication time of the parent subpopulation (2.28 h). Yeast populations growing at different specific growth rates and double-tagged with the procedure described can be analysed (Table 1). The data shown in the Table indicate that (*i*) the cell size increase rate (CSIR; *i.e.* the specific growth rate) of the daughter cell subpopulation is almost identical to the specific growth rate of the overall population as determined by the increase of the cell number concentration. Furthermore, (*ii*) since the specific growth rate value for the daughter subpopulation and for the overall population is the same in all the tested conditions, it follows that the parent subpopulation grows at the same average specific growth rate.

Similar data were obtained using different yeast strains (data not shown).

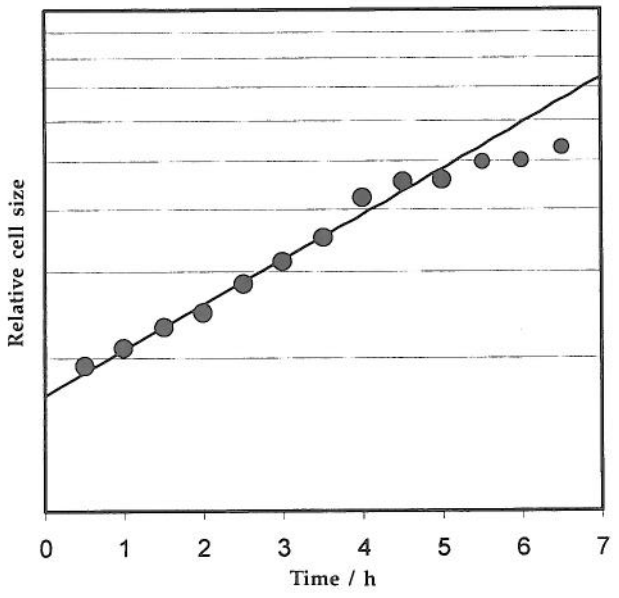

Fig. 3. Average cell protein content (logarithm of the TRITC signals) of a selected population of partially stained daughter cells as a function of time after birth. The gate r3 in Fig. 2 shows an example for selection of the same cohort of partially stained daughter cells over time.

Table 1. Comparison between the specific growth rate of the whole yeast population and of a single cohort of daughter cells. Yeast cells have been grown in YNB-based medium till exponential phase, stained, resuspended in the same fresh media and analysed as described in Figs. 2 and 3. Glucose or galactose or raffinose were used as carbon source.

Specific growth rate/ $\mu m\ h^{-1}$	
Cohort of daughter cells*	Whole population**
0.188	0.182
0.206	0.215
0.207	0.211
0.213	0.215
0.226	0.224
0.251	0.250

* Specific growth rates of single-cell size have been determined on cohorts of growing daughter cells of the same age.

** Specific growth rates of the whole population have been determined from the relative cell number increase.

Identification of the daughter and parent subpopulations

The transient behaviour shown in Fig. 2 allows the estimate of the cell protein content distributions and frequencies of the daughter and parent subpopulations during balanced cell growth. In fact, the cell protein content distribution of the newly originated daughter cells determined after a period of time corresponding to the determined generation time of the daughter cells subpopulation (*i.e.* 4.4 h; *(13)*) should correspond to the situation detectable during balanced cell growth. In accordance with this, the fraction of this cellular subpopulation determined by flow cytometry 4.5 h after resuspension of the stained cells and the fraction of daughter cells determined by bud scars analysis on the whole population are very similar, 66% against 63%, respectively *(13)*. Furthermore, if after such time, the new partially stained and unstained cells represent the daughter subpopulation, clearly all the other cells should belong to the parent subpopulation (Fig. 4).

Identification of the complex structure of an asynchronously growing yeast population

Table 2 summarises most of the data obtained using this novel flow cytometric procedure. Specifically, in the

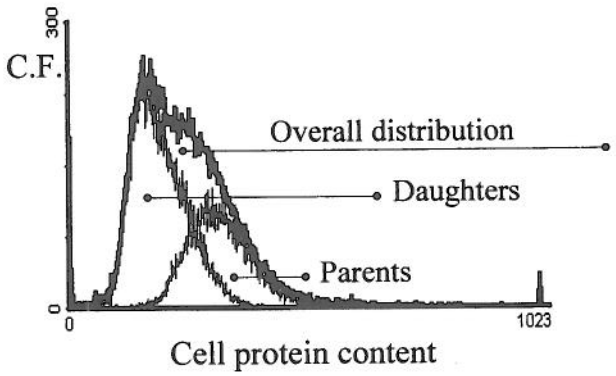

Fig. 4. Comparison of the experimental cell protein content distributions of the daughter and parent subpopulations with the overall cell protein content distribution. The cell protein contents are expressed as channel number against cell frequency (C.F.).
Following resuspension of stained cells, the distributions of the two subpopulations were determined after a period of time equal to the determined generation time of the daughter subpopulation (see also text).

Table 2. Comparison between coefficients of variation (CV) for different yeast (sub)populations.

Yeast (sub)population:	Data from the double-tag approach	Other teams
Overall	37.73	36[a] – 24.1[b]
Daughters	29.44	36[a]
Daughters newborn	19.00	15[a] – 18.2[b]
Daughters in G1	23.36	
Daughters in S+G2+M+G1*	21.54	
Daughters dividing	18.27	13[a]
Parents	23.21	25[a]
Parents newborn	n. d.	16.4[b]
Parents in G1	n. d.	
Parents in S+G2+M+G1*	n. d.	
Parents dividing	27.39	

n. d. – not detectable following the double-tag approach

[a] data from ref. *31* (the data about the daughter and parent subpopulations have been determined analysing the cell volume by time-lapse studies of a yeast population growing on a solid support, while the data of new-born and dividing daughter cells have been inferred by calculation).

[b] data from ref. *42* (the data have been obtained following a flow cytometric extrapolation of the cell volume).

table are reported the CV values for the different parent and daughter subpopulations identified. The CV value for the distribution ranges between 0 and 100. The CV value represents the ratio of the standard deviation and the mean and indicates the dispersion of the protein content distribution around the average protein content value (*i.e.* a protein content distribution of cells having identical size has a CV value equal to zero). Therefore, excluding the experimental mistake and noise, the CV value of a cell protein content distribution represents the heterogeneity in cell size of the different cells belonging to the yeast (sub)population. At least two different CV behaviours deserve some comments. On the one hand, it is interesting to note that the dispersion of the protein content for the daughter subpopulation (29.44 see also Fig. 4) is higher than that for the parent one (23.21). On the other hand, the CV value for daughter cells at division (18.27) is considerably lower than the CV value for the dividing parent (27.39) cells. Since all the cells of the population grow at very similar specific growth rates *(13)* and share the same length of the budded phase *(12, 23–24)*, the different dispersion for daughter and parent cells around the average protein content value can be explained considering the much longer G1 phase of the daughter cells in comparison to the G1 phase of the parent cells. On the other hand, taking into consideration that DNA replication occurs when a yeast cell reaches a critical cell size at Start *(24–36)*, that such critical value increases at each new generation *(24, 31–34)* and that the budded period is of constant duration for all the cells of the population *(12,23–24)*, the higher dispersion for the dividing parent cells can be easily explained considering that parent cells of different genealogical age enter in S phase and then divide having very different cell protein contents. On the contrary, the lower dispersion for the dividing daughter cells could be explained assuming that all the daughter cells begin to bud and then divide having more similar protein contents (i.e. independently of the genealogical age of the parent from which have been originated). These considerations have been tested following simulations of cell size distributions and relative cell frequencies of daughter and parent cells belonging to different cell cycle positions *(15)*. Simulations have been obtained using a mathematical model previously developed in our laboratory which allows the deconvolution or the simulation of a cell protein content distribution (*24*, *37* and reviewed in *6*). For the simulations the computer must be fed with the specific growth rate value, fraction of budded cells in the population, percentage of increase of the critical size at Start for the parent cells of first generation and momentary coefficient of variation for the daughter and parent subpopulations. The simulated data are very close to the experimental findings (data not shown) *(14–15)* not only for the whole population, but also for the fraction of different subpopulations and for the CVs of the protein content distributions.

Finally, Fig. 5 summarises the main information obtained using this new procedure. All daughter cells belonging to a yeast population begin to bud and then divide at the same relative protein content, independently of the genealogical age of their parents. A similar process of resetting of the individual cell cycles for daughter cells is also operative during transient steady-states of growth *(16)*. On the contrary, the critical protein content required for the parent cells to bud increases at each new generation. Since the budded period is of constant duration for all the cells, genealogically older parent cells are larger at division and result in larger new-born daughter cells.

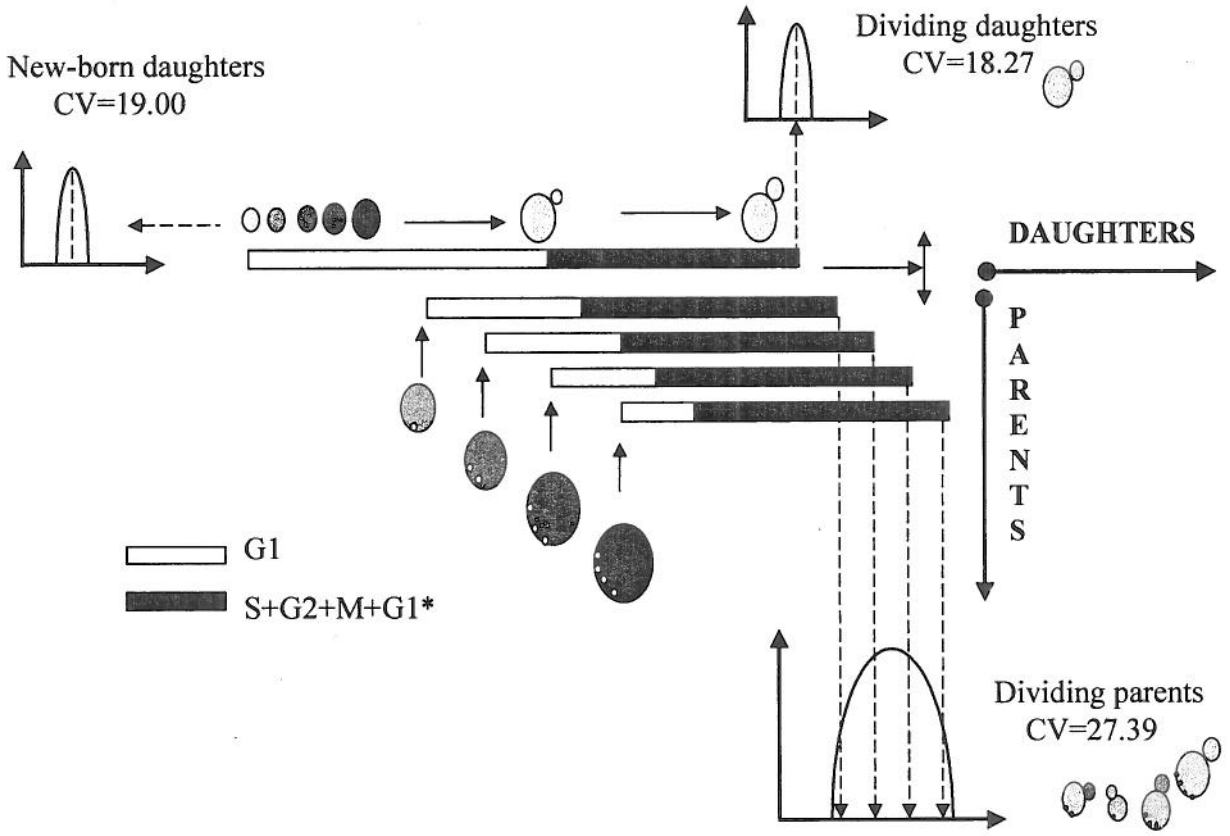

Fig. 5. Designing the complex structure of an exponentially growing yeast population. All the experimental information obtained was used to design the complex structure of an exponentially growing yeast population.

Conclusions

Studies of the kinetics of the cell cycle progression of the yeast *Sacch. cerevisiae* require the analysis of individual cells growing under both balanced and transient growth conditions. Both time-lapses and flow cytometric applications are required for successful investigations. For example, time lapse studies were used to verify that the budded phase of cells having different genealogical age is of constant duration *(38)*, to determine the cell volume increase at Start for cells of different genealogical age *(31)*, to determine the specific growth rate of individual cells *(39)*, to analyse the dimorphic behaviour of laboratory strains *(40)* as well as to monitor the life-span of a yeast cell *(41)*. On the other hand, flow cytometry allows the determination of the cell protein content which represents a good estimation of the cell size and, analysing a wide sample of cells (*i.e.*, up to 10^5 cells) *(3)*, allows the measurement of the heterogeneity and variability of the growing population.

The flow cytometric procedure described here allows the direct determination of the properties (*i.e.*, growth dynamics, relative fractions, CV values of the protein content distributions) which constitute a growing yeast culture without any assumption and/or any indirect calculation.

Data obtained during both balanced and transient growth conditions indicate (*i*) an exponential growth for individual cells during cell cycle progression, (*ii*) that each cell in the population grows at the same average specific growth rate, (*iii*) that the cell size increase rate (CSIR) of each individual cell reflects the specific growth rate of the overall population, (*iv*) the opportunity to determine the budded phase length of the growing population and (*v*) the cell cycle length of the daughter subpopulation, (*vi*) the opportunity to extrapolate the cell cycle length of the parent subpopulation and (*vii*) the G1 length for both the daughter and parent subpopulations as well as (*viii*) singling out the cell size distributions of the parent and daughter subpopulations belonging to different cell cycle phases from the overall distribution (*i.e.* new-born, G1, S + G2 + M + G1* and dividing cells) and finally (ix) to determine the complex structure of a growing yeast population *(12–16)*. Usually, these dynamic properties cannot be determined. Only for cells growing exponentially, it is possible to calculate some of these values (*i.e.* the length of the budded phase and of the duplication times for the daughter and parent subpopulations) by inference from other data using equations and/or mathematical models (summarised in ref. *6*).

The characterisation reported in this short overview evidences the extreme complexity of the processes controlling the co-ordination between growth dynamics and division events of a growing yeast population. In fact, there is a large overlap between the cell protein content distributions of daughter and parent cells as well as between cells of the same subpopulation but belonging

to different stages of the cell division cycle. Clearly, the situation shown depends on the specific growth rate of the yeast population; in fact, yeast cells divide asymmetrically with slow growing populations dividing more asymmetrically *(35–36)*. Finally, because the presented procedure is very rapid it could be useful for the isolation/characterisation of yeast mutants as well as for studying the physiological reactions of *Sacch. cerevisiae* to modulation of the cellular environment *(16)*. Furthermore, such a procedure could be useful for other interesting applications. In fact, *Sacch. cerevisiae* is a microorganism of wide scientific interest not only for conventional biotechnological productions (*i.e.* biomass, ethanol, vitamins, enzymes), but also for the studies of the cell cycle control in eukaryotic cells, for the analyses of the mechanisms controlling the glycolytic pathway and for advanced biotechnological applications (*i.e.* heterologous proteins, vaccines, chemicals).

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References

1. A. G. Fredrickson, D. Ramkrishna, H. M. Tsuchiya. *Math. Biosci. 1* (1967) 327.
2. D. Ramkrishna: Statistical models of cell populations. In: *Advances in Biochemical Engineering, Vol. 11*, T. K. Ghose, A. Fiechter, N. Blakebrough, (Eds.), Springer-Verlag, Berlin (1979) pp. 1–47.
3. H. M. Shapiro. *Practical Flow Cytometry*, A. R. Liss (Ed.), New York (1988).
4. D. W. Agar, J. E. Bailey, *Biotechnol. Bioeng. 23* (1981) 2315.
5. F. Srienc, S. Dien, *Ann. N.Y. Acad. Sci. 665* (1992) 59.
6. L. Alberghina, D. Porro, *Yeast, 9* (1993) 815.
7. P. Eitzman, F. Srienc, *J. Biotechnol. 21* (1991) 63.
8. P. Skowronek, G. Krummeck, O. Haferkamp, G. Rodel, *Curr. Genet. 18* (1990) 265.
9. C. Smeraldi, E. Berardi, D. Porro, *Microbiology, 140* (1994) 3161.
10. D. Porro, C. Smeraldi, E. Martegani, B. M. Ranzi, L. Alberghina, *Biotechnol. Progr. 10* (1994) 193.
11. J. W. May, J. M. Mitchison, *Nature, 322* (1986) 752.
12. D. Porro, F. Srienc, *Biotechnol. Progr. 11* (1995) 342.
13. D. Porro, B. M. Ranzi, C. Smeraldi, E. Martegani, L. Alberghina, *Yeast, 11* (1995) 1157.
14. D. Porro, L. Alberghina, Protein distribution to analyse budding yeast population structure and cell cycle progression. In: *Flow Cytometry Applications in Cell Culture*, M. Al-Rubeai, A. N. Emery (Eds.), Marcel Dekker, Inc., New York (1996) pp. 225–240.
15. D. Porro, E. Martegani, B. M. Ranzi, L. Alberghina, *Res. Microbiol. 148* (1997) 205.
16. L. Alberghina, C. Smeraldi, B. M. Ranzi, D. Porro, *J. Bacteriol. 180* (1998) 3864.
17. C. E. Ballou, Yeast cell wall and cell surface. In: *The Molecular Biology of the Yeast Saccharomyces cerevisiae. Metabolism and Gene Expression*, J. N. Strathern, E. W. Jones, J. R. Broach (Eds.), Cold Spring Harbor Laboratory, New York (1982) pp. 335–360.
18. E. Cabib, R. Roberts, B. Bowers, *Ann. Rev. Biochem. 51* (1982) *763*.
19. I. J. Goldstein, L. L. So, *Arch. Biochem. Biophys. 111* (1965) 407.
20. J. S. Tkacz, E. B. Cybulska, J. O. Lampen, *J. Bacteriol. 105* (1971) 1.
21. K. L. Chung, R. Z. Hawirko, P. K. Isaac, *Can. J. Microbiol. 11* (1965) 953.
22. C. E. Ballou, Organization of the *Saccharomyces cerevisiae* cell wall. In: *Self-Assembly Architecture*, J. E. Varner (Ed.), Alan R. Liss, Inc., New York (1988) pp. 105–117.
23. P. G. Lord, A. E. Wheals, *J. Bacteriol. 142* (1980) 808.
24. M. Vanoni, M. Vai, L. Popolo, L. Alberghina, *J. Bacteriol. 156* (1983) 1282.
25. B. L. A. Carter. The control of cell division in *Saccharomyces cerevisiae*. In: *The Cell Cycle*, P. L. C. John (Ed.), Cambridge University Press (1981) pp. 99–119.
26. B. L. A. Carter, M. N. Jagadish, *Exp. Cell. Res. 112* (1978) 15.
27. L. H. Hartwell, M. Unger, *J. Cell. Biol. 75* (1977) 422.
28. G. C. Johnston, R. A. Singer, S. O. Sharrow, M. L. Slater, *J. Gen. Microbiol. 118* (1980) 479.
29. A. Lorincz, B. L. A. Carter, *J. Gen. Microbiol. 113* (1979) 281.
30. J. R. Pringle, L. H. Hartwell, *The Saccharomyces cerevisiae Cell Cycle*, Cold Spring Harbor Monogr. Ser. (1981) pp. 97–142.
31. C. L. Woldringh, P. G. Huls, N. O. E. Vischer, *J. Bacteriol. 175* (1993) 3174.
32. G. C. Johnston, J. R. Pringle, L. H. Hartwell, *Exp. Cell. Res. 105* (1977) 79.
33. P. G. Lord, A. E. Wheals, *J. Cell. Sci. 59* (1983) 183.
34. A. E. Wheals, *Mol. Cell. Biol. 2* (1982) 361.
35. P. G. Lord, A. E. Wheals, *J. Cell. Sci. 50* (1981) 361.
36. P. W. Thompson, A. E. Wheals, *J. Gen. Microbiol. 121* (1980) 401.
37. E. Martegani, M. Vanoni, D. Delia, *Cytometry, 5* (1984) 81.
38. P. G. Lord, A. E. Wheals, *J. Bacteriol. 142* (1980) 808.
39. A. E. Wheals, P. G: Lord, *Cell. Prolif. 25* (1992) 217.
40. S. J. Kron, C. A. Styles, G. R. Fink, *Mol. Cell. Biol. 5* (1994) 1003.
41. B. K. Kennedy, N. R. Austriaco, L. Guarente, *J. Cell. Biol. 127* (1994) 1985.
42. D. E. Block, P. D. Eitzman, J. D. Wangensteen, F. Srienc, *Biotechnol. Progr. 6* (1990) 504.

Novi protočni citometrijski postupak za određivanje rasta jednostaničnih organizama u asinkronoj populaciji *Saccharomyces cerevisiae*

Sažetak

Prikazan je novi protočni citometrijski postupak kojim se mogu odrediti svojstva rasta pojedinih stanica Saccharomyces cerevisiae u asinkronim kulturama. Postupak se sastoji u označavanju staničnog zida s konkavalinom A na koji je vezan fluorescein izotiocijanat (FITC) i utvrđivanje stanične fluorescencije protočnom citometrijom nakon izlaganja stanica uvjetima rasta. Budući da je oblikovanje novog staničnog zida u pupajućim stanicama ograničeno na vrh pupa, izlaganje obojenih stanica uvjetima rasta davat će tri tipa stanica: (i) obojene, (ii) djelomično obojene i (iii) neobojene stanice. Istodobno je određena količina proteina u pojedinim stanicama, što daje dobru procjenu veličine stanica. Analiza dvostruko obojenih uzoraka tijekom vremena omogućava određivanje starosti stanice i specifične brzine rasta pojedinih staničnih skupina. Postupak je bio provjeren na populaciji kvaščevih stanica pri različitim brzinama rasta. U radu je iznesen kratki pregled dobivenih podataka.