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Parameters controlling yeast hybrid yield in electrofusion: the relevance of pre-incubation and the skewness of the size distributions of both fusion partners

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A method was sought to predict the success of electrically induced hybridization of yeast protoplasts prior to electrofusion. It was assumed that the protoplast volume would play a significant role in hybrid yield, because this volume influences the dielectrophoretic positioning as well as the electrical breakdown of the membrane. This was shown to be the case when fusing protoplasts of the two yeast strains AH22(pADH040-2) and AH215 with the same mating type. The yield of hybrids could be predicted on the basis of volume distributions, but not on the basis of culture conditions. A 'protoplast index' was therefore developed. It is the sum of several factors which are decided depending on the protoplast volume distributions. These factors are the modal volume, the 'debris-to-cell ratio' and the 'modal volume + half-width' of the volume distributions of each of the fusion partners. The 'debris-to-cell ratio' and the 'modal volume + half-width' take into account the relative proportions of undersized protoplasts (and of debris) and of very large cells in the population, respectively. It could be demonstrated that there is a very good correlation between hybrid yield and the protoplast index. In particular, it could be shown that the protoplast index of fresh protoplast preparations leading to poor yields could be improved by incubation of the protoplasts in a special pre-incubation medium. Pre-incubation, however, has no effect on yield if the protoplast index of the fusion partners is optimum just after preparation of the protoplasts. The calculation of a protoplast index from the measurements of the volume distribution of the fusion partners is a rapid and quantitative procedure which allows optimization of the field parameters, from which it can be decided whether enzymatic pre-treatment or pre-incubation of the protoplasts is required. The implementation of this index method should allow superior optimization of hybridoma yields by electrofusion.

Introduction

Electrofusion of prokaryotic and eukaryotic cells leads to high yields of viable hybrids under the appropriate experimental conditions (see review articles Refs. 1–10). In many cases, the electrofusion method produces higher yields of hybrids than may be produced by conventional fusion methods (i.e., polyethylene glycol or Sendai virus). For both chemical fusion and electrofusion, variations in the yield of hybrids are reported [1–11]. An increased quantitative understanding and optimization of system and cell parameters might allow more reproducible results, an increase

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in fusion efficiency and the prediction of absolute hybrid yield.

In this communication we describe the determination of cell parameters which provide quantitative criteria for the prediction of hybrid yields in electrofusion. Using the electrofusion of protoplasts from two different strains of *Saccharomyces cerevisiae* carrying different genetic markers [11] as a model system, we demonstrate that the electronic determination and characterization of the usually skewed cell volume distribution of the two strains permits the definition of a 'protoplast index' of the cell populations to be fused. With the aid of this index, which is calculated from volume distributions measured by a hydrodynamically focusing particle analyzer [12], it is possible to predict the absolute hybrid yield.

Experiments in this laboratory show that modal volume and skewness of the yeast protoplast volume distribution can vary considerably, in spite of apparent constant culture, preparation and pre-incubation conditions, and therefore it can influence hybrid yield. The reason for this is most likely the variability of the biological material and has probably accounted for the fluctuations in the hybrid yield after electrofusion.

The method of determination of the cell parameters described here may find application in electrofusion of other cells and perhaps even in polyethylene glycol fusion. It replaces the previous, very qualitative and subjective methods of predicting the number of viable cell hybrids by microscopical counting of fusion products (cited in Ref. 13).

Principles and Objective

The field strength required for electrical membrane breakdown of at least two attached spherical cells and for subsequent fusion can be calculated to a first approximation by use of the integrated Laplace equation derived for single spherical freely suspended cells [14,15]:

$$V_m = 1.5\, a\, E \cos\alpha \qquad (1)$$

where V_m is the membrane potential built up in response to an external field pulse of strength E; a is the radius of the cell and α is the angle between a given membrane site and the field direction. The radius dependence of the membrane voltage and breakdown field strength explains why the electrofusion of small cells requires pulses of higher field strength than that of larger cells. The angular dependence of the membrane voltage, V_m, means that at a given field strength the size of the membrane area in which breakdown is initiated differs according to cell size. Let us assume that field pulses are applied with an intensity such that the breakdown voltage of the smallest cells in the suspension is reached only in those membrane sites oriented in the field direction ($\alpha = 0°$). Under these conditions, the larger cells in the suspension would have larger membrane areas subjected to breakdown because of the radius and angular dependence of the membane voltage. If the size of the membrane area affected by breakdown reaches a certain proportion of the total membrane surface area, irreversible destruction of the cells occurs. The release of intracellular substances into the medium significantly disrupts the fusion process [2,10]. On the other hand, the injection of field pulses of lower field strength also prevents fusion between attached cells of different size because the membrane of the smaller cells fails to break down.

These considerations hold if the breakdown voltage and the intrinsic membrane potential (see Ref. 11) of all cells in a suspension are identical and the steady state potential according to Eqn. 1 is reached [2,7]. These conditions are fulfilled for the cells of the yeast strains used here.

Optimum fusion would, therefore, be achieved if all cells in a suspension subjected to electrofusion were of the same volume. Identical volume of fusion partners would also be the optimum prerequisite for establishing membrane contact between cells of different strains by means of dielectrophoresis [15–17]. A rough calculation of the dielectrophoretic force, F, on an insulating dielectric sphere of radius a and dielectric constant ε_2 in an insulating dielectric fluid (dielectric constant ϵ_1) with imposed electric field, E, is given by [17]:

$$F = 2\pi a^3 \epsilon_1 \left(\frac{\epsilon_2 - \epsilon_1}{\epsilon_2 + 2\epsilon_1} \right) \nabla E^2 \qquad (2)$$

Therefore, the dielectrophoretic force is propor-

tional to cell volume and substantially different volumes of the two fusion partners could lead to segregation by dielectrophoresis in the converging field [17]. This leads to a reduced chance of hybrid formation. Furthermore, the gravitation force separates different sized protoplasts according to the square of the radius. Identical volumes or narrow volume distribitions with the same modal volume for both fusion partners are rare. It is more likely for yeast cell protoplasts of the two strains to have volume distributions with a different modal volume, width and skewness (Figs. 1 and 2). In addition, in spite of apparent constant culture and preparation conditions, these parameters are highly variable for the parental fusion partners, so that hybrid yield can vary considerably under standardized conditions of the electric field.

Experimentally, the shape of the volume distribution of a certain cell line can be changed by enzyme pretreatment [1–9] and/or preincubation

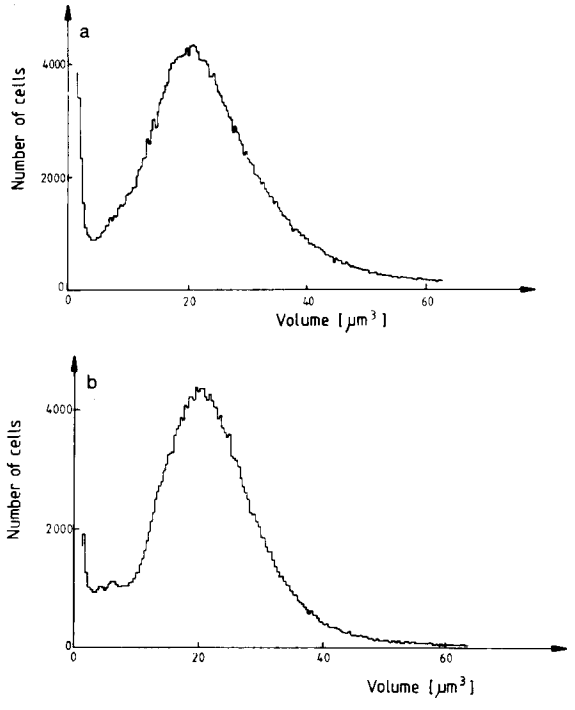

Fig. 1. Volume distributions of protoplasts of the two yeast strains AH22(pADH040-2) (a) and AH215 (b). Both volume distributions show a small amount of debris and undersize protoplasts, a small half-width and the same modal volume. The fusion of protoplasts from these preparations led to a high yield of hybrids (10922 hybrids per helical chamber).

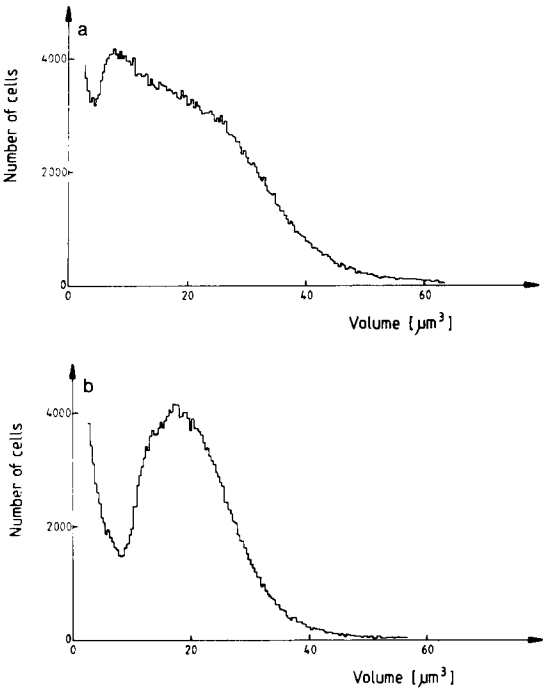

Fig. 2. In contrast to Fig. 1 these volume distributions show a large amount of small protoplasts and a marked skewness to the right, especially for the strain AH22 (a). The modal volume (8 μm^3) of the strain AH22 is different from the optimal volume of about 20 μm^3.

of the protoplasts in media of a special composition. It is, therefore, usually possible to change and match the volume distributions of the two fusion partners in such a way that optimum hybrid yields are obtained under standardized fusion conditions.

In order to describe the volume distributions of the two cell populations to be fused, these distributions must be characterized by parameters (Fig. 3) which are then used to calculate the protoplast index. This protoplast index permits definite statements to be made about the expected hybrid yield.

The volume distributions of yeast protoplasts differ greatly from the normal distribution, and it is therefore difficult to impose a simple classification scheme [18].

Measurements in a hydrodynamically focusing particle analyzer show that there are distributions which, depending on the strain and the culture and the preparation and pre-incubation conditions, may be positively or negatively skewed or

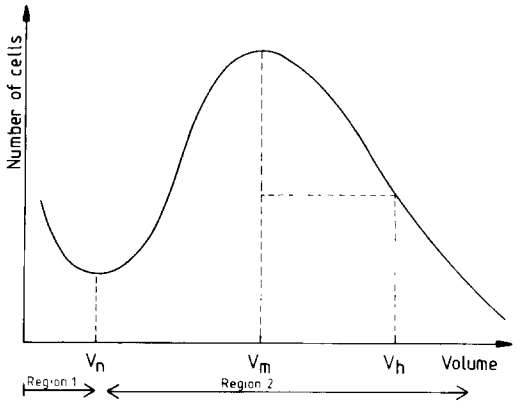

Fig. 3. Scheme of a volume distribution to explain the parameters which are necessary to calculate the protoplast index according to Eqn. 3. According to Figs. 1 and 2, the volume distribution is composed of the volume distribution of the protoplasts (region 2) and the distribution which is due to debris, tiny protoplasts and electronic noise (region 1). The amplitude of the volume, V_n, at the local minimum between regions 1 and 2 is a measure of the number of small particles. V_m is the modal volume and V_h is the 'modal volume + half-width', which is a measure of the skewness of the curve indicating the proportion of larger protoplasts.

bimodal. Figs. 1 and 2 each show histograms of volume distributions for yeast protoplasts of strain AH22 and AH215 which were selected for the experiments described below. When protoplasts of AH22 and AH215 exhibiting volume distributions as shown in Fig. 1 are fused, very high yields of hybrids are obtained. Much lower yields are seen when the preparations exhibit the volume distributions shown in Fig. 2. Comparison of Figs. 1 and 2 leads to the definition of the parameters characterizing the distribution (Fig. 3) and, in turn, to a protoplast index which correlates well with the expected hybrid yield. All the curves exhibit a 'bimodality' which is particularly prominent for strain AH22 in Fig. 2a.

The first peak (region 1 in Fig. 3) is the result of varying amounts of debris and tiny cells present in the protoplast population of each strain and of a varying amount of superimposed electronic noise [19]. The second peak (region 2 in Fig. 3) is attributed to the intact protoplasts of average size.

It is evident that the hybrid yield is reduced when more debris is present because it disturbs the close membrane contact of the fusion partners. As we have also already pointed out, small cells can disrupt fusion, because of segregation during dielectrophoresis or their failure to reach the breakdown voltage.

The electronic noise in the volume distribution curve predominates towards very small volumes. Therefore, the amplitude of the volume, V_n (Fig. 3) at the local minimum between regions 1 and 2 was used as a measure for the number of debris and tiny cells present in the protoplast preparations. The ratio of the amplitudes of V_n-to-V_m is a measure of the quality of the protoplast preparation in respect to electrofusion.

For brevity, this ratio is referred to as the debris-to-cell ratio. The higher this ratio is, the lower is the observed yield of hybrids. In Fig. 1a, the debris-to-cell ratio of strain AH22 is 0.206 and that of AH215 is 0.220; in Fig. 2 it is 0.787 and 0.386, respectively. Experimentally, increasing the debris-to-cell ratio greatly reduce hybrid yield. According to a standard method in statistics [20], the protoplast index is calculated by adding the values of the step function, Θ, with different arguments. Each argument contains a parameter characterizing the shape of the volume distribution. The step function, Θ, is the defined by $\Theta(x > 0) = 1$ and $\Theta(x < 0) = 0$. The influence of the debris-to-cell ratio on the protoplast index is, therefore, given by $\Theta(\mathrm{DCR} - 0.25) + \Theta(\mathrm{DCR} - 0.5)$, where DCR is the debris-to-cell ratio. The values 0.25 and 0.5 were empirically derived from the experiments shown below by comparing preparations of protoplasts which had similar volume distributions within region 2. Debris-to-cell ratios which are high correspond to low hybrid yields and vice versa. It is possible to classify the hybrid yields as low, medium or high, and to determine the corresponding boundaries of debris-to-cell ratios between these classes.

The modal volume of the cell size distribution is the second parameter which can characterize the 'fusogenicity' of a cell population.

If the distributions of the two protoplast populations exhibit approximately the same modal volumes (see Fig. 1), there should be optimal fusion if the field conditions for dielectrophoresis and for the electric breakdown pulse are ap-

propriate. If the modal volumes of the two cell populations differ (as in Fig. 2, AH22, 8 μm^3; AH215, 18 μm^3), fusion yield and hybrid yield are reduced. For the two strains under investigation the modal volume is on average 20 μm^3 and field conditions were, therefore, optimized for this volume range.

Using a breakdown voltage of 2.3 V across the membrane for these yeast strains (room temperature, unpublished results) and a corresponding electrical field strength of about 10 kV/cm for the breakdown pulse, it is possible to calculate (according to Eqn. 1) that cell volumes below 15 μm^3 no longer break down, while cell volumes exceeding 30 μm^3 lead to breakdown of such a large proportion of the membrane surface (more than 21%) that the cells are irreversibly destroyed, as confirmed experimentally. This is taken into account by adding $\Theta(15\ \mu m^3 - V_m) + \Theta(V_m - 30\ \mu m^3)$ to the protoplast index.

A volume distribution skewed to the right (larger cells) indicates that the population has too high a proportion of large cells in relation to 'normal' protoplasts (volume = 20 μm^3). For reasons already outlined above, this leads to a reduction in hybrid yield. In order to take into account these larger protoplasts, we introduce the parameter 'modal volume + half-width', abbreviated to V_h (see Fig. 3). For the volume distributions in Fig. 1, this parameter has the value 32 μm^3 (AH22) and 29 μm^3 (AH215) and for those in Fig. 2 33 μm^3 (AH22) and 28 μm^3 (AH215). It can be shown experimentally that V_h values exceeding 40 μm^3 greatly reduce hybrid yield. Therefore, the protoplast index is expanded by adding $\Theta(V_h - 40\ \mu m^3)$.

A marked skewness to the right ($V_h > 30\ \mu m^3$) has a positive effect on hybrid production if the modal volume is less than 15 μm^3. This is because extreme skewness to the right means that a considerable proportion of those volumes which are only slightly larger than the modal volume are in the optimum volume range for fusion. In this case, the value $\Theta(15\ \mu m^3 - V_m)$ must be multiplied by $\Theta(30\ \mu m^3 - V_h)$.

The final expression for the protoplast index, I, which takes into account that the volume distributions of both fusion partners (AH22, $n = 1$; AH215, $n = 2$) influence the hybrid yield, is calculated in the following way:

$$I = \sum_{n=1}^{2} \Theta_n(15\ \mu m^3 - V_m) \cdot \Theta_n(30\ \mu m^3 - V_h) + \Theta_n(V_m - 30\ \mu m^3) + \Theta_n(V_h - 40\ \mu m^3) + \Theta_n(DCR - 0.25) + \Theta_n(DCR - 0.5) \quad (3)$$

where DCR is the debris-to-cell ratio. According to this method, a protoplast index, I, of 8 leads to an extremely low hybrid yield, whereas a protoplast index of 0 leads to a large number of clones.

Materials and Methods

The strains *S. cerevisiae* AH22 and AH215 are of the same mating type. Strain AH22 is characterized by the double mutation Leu2-3, Leu2-112, and the single mutation His4-519. Strain AH22 is transformed with plasmid pADH040-2 which carries the Leu2 gene of yeast and the β-lactamase gene from *Escherichia coli*. Strain AH215 carries the same double mutation Leu2-3, Leu2-112, and in addition the double mutation His3-11, His3-15. Fusion products of AH215 and AH22(pADH040-2) are able to grow in selective agar.

Cells were harvested in logarithmic growth phase. Strain AH215 was cultivated in YEP medium (1% yeast extract, 2% peptone and 2% glucose). The minimal YNB medium (0.67% yeast nitrogen base w/o, 2% glucose supplemented with 30 μg/ml histidine) was used for the culture of AH22.

The preparation of protoplasts has been described elsewhere [21]. After treatment in enzyme solution (0.1 mM Ca^{2+}-$(acetate)_2$, 0.5 mM Mg^{2+}-$(acetate)_2$, 50 mM Tris (pH 7.5), 1.2 M sorbitol, 0.6 mg/ml zymolyase (100 000 U/g)), yeast protoplasts were washed once in 1.2 M sorbitol and divided into 2 aliquots. The first was suspended at a suspension density of $3 \cdot 10^8$ protoplasts per ml in pre-incubation medium of the following composition: 1 M sorbitol, 50 mM Tris (pH 7.5) and 1% yeast extract. These protoplasts were then incubated for up to 2 days at 29 °C on a rotator (8 min^{-1}) to prevent aggregation and sedimentation of the protoplasts. The second sample was im-

mediately prepared for fusion by washing three times in 1.2 M sorbitol.

In all experiments, the two strains were mixed at a ratio of 1:1 and resuspended to a final suspension density of $1.2 \cdot 10^9$ protoplasts per ml. The fusion medium consisted of 1.2 M sorbitol, 0.1 mM Ca^{2+}-(acetate)$_2$ and 0.5 mM Mg^{2+}-(acetate)$_2$.

For electrofusion 385 μl of this suspension were pipetted into each helical chamber (length of electrodes = 87 cm, electrode distance = 200 μm, distance of the electrodes to the jacket wall = 200 μm) [21]. Two helical chambers were used in each experiment.

Dielectrophoresis and associated membrane contact were achieved with an alternating field of 275 V/cm intensity and a frequency of 2 MHz. The collection time was 2 min.

Fusion was initiated by two pulses of 10 kV/cm strength and 10 μs duration. The interval between the two pulses was 0.5 s. The alternating field was automatically switched off for 10 ms during pulse application.

After application of the two breakdown pulses, the cells and hybrids were treated as previously described [11,21]. The number of hybrid colonies grown on selective agar (3% agar, 0.67% YNB, 2% glucose, 1.2 M sorbitol) was counted after 14 days.

For the control experiments, cell mixtures were exposed to the same experimental conditions except for the application of the alternating field and the field pulses.

The treatment of the pre-incubated protoplasts was performed in the same way as was described for the non-incubated protoplasts after dividing into two probes. The media and ingredients used here were supplied as specified in Refs. 11 and 21.

Willsky [22] found that ATPase in the plasma membrane of yeast protoplasts is inhibited by sorbitol. However, if KCl is used as the osmotic agent the ATPase remains active. Therefore, in some experiments protoplasts were prepared using 0.6 M KCl as the osmotic agent instead of 1.2 M sorbitol. In the incubation medium 0.5 M KCl instead of 1 M sorbitol was used.

The volume distributions of all yeast protoplast preparations were measured with a hydrodynamically focusing particle analyzer (AEG Telefunken Model TF) [12]. The hydrodynamical focusing is necessary to avoid artifact signals from protoplasts passing the orifice near the wall [23]. For the measurements, the protoplasts were transferred to a mixture of 0.6 M KCl and 1.2 M sorbitol (ratio, 2:7; conductivity, 12 mS/cm at 21° C). A cylindrical orifice of 60 μm in length and diameter was used. The adjustment of the particle analyzer for the measurement of the volume distributions was: orifice current 0.5 mA, gain 160, pressure difference 0.02 MPa. The counting rate of the protoplasts was adjusted to 2000–4000 counts/s to reduce the probability of coincidence within the orifice.

Results

Freshly prepared protoplasts of both strains exhibit marked variability in modal volume. Pre-incubation of protoplasts in YEP medium at 29° C for different periods of time leads to changes in the modal volume. Fig. 4a and b each shows three typical measurements of the modal volume for AH22(pADH040-2) and AH215, as a function of pre-incubation time. For each strain, the time dependence of the corresponding mean values for the modal volume of eight independent measurements is illustrated in the insets of Fig. 4a and b, together with their standard deviations.

The variability in modal volume of the different protoplast preparations is larger for strain AH22 than for strain AH215. For freshly prepared protoplasts of AH22 the mean values are 18 μm^3 ($\pm$48%) and for AH215 24 μm^3 ($\pm$26%). During the course of pre-incubation, the modal volume of protoplast preparations of AH22 and AH215 increases. This increase can be particularly marked when the modal volume of the freshly prepared protoplasts is initially low (graph □ in Fig. 4a). On the other hand, there is often no change in the modal volume if the freshly prepared protoplasts are relatively large (e.g., graph ○ in Fig. 4a). In general, we can state that differences in the mean value of the modal volume are still relatively high ($\pm$34% or $\pm$50%) for protoplasts of strain AH22 even after 1–2 days of pre-incubation, whereas for protoplasts of strain AH215 they remain relatively low ($\pm$14% or $\pm$12%). The measurements show, however, that pre-incubation for a day (or sometimes 2 days) can lead to an optimum modal

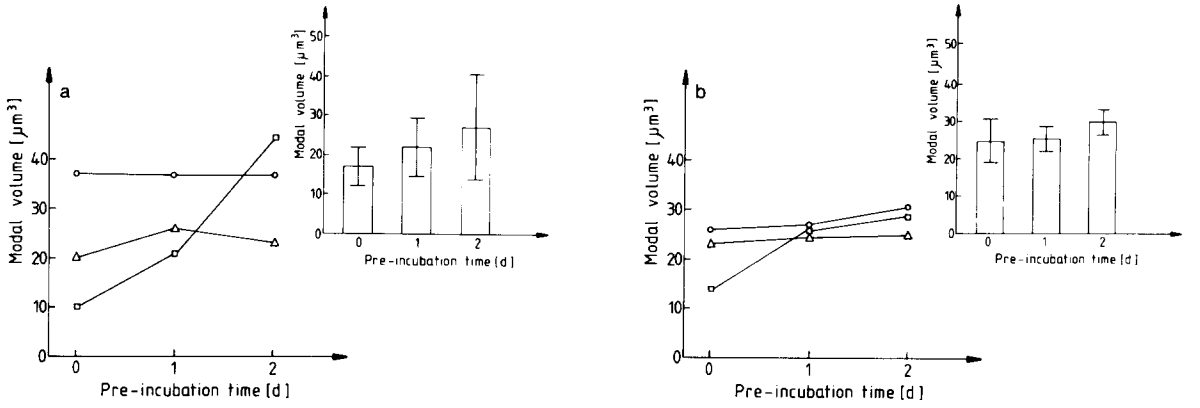

Fig. 4. The relationship between the modal volume of protoplasts from the yeast strains AH22 (a) and AH215 (b) and pre-incubation time (○, □, △ = three individual protoplast preparations). If the modal volume of freshly prepared protoplasts is very small there is often a marked increase in volume during pre-incubation (□). A nearly constant modal volume is observed if the modal volume of the freshly prepared protoplasts is large (○). This is valid for the protoplasts of both strains. The mean value and the standard deviation of the modal volumes from eight experiments are shown by the insets. Modal volumes of 15–30 μm^3 favour the fusion process. The mean value of the modal volume of the protoplasts of strain AH22 increases during pre-incubation (see inset of a) and the standard deviation is large compared with AH215 (see inset of b).

volume for the individual protoplast preparations of about 20–25 μm^3. This applies especially to the protoplasts of AH22. The decision whether or not to carry out pre-incubation must be made according to the value of the modal volume of the freshly prepared protoplasts.

Fig. 5a and b shows the individual measurements of the debris-to-cell ratio of the volume distributions corresponding to Figs. 4a and 4b, as a function of the pre-incubation time. In the case of strain AH22, it is noticeable that the debris-to-cell ratio for both freshly prepared and pre-incubated protoplasts is subject to considerable variation. This indicates that the proportion of debris, dead cells and living cells of inadequate size can vary in an irreproducible manner, in spite of apparently constant experimental conditions. Although the mean of the debris-to-cell ratio decreases with the duration of pre-incubation (inset in Fig. 5a), the standard deviation rises dramati-

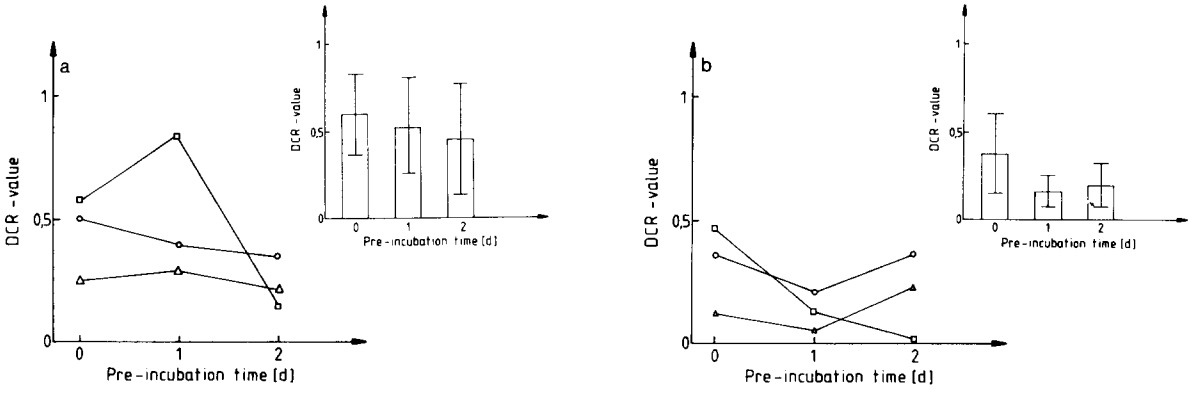

Fig. 5. Relationship between the 'debris-to-cell ratio' of protoplasts of the yeast strains AH22 (a) and AH215 (b) and pre-incubation time. The data are devided from the same volume distributions as in Fig. 4. The debris-to-cell ratio for freshly prepared and pre-incubated protoplasts is subject to considerable variation in the case of strain AH22 and AH215. The debris-to-cell ratios of strain AH215 are lower than those of strain AH22. This means that the fusion process is less inhibited by cell debris and small protoplasts.

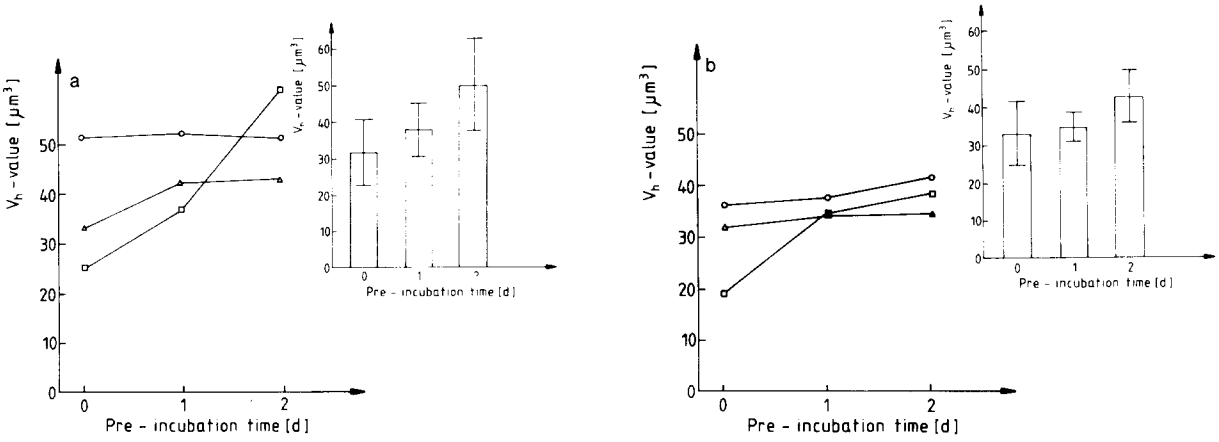

Fig. 6. Relationship between the V_h value ('modal volume + half-width') of protoplasts of the yeast strains AH22 (a) and AH215 (b) and pre-incubation time. The data are derived from the same volume distributions as in Fig. 4. If the V_h value of freshly prepared protoplasts is very small there is often a marked increase in this value during pre-incubation (□ in a and b). A nearly constant V_h value is observed if the V_h value of the freshly prepared protoplasts is large (○ in a and b). The mean value of the volume V_h of both strains is increased during pre-incubation (see inset of a and b). There is less variation in the V_h value of strain AH22 than in the modal volume (see Fig. 4) which indicates that the upper volume of the protoplasts of this strain is relatively fixed.

cally. In the case of strain AH215, the mean value of the debris-to-cell ratio for freshly prepared protoplasts is about half that of strain AH22, and the ratio is considerably reduced after 1 day of pre-incubation, particularly if the initial debris-to-cell ratio were very high. This indicates that those cells that were too small increase in volume during pre-incubation time and, therefore, shift the debris-to-cell ratio towards a more favourable level, which is also true if the protoplasts were prepared and pre-incubated in isotonic KCl solution (not shown).

Fig. 6a and b shows the corresponding measurements of the V_h value as a function of pre-incubation time. The V_h value (modal volume + half-width) characterizes the right side of the volume distribution. As demonstrated by the individual measurements and the standard deviation of the mean value (see inset of Fig. 6a), there is much less variation in the V_h value of strain AH22 than in the modal volume. This indicates that the protoplasts of this strain have relatively fixed upper and lower volume limits, although the modal volume can vary considerably within these limits.

In the case of strain AH215, the variations in both the V_h value and the value of the modal volume are roughly the same. In general, we can state that protoplasts prepared from strain AH22 have a volume distribution which deviates much more from the normal distribution than that of strain AH215.

The V_h value usually increases with pre-incubation time (however, see Fig. 6a and b for exceptions) and the increase is more marked for strain AH22 than strain AH215. After 2 days of pre-incubation, extremely unfavourable values are obtained. However, it should be noted that after 2 days of pre-incubation the mean V_h value of strain AH215 is no longer favourable either (43 μm^3) according to the upper limits given above. Experiments with protoplasts which were prepared and pre-incubated in isotonic KCl solution show the same trend in the V_h value according to the duration of pre-incubation.

Fig. 7 shows the hybrid yields obtained by fusion of the two parental strains as a function of pre-incubation time. The hybridization was performed between the appropriate protoplast preparations of strains AH22 and AH215, following the periods of pre-incubation given in Figs. 4–6. The hybrid yield is the mean value of the clones from two identically treated helical chambers, each containing aliquots of the same protoplast mix-

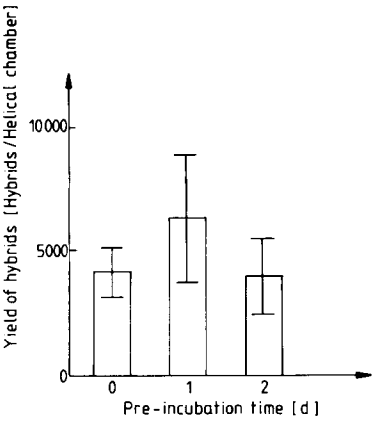

Fig. 7. The effect of pre-incubation of protoplasts of the strains AH22 and AH215 on the hybrid yield. Aliquots from the protoplast preparations which were used for the fusion experiments on day 0 (freshly prepared protoplasts) were pre-incubated and used for the fusion experiments on days 1 and 2. Each mean value in this histogramm is calculated from eight fusion experiments. After 1 day of pre-incubation, the mean value of the hybrid yield is higher than the mean value of freshly prepared protoplasts; however, the standard deviation is around 50%. This means that the hybrid yield after 1 day of pre-incubation is equal to or higher than the fusion yield obtained with non-incubated protoplasts.

ture. Fig. 7 shows that on average a 1-day pre-incubation period results in an increase in hybrid yield, but that it is not possible, because of the high standard deviation ($\pm 40\%$), to establish a clear correlation between absolute hybrid yield and the duration of pre-incubation.

If we evaluate the individual parameters of the volume distribution curves corresponding to AH22 and AH215 and substitute them into Eqn. 3 used to calculate the protoplast index, we find a definite correlation between protoplast index and absolute yield which is in the range of 2000–10000 hybrids per helical chamber (Fig. 8). The mean error in the yield as a function of the protoplast index is about 15%. This is a small error which approximates the error introduced by the varying geometry of the manually produced helical chambers and inaccuracies in pipetting.

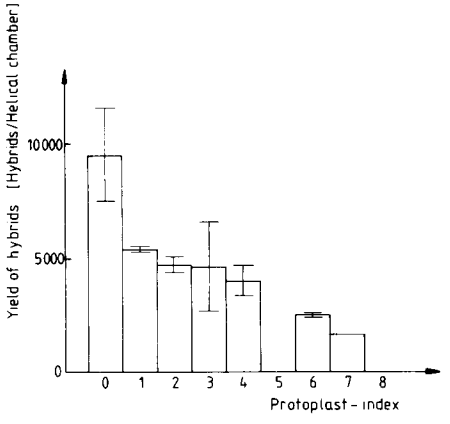

Fig. 8. The strong correlation between hybrid yield and protoplast index calculated from the volume distributions of both fusion partners (see Eqn. 3), indicates that the shape and the modal volume of the volume distribution of each fusion partner strongly affects the hybrid yield. Most protoplast preparations yield a protoplast index number in the range of 1–4; index numbers 5 and 8 have not been observed.

Discussion

The results reported here demonstrate that the volume distributions of yeast protoplast preparations determine the absolute yield of hybrids obtained by electrofusion. Measurement of the volume distributions of the fusion partners and their evaluation by means of the protoplast index method developed here, provides an objective means of determining whether a high yield of hybrids can be expected and, therefore, whether one should make appropriate modifications prior to carrying out fusion. In principle, one might increase the yield of hybrids from the fusion of given protoplast preparations using the protoplast index as a guide. For the two yeast strains discussed here, we have been able to show that considerable increases in yield can be obtained by preincubating the protoplasts in solutions of appropriate composition, provided that the volume distributions and/or the proportion of debris and cells of inadequate size were unfavourable in the freshly prepared protoplasts. Depending on the nature of the original protoplast volume distribution, pre-incubation can favourably influence the hybrid yield.

Another way of optimizing the yield of hybrids, even when the protoplast index is high, is by adapting the electrical parameters to the measured volume distributions as described previously [1–10]. Eqn. 1 represents the basis for the recalculation of the electrical parameters. Which of these procedures is chosen will depend on the nature of

the cells used and the aims of the research.

Some preliminary volume distribution measurements in our laboratory as well as FACS cell size measurements (G.A. Neill, Research Institute of Scripps Clinic, La Jolla, U.S.A., personal communication) carried out on stimulated lymphocytes and myeloma cells demonstrate that the index method can also be applied to this important fusion system by using the adequate limits of Eqn. 3. It can be shown that these fusion partners are also subject to considerable variation in the shape of their volume distributions, so that it is easy to understand why the yield of hybridomas using electrofusion has been reported to be highly variable [10].

These preliminary experiments show that by varying the culture or pre-incubation conditions, the modal volume and skewness of the volume distributions of myeloma cells (but also of lymphocytes) can be changed in a reproducible manner optimal for hybrid yield.

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References

1 Zimmermann, U., Scheurich, P., Pilwat, G. and Benz, R. (1981) Angew. Chem. 93, 332–351, Int. Edn. 20, 325–344
2 Zimmermann, U. (1982) Biochim. Biophys. Acta 694, 227–277
3 Zimmermann, U. and Vienken, J. (1982) J. Membrane Biol. 67, 165–182
4 Zimmermann, U., Vienken, J. and Pilwat, G. (1984) in Investigative Microtechniques in Medicine and Biology (Chayen, D. and Bitensky, J., eds.), pp. 89–167, Marcel Dekker, New York
5 Zimmermann, U., Büchner, K.-H. and Arnold, W.M. (1984) in Charge and Field Effects in Biosystems (Allen, M.J. and Usherwood, P.N.R., eds.), pp. 293–318, Abacus Press, Tunbridge Wells
6 Zimmermann, U., Vienken, J., Pilwat, G. and Arnold, W.M. (1984) in Cell Fusion (Evered., D. and Whelan, J., eds.), pp. 60–85, Pitman Books, London
7 Arnold, W.M. and Zimmermann, U. (1984) in Biological Membranes (Chapman, D., ed.), Vol. 5, pp. 389–454, Academic Press, London
8 Zimmermann, U. and Vienken, J. (1984) in Agricultural and Veterinary Research (Stern, W.J. and Gamble, H.R., eds.), pp. 173–200, Rowman and Allanheld, Totowa
9 Zimmermann, U., Vienken, J., Halfmann, J. and Emeis, C.C. (1985) in Advances in Biotechnological Processes 4, (Misrahi, A. and Van Wenzel, A.L., eds.), pp. 79–150, Alan R. Liss, New York
10 Zimmermann, U. (1986) Rev. Physiol. Biochem. Pharmacol. 105, 175–256
11 Schnettler, R. and Zimmermann, U. (1985) FEMS Microbiol. Lett. 27, 195–198
12 Pilwat, G. and Zimmermann, U. (1985) Biochim. Biophys. Acta 820, 305–314
13 Peters, J.H., Baumgarten, H. and Schulze, M. (1985) Monoklonale Antikörper, Springer Verlag, Berlin
14 Jeltsch, E. and Zimmermann, U. (1979) Bioelectrochem. Bioenerg. 6, 349–384
15 Schwan, H.P. and Sher, L.D. (1969) J. Electrochem. Soc. 116, 170–175
16 Takashima, S. and Schwan, H.P. (1985) Biophys. J. 47, 513–518
17 Pethig, R. (1979) Dielectric and Electronic Properties of Biological Materials, John Wiley and Sons, Chichester
18 Downie, N.M. and Heath, R.W. (1970) Basic Statistical Methods, Harper International Edition, New York
19 Kachel, V. (1979) in Flow Cytometry and Sorting (Melamed, M.R., Mullaney, P.F. and Mendelsohn, M.L., eds.), pp. 61–104, Thomas Wiley, New York
20 Kendall, M.G. and Buckland, W.R. (1982) in A Dictionary of Statistical Terms, Longman Group, London
21 Schnettler, R., Zimmermann, U. and Emeis, C.C. (1984) FEMS Microbiol. Lett. 24, 81–85
22 Willsky, R.G. (1981) in Yeast Cell Envelopes: Biochemistry, Biophysics, and Ultrastructure (Arnold, W.N., ed.), Vol. II, CRC Press, Boca Raton
23 Kachel, V. (1976) J. Histochem. Cytochem. 24, 211–230