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# CELL-CYCLE DEPENDENT TRANSFORMATION COMPETENCE IN *DICTYOSTELIUM DISCOIDEUM*

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We describe a modification of the transformation procedure for *Dictyostelium* which allows for a more exact estimate of transformation efficiency and the isolation of primary transformants. Investigations of transformation competence revealed a negative correlation to cell density and a distinct distribution during the cell-cycle. In synchronized cells, transformation efficiency is 2-3 fold higher during mitosis when compared to unsynchronized cells. 

Transformation in *Dictyostelium* has become a routine method to study the regulation of gene expression (e.g. 1) and to investigate the function of individual genes by antisense mutagenesis (2, 3) or gene disruption (4, 5). In these experiments, transformation efficiency is of minor importance since a few clones will yield the desired information. However, for shot-gun transformation, e.g. for complementation of a mutant strain, a considerably larger number of independent transformants is needed. Here we specify conditions which allow for the isolation of primary transformants and improve the yield of transformants.

## Material and Methods

Cells of the axenic strain AX2 were grown in HL-5.medium (6) as described. For synchronization, cultures were grown to a density of $5x10^6$, transferred to 4°C and shaken at 100 rpm over night. Synchronous growth was initiated either by plating the cells onto petri dishes at 22°C or by shaking the culture at 22°C (150 rpm). Cell growth was

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monitored by counting cells in a Neubauer chamber (synchronization in suspension) or in 1 $mm^2$ squares on petri dishes (synchronization on plates). In the first case, equal numbers of cells were plated before each transformation. In the second case, three different dilutions were plated at the beginning of the experiment in order to roughly compensate for cell growth. According to the growth kinetics of the culture, the appropriate dilution was used for the transformation. Transformation efficiency was then calculated relative to the acctual number of cells on the plate.

In general, transformation was done as described previously. AX2-medium or HL5-medium was used for growth and selection, in some cases dextrose was omitted from HBS. Other modifications of the procedure are outlined in table 1. pB10SX (7) or pDNeo2 (5) were used as integrating transformation vectors, pBMW-Neo1 (Knecht, unpublished) as an extrachromosomal vector. The latter is a derivative of the endogenous *Dictyostelium* plasmid Ddp2.

Transformation experiments were done under L1 conditions.

## Results

We used a modification of the original transformation protocol (8) which is summarized in table 1. This procedure is similar to the one independently developed by Early and Williams (9) and enabled us to count primary transformants immediately on the transformation plate and thus get a reliable estimate for transformation frequencies under different conditions. In addition, large numbers of independent transformants can be easily isolated.

The formation of sister colonies (siblings of one primary transformant) can be largely excluded until the clones increase in size and some cells will eventually detach. Some of the clones that grow up early after transformation appear to be unstable and are finally lost. Others grow up late suggesting a lag phase before the resistance gene is efficiently expressed (data not shown).

To investigate the influence of cell density on transformation efficiency, cells were grown to $5x10^6$/ml, plated at different densities and transformed; we then calculated the relative

**Table 1**

**Transformation protocol**

1. Grow cells to approx. $5x10^6$/ml in GM-0. For synchronization incubate cells over night shaking at 100 rpm, 4°C.
2. Plate cells (1 to $5x10^7$) on 9 cm petri dishes, let adhere for at least 10 minutes.
3. Wash cells with MES-HL5, remove medium.
4. Add DNA precipitate (12 µg DNA), incubate for 20 minutes, add 10 ml MES-HL5.
5. After three hours remove medium, add 2 ml of 18% glycerol/HBS solution, incubate for 5 to 9 minutes.
6. Remove glycerol, add 10 ml GM-0; recovery over night.
7. Change medium to GM-20, incubate for two days.
8. Change medium to GM-20, incubate for 1 day.
9. Change medium to GM-5; at this stage clones of transformed cells become microscopically visible.
10. After one or two more days clones can be picked with a pasteur pipette or a toothpick after removing most of the medium. Transfer to GM-10 into microtiter wells or onto a bacterial lawn. Recloning to purify transformants is advised.

Media and solutions are as described (Nellen *et al.*, 1984). GM (growth medium) is HL-5 medium containing the amount of G418 indicated (µg/ml).

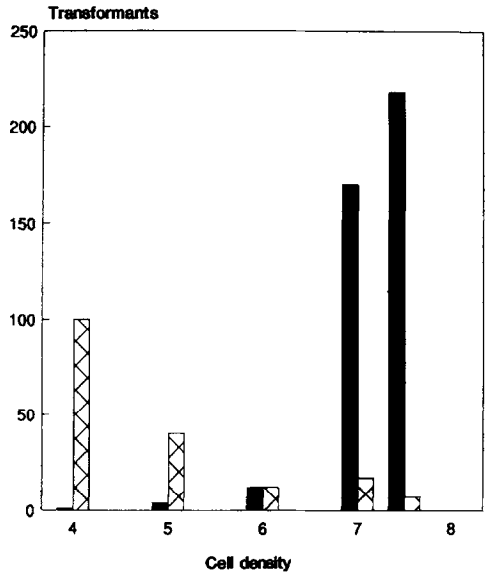


Figure 1
Density dependence of transformation efficiency.
Black bars represent the number of transformants per plate, hatched bars the number of transformants calculated per $10^6$ input cells. Numbers on the x-axis indicate cell density (log 10), numbers on the y-axis arbitrary units for the number of transformants.

number of transformants. Though the absolute yield of transformants increases with higher cell densities, the transformation efficiency in terms of transformants per $10^6$ cells decreases (figure 1).

To evaluate transformation competence during the cell-cycle, cells were synchronized by a modification of the method of Weijer *et al.* (10) and Maeda (11). An axenic suspension culture was grown to a density of 1 to $5x10^6$ cells/ml at 22°C and then transferred to 4°C and shaken over night. A dilution of the culture was kept under growth conditions at 22°C and used as an unsynchronized control. At 4°C, cells are arrested late in the G2 phase of the cell-cycle (10). When the culture is then transferred to 22°C, the cells divide synchronously after a lag of approximately two hours. Aliquots were taken at 1-2 hr intervals, plated on petri dishes at a density of $10^7$ cells/dish and transformed. The results shown in figure 2 demonstrate that transformation efficiency is strongly correlated with cell-cycle phase: a distinct peak of transformation efficiency is reached during the first cell division. During G2 phase, the number of transformants decreases below the value in unsynchronized cells. The synchronization and transformation were carried out several times using different vectors. Though variations were observed in the absolute numbers of transformants from experiment to experiment (up to a factor of 10), the results were qualitatively identical: the peak of transformation competence was always found at the end of the first cell division period. When cells are synchronized on plates, the peak of transformation competence stays high for a longer period of time (Fig. 2c). In suspension culture, the cell-cycle takes about 6 hours, on plates about 4 hours. Peaks of transformation competence are also observed in the subsequent cycles (Fig. 2d), even though synchrony is

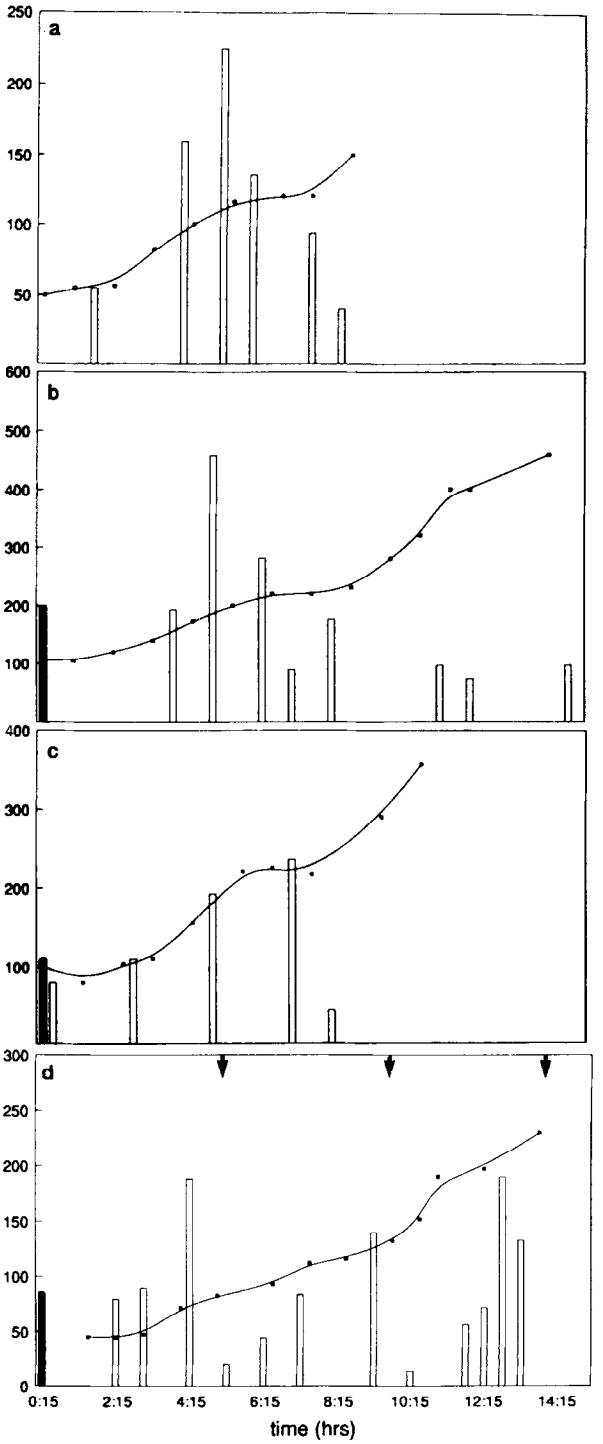


Figure 2
Cell-cycle dependence of transformation efficiency.
The curve represents the cell density, white bars the yield of transformants (relative to the number of input cells) at the respective time. The black bar gives a comparison to the transformation efficiency with unsynchronized cells. The x-axis indicates the time after release from cell-cycle arrest, the y-axis the yield of transformants in arbitrary units. In a and b cells were synchronized in suspension, in c and d on plates; transformation vectors used are: pBMWNeo1 (a), pDNeo2 (b and d), pB10SX (c). Cell growth is plotted on a linear scale, for clarification, completion of cell-cycles (doubling of cell number) is indicated by arrows in d.

gradually lost. In this experiment the growth curve is somewhat obscured because of difficulties in counting large numbers of cells on the petri dish.

If the cell-cycle dependence of transformation efficiency is due to varying accessibility of chromosomal DNA for vector integration, transformation with an extrachromosomal vector should be independent of the cell-cycle phase. This, however, appears not to be the case. As shown in figure 2a, transformation competence was also observed to be cell-cycle dependent when the extrachromosomal vector pBMWNeo1 is used.

## Discussion

We have used a modification of the *Dictyostelium* transformation protocol to study transformation competence of *Dictyostelium* cells in relation to cell density and to cell-cycle phase.

The new protocol is faster, easier, and allows for clonal isolation of primary transformants. Single colonies can be picked either into microtiter wells or onto a bacterial lawn and can be screened by the colony blotting technique for the expression of specific proteins (12) or RNAs (Maniak, Saur and Nellen, submitted for publication).

In contrast to Early and Williams (9) we find that the AX2 strain can be transformed in the same way as the KAX3 (AX4) strain and with an even better efficiency. We do not know the reasons for this discrepancy but it could be due to subtle differences in the composition of growth media or growth conditions.

Density dependent transformation competence is not due to limiting DNA concentrations (data not shown). We assume that at high densities cells, which cannot attach to the plastic surface, take up DNA less efficiently and that the vector DNA is more rapidly degraded by nucleases released from lysed cells. Unfortunately, the observation that lower cell densities increase transformation efficiency is only of limited practical use, higher absolute numbers of transformants can only be obtained when using high cell densities.

Transformation competence changes considerably during the cell-cycle. Low rates during the first hour after the release from cell-cycle arrest as well as the lag period before the cells start to divide could be due to recovery from cold shock (13). Transformation competence then increases and the highest rate is observed at the end of the first cell division, probably during S or early G2 phase. (As described by Weijer *et al.*, (10), there is no detectable G1 phase in the *Dictyostelium* cell-cycle.) After mitosis, competence drops to 20% or less of the peak value.

It can be excluded that the increase in transformation efficiency is only due to recovery from the synchronization conditions (cold shock) since a cell-cycle dependent decrease is observed after mitosis. In addition we have shown that cyclic changes in transformation competence can also be observe in a second and third round of the cell-cycle, i.e. 15 hours after the cold shock. We assume that during cell division, the physical uptake of DNA is enhanced or that cell-cycle dependent transformation competence is coupled to the S phase

in that recombination and integration of the vector DNA is facilitated during synthesis of the genomic DNA.

The latter interpretation does not agree with the observation that the extrachromosomal transformation vector pBMWNeo1 also shows cell-cycle dependent transformation efficiency. It could, however, be that the initial transformation event with this vector requires a transient integration into the genome.

Even though other, unknown factors cause variations in transformation efficiency, the use of cells during mitosis reproducibly increases the yield of transformants by a factor of 2 to 3. Since synchronization can be done easily and reproducibly, this procedure can be included in the transformation protocol with no additional effort.

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