matters arising

Transition probability and cell-cycle initiation in yeast

SHILO *et al.*¹ have suggested that the transition probability model, devised to explain the regulation of proliferation of mammalian cells², can also be applied to yeast. They propose that traverse of an early G1 event called 'start'³ is probabilistic and is the rate-limiting step of the cell cycle. This suggestion arose from their investigation of the kinetics of bud emergence in cells released from a block at 'start', imposed either by using a strain conditionally defective in *cdc 25* or by using the mating hormone α factor. After release from a block at

Fig. 1 The kinetics of bud emergence according to Fig. 1 in ref. 1, replotted. The proportion of unbudded cells (ordinate) is plotted on a logarithmic scale against time after release of the block (abscissa). ▲, strain 352/1 (*cdc 25*) after release of temperature block; △, same strain after release of α factor block.

'start', the proportion of unbudded cells seemed to fall exponentially, indicative of a probabilistic traverse of 'start'. Unless the proportion of unbudded cells is followed to a low level, however, it is difficult to distinguish between an exponential line and the lines derived from other distributions of cell generation times, such as reciprocal normal (A. E. Wheals, personal communication). Of the six semi-logarithmic plots given by Shilo *et al.*¹ only one continues below a level of 10% of the starting value, and in this case (Fig. 1 in ref. 1) the line curves gradually downwards. There is also scatter of the points around the fitted exponential lines, particularly in Figs 2 and 4. Therefore the evidence in

favour of a probabilistic traverse of 'start' is not convincing.

Shilo *et al.* also suggest that the kinetics of bud emergence after release from a block at 'start' are the same as in a steady state culture, indicating that 'start' is the rate-limiting step in the cell cycle. This interpretation is only possible if the kinetics of bud emergence after release from the block are due solely to the traverse of 'start' and not to physiological recovery from the block. In the examples given this is not in fact so: although it is claimed that bud emergence shows similar first-order kinetics whether the block is imposed by α factor or by *cdc 25*, this only seems to be so because the data are on linear plots. If the data are replotted semi-logarithmically (Fig. 1), the lines can be seen to be different, the half life of the unbudded state for cells after release from the α factor block being 42 min, whereas after release from the *cdc 25* block, the half-life is 29.5 min. Corresponding transition probabilities (*P* values—see ref. 2 for definition) are 0.63 and 0.76 h^{-1} . Clearly the nature of the block at 'start' is affecting the kinetics of bud emergence, which nullifies any comparison with a steady state culture.

Interpretation of these experiments is further complicated by the use of *cdc 25*. Mutants in this gene are unusual as they stop growth at the restrictive temperature, unlike mutants in most other *cdc* genes⁴. For this reason Johnston *et al.*⁵ consider it likely that *cdc 25* is affected in intermediary or macromolecular metabolism and that its arrest before 'start' is only a secondary pleiotropic effect. If this is so then the experiment plotted in Fig. 2 only determines the kinetics of recovery from this block in metabolism and is not informative about the rate limiting step of the cell cycle.

Note added in proof: Variability in event traverse is not evidence for its rate-limiting nature unless it can be established that event traverse is probabilistic. Thus, knowledge of the exact kinetics of cycle initiation (see reply by Shilo *et al.*) is essential for determining whether 'start' is the rate-limiting step of the yeast cell cycle.

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- ¹ Shilo, B., Shilo, V. & Simchen, G. *Nature* **264**, 767–770 (1976).
- ² Smith, J. A. & Martin, L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1263–1267 (1973).
- ³ Hartwell, L. H. *Bact. Rev.* **38**, 164–198 (1974).
- ⁴ Hartwell, L. H., Mortimer, R. K., Culotti, J. & Culotti, M. *Genetics* **74**, 267–286 (1973).
- ⁵ Johnston, G. C., Pringle, J. R. & Hartwell, L. H. *Expl Cell Res.* **105**, 79–98 (1977).

SHILO *et al.*¹ claim that their data support the concept of transition probability² in the yeast cell-cycle. However, evidence that fails to distinguish between two alternative hypotheses cannot be regarded as evidence in support of one of them. There are at least two contrasting models to account for the transition from an expandable part of the cell-cycle to a determined phase. The transition probability hypothesis argues that it occurs at random, there being a constant probability per unit time that a cell will undergo the transition^{2,3}. The cell size hypothesis suggests that cells monitor their own size and as they grow they undergo the transition when they have reached a critical cell size⁴.

Rate of entry into the budded phase is reported as showing first-order kinetics¹, but the measurements of the fraction of unbudded cells were not continued below 2%. Above these values there is virtually no measurable difference between first-order kinetics (α -curve), a lognormal distribution⁵ and a reciprocal normal distribution⁶ as can most clearly be seen when the same data are plotted equally successfully in quite different ways, for example HeLa cells^{7,8} and *Euglena*^{9,10}. Whereas first-order kinetics is consistent with the transition probability hypothesis, both lognormal and reciprocal normal distributions are consistent with critical cell-size hypotheses and the rate of entry could be interpreted as reflecting the heterogeneity in the size of the unbudded cells and the variation in time needed to grow to a critical cell size. Concomitant measurements of size were not made. Where this has been done, attainment of a critical cell size has been shown to be an important factor in the initiation of division in, for example, *Physarum polycephalum*¹¹, *Stentor*¹², *Amoeba*¹³, *Schizosaccharomyces pombe*¹⁴, and *Saccharomyces cerevisiae* (refs 15,16) and Wheals's unpublished data.

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