The relative significance of growth rate and interdivision time in the size control of cultured mammalian epithelial cells

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Summary

Genealogies of a line of mammalian epithelial cells (GPK) have been constructed from time-lapse film of monolayer cultures and measurements made of initial (post-divisional) cell size, final (pre-divisional) cell size and interdivision time (IDT). The mean initial cell volume was 2696 ± 404 (S.D.) μm^3, the mean final volume was $5247 \pm 696\ \mu m^3$ and the mean IDT was 985 ± 84 min.

Cell size regulation must be by modulation of either the growth rate or the length of the growth period. Increase in size was strongly correlated with the average rate of growth (increase in volume per unit time) ($R = 0{\cdot}94$, $P \ll 0{\cdot}001$), whilst no correlation was found between increase in size and IDT.

Although a negative correlation was found between initial volume and IDT ($P < 0{\cdot}02$), this appeared to be due to differences in IDT between sister cells being correlated with differences in their initial volumes ($P < 0{\cdot}02$), as indicated by the lack of correlation between mean sister IDT and mean sister initial volume.

The regulatory effect of growth rate was demonstrated by a negative correlation between growth rate and the initial volume of the cell ($P < 0{\cdot}005$), which is independent of differences between siblings. The mean growth rate of sibling cells was found to be negatively correlated with both the maternal growth rate ($P < 0{\cdot}01$) and the maternal volume increase ($P < 0{\cdot}005$). This implies that the growth rate of division products (which manifest similar growth rates) is influenced by the growth of the progenitor cell.

Key words: cell size regulation, growth rates, interdivision times.

Introduction

Whilst there is abundant evidence for the existence of size regulation in eukaryotic cells (Yen *et al.* 1975) the nature of the mechanism that controls cell volume in eukaryotes is not known.

In studies of so-called 'piggyback' divisions (Riley & Hola, 1980*a*), in which the daughter cells are separated vertically to the substratum instead of in the plane of the monolayer, a correlation has been observed between the duration of the interdivision time (IDT) and the relative orientation of the sister cells at cytokinesis; the gravitationally lower cell exhibiting the shorter IDT (Riley & Hola, 1983). Although there are complications with regard to the effects of delayed substratum attachment and spreading on the subsequent growth (volume increase) of the cells, the data are consistent with the view that the birth volume is an important determinant of subsequent IDT. However, it has been repeatedly shown that there is a lack of an inverse correlation between IDTs of mother and daughter (Collyn-d'Hooghe *et al.* 1977; Dawson *et al.* 1965; Froese, 1964; Miyamoto *et al.* 1973; Sisken, 1963; Sisken & Kinosita, 1962; van Wijk & van de Poll, 1979), which would be expected if the growth rate were constant and size regulation therefore reflected by the duration of the interdivision time.

We have made measurements on cell lineages of a line of mammalian epithelial cells (GPK) propagated in layer culture from which estimates of the initial and final cell volumes were made, and report evidence that indicates that the growth rate (average rate of increase of cell volume during interphase) varies among cells and is inversely related to the growth of the preceding generation.

Materials and methods

Cells and medium

The cells used in this study were a line of mammalian epithelial cells (GPK cells). The cells were routinely propagated in polystyrene flasks and subcultured by trypsinization on a weekly basis. Routine autoradiographic and broth inoculation tests were made to exclude contamination by mycoplasma and stocks of cells were maintained in dimethyl sulphoxide (DMSO)-containing medium frozen in liquid nitrogen. A culture from passage 129 was examined. The cells were grown at 37°C in 2·5 mM-Hepes-buffered Minimum Essential Medium (Eagle), supplemented with 10% foetal bovine serum (Flow Laboratories, Irvine, UK), penicillin (1000 units%) and streptomycin (1 mg%). Cells to be observed were inoculated into a polystyrene flask (25 cm^2 growth area; Falcon, Scientific Supplies Ltd, UK) in fresh medium at a mean density of 400 cells cm^{-2} and allowed to settle for 3 h before filming.

Filming and analysis

Time-lapse filming was carried out as described (Riley & Hola, 1980*b*). The data analysed were from the initial part of the film and represented the exponential portion of the growth curve. The film was analysed to give data on the pedigree and interdivision time of cells from seven clones.

Estimation of cell volume

Cell volumes were estimated at mitosis, assuming the cells to be spherical. Cell shape was investigated by stereo photogrammetry on phase-contrast images of dividing cells using a stage movement of 100 μm. The photographic prints were examined with a vernier stereophotogrammeter (Gordon Enterprises, Hollywood, CA, USA) and the maximum cell height, estimated from the culture surface, compared with the maximum cell diameter and expressed as a ratio. The mean of the values obtained for the ratio of cell height to diameter was 0·97 ± 0·15 (S.D.) (n = 11). A ratio ($P^2/4\pi A$) of the perimeter (P) to the area (A) was calculated as a method of estimating the circularity of the projected images of the cells (Bradbury, 1979). A perfect sphere would have a projection with a $P^2/4\pi A$ ratio equal to 1. Values greater than unity reflect deviations from circularity. The ratio was determined for tracings of cell projections, which had first been enlarged four times by a Tecnostyl 61 Pantografo. The perimeters were measured using a Stesco 45 map measurer and the areas determined with a Haff 315 planimeter. The mean of the values obtained was 1·045 ± 0·015 (n = 10). On the basis of these data we regard the assumption of sphericality as reasonable.

To minimize the influence of deviations from circularity of the projected images on the estimation of the volumes we employed a method based on the measurement of the projected area. The volume of mitotic cells was estimated by projecting the film onto a screen (giving a total magnification of ×2100) and making three separate tracings of the projected area of the cells onto paper of known weight (Crown Imperial; 1 cm^2 weighs 7·175 mg). The margin of the cells was taken to be the outer edge of the brightest ring of the phase-contrast image. This corresponded to the edge observable in direct transmitted light. The tracings of the projected images were cut out and weighed. The projected area was taken to be equivalent to the median section and, on this basis, the volumes (in μm^3) were estimated as follows:

$$V = (4\pi/3)\,(kW/\pi)^{3/2},$$

where V = the estimated spherical volume, W = the mean weight (in mg) of the projected area tracings, and k = conversion factor (= 3·1567). Measurements were made on pre-mitotic cells as soon as they were rounded-up and on post-mitotic cells when they had separated to the greatest extent before either of them exhibited signs of flattening. Thus, the volume estimates of post-mitotic cells were considered less reliable because the cells are smaller and their projections usually not circular in outline.

Post-mitotic cell volumes were therefore calculated by using the estimated post-mitotic cell volumes as a ratio and using this ratio to calculate corrected volumes, using the pre-mitotic volume as the standard. Thus, the corrected volumes of the two sister cells (V_1^* and V_2^*) were calculated as:

$$V_1^* = PV_1/(V_1 + V_2)$$

and

$$V_2^* = PV_2/(V_1 + V_2),$$

where P, V_1 and V_2 are the estimated volumes of the pre-mitotic and post-mitotic cells, respectively.

The reproducibility of the method was assessed by estimating the volume of a premitotic cell on four separate occasions. The coefficient of variation (c.v.) was 2·45% and the range (expressed as a percentage of the mean) was 5·82%. The coefficient of variation of the 12 individual measurements was 2·81% and the range was 9·82%. This is less than the variability of the estimated volumes of pre-mitotic (c.v. = 13·2%, range = 75·0%) and post-mitotic cells (c.v. = 15·0%, range = 86·2%).

The accuracy of the method was examined using polystyrene divinyl benzene latex beads (Coulter Electronics Ltd, Luton, UK) that are normally used for calibrating a Coulter Counter. The beads, which are supplied in a buffered saline solution, were resuspended in 10 ml of culture medium in a Falcon flask and allowed to settle on to the culture surface. The beads were subsequently photographed using the phase-contrast optics of a Nikon M35S inverted microscope. The resulting photographic negative was mounted and projected to give a final magnification of ×2080. The margin of the beads was taken to be the outer edge of the brightest ring of the projected phase-contrast image. This corresponded to the edge indicated by the images of abutting beads. The volumes of 16 beads were estimated by the above method and the mean volume was found to be within 3·5% of the median volume of the beads (value derived from an assay sheet supplied with the beads).

Statistical methods

The statistical tests employed in the analysis of the data were as previously described (Riley & Hola, 1983). In addition we have made use of the comparison of intra- and inter-class correlation using the method of Fisher (1946), linear regression analysis and analysis of variance. Validation of the linear regression analysis of growth rate *versus* initial volume was by analysis of the residuals. We thank Dr Steve Gallivan

Table 1. *Analysis of clonal variance*

Variable	Variance ratio	*P* value
Initial volume	3·614	<0·01
Final volume	3·405	<0·01
IDT	2·663	<0·05
Volume increase	1·439	NS*
Growth rate	1·837	NS

Degrees of freedom: numerator 6, denominator 95.
* NS, not significant at the 5 % level.

of the Department of Statistical Science, University College London, for advice and for examining this aspect of the data.

Results

The measured parameters were the initial cell size (i.e. the corrected post-divisional volume), the final cell size (i.e. the estimated pre-divisional volume) and the interdivision time (IDT). From these data the total increase in volume during interphase was obtained and the average rate of volume increase was calculated. Throughout this paper we refer to growth rate as this average rate of volume increase, but we have not assumed linear growth characteristics. Indeed, there is evidence from the data of Prescott (1956) to suggest non-linearity in the rate of acquisition of mass in *Amoeba*, although Wheatley & Inglis (1986) have shown that net incorporation of labelled amino acids into protein in HeLa S-3 cells approximates to linearity. However, the results and conclusions presented here do not depend on an assumption about the growth curves of individual cells.

Size regulation in this system is illustrated by a consideration of the ranges of initial and final cell volumes found in our study. The values of the initial cell volumes fell between 1861 and 4185 μm^3, and had a range of 2324 μm^3. The final cell volumes were found to lie between 4147 and 7029 μm^3, and had a range of 2882 μm^3. Assuming that the premitotic cells divide exactly in two, a population with a range of 2073·5–3514·5, i.e. 1441 μm^3, would be generated. The range of this hypothetical set of initial volumes is only 62 % of the range of the measured initial volumes. As the size distribution of cells in logarithmic growth remains constant, the range of volumes of post-mitotic cells being produced would be expected to remain stable. Thus a 38 % increase in range would be necessary in order to obtain the range of the measured initial volumes. This increase in range could be accomplished by unequal distribution of cytoplasm at cytokinesis and in this case would be generated by a 10 % decrease in the size of one of the smallest cells in the hypothetical set of initial volumes (i.e. as would result from a 45:55 division of the cytoplasm of the progenitor cell) and a 20 % increase in the size of one of the largest (i.e. as would result from a 60:40 split).

For the entire population examined initial cell volume (mean ± S.D.) was 2696 ± 404 μm^3 (coefficient of variation (c.v.) = 15·0 %), the mean final volume was 5247 ± 696 μm^3 (c.v. = 13·2 %) and the mean interdivision time was 985 ± 84 min (c.v. = 8·6 %). The mean volume increase was 2250 ± 726 μm^3 (c.v. = 32·22) and the mean growth rate was 2·59 ± 0·76 $\mu m^3 min^{-1}$ (c.v. = 29·3 %). For a more than twofold range of initial volumes in our sample (1861–4185 μm^3) the growth rates recorded varied over a sixfold range (1·06–5·904 $\mu m^3 min^{-1}$) in comparison with a less than twofold range of interdivision times (768–1294 min). Growth rates thus showed greater variability than interdivision times.

For sister pairs we found the within-pair variance of the data to be less than the between-pair variance, giving variance ratios of 2·05, 4·22, 2·52, 3·73 and 3·30, respectively, for initial volumes, final volumes, interdivision times, volume increases and growth rates. These *F* ratios are all significant at the 1 % level. Thus in all these respects sibling cells were more similar to each other than to the population as a whole.

An analysis of clonal variance (Table 1) shows that whilst significant differences exist between clones with regard to initial volume, final volume and interdivision time, volume increase and growth rate are not clonally determined. Table 2 gives the mean clonal values for the measured variables.

Table 2. *Mean clonal values for the measured variables*

Clone*	Number of observations	Initial volume (μm^3, ±S.D.)	Final volume (μm^3, ±S.D.)	IDT (min, ±S.D.)	Volume increase (μm^3, ±S.D.)	Growth rate ($\mu m^3 min^{-1}$, ±S.D.)
1	4	2801 (192)	4489 (268)	1083 (142)	1688 (211)	1·561 (0·103)
3	24	2921 (516)	5476 (782)	979 (83)	2555 (835)	2·606 (0·782)
4	24	2652 (357)	5282 (600)	1012 (101)	2629 (668)	2·602 (0·637)
5	4	2969 (251)	5888 (939)	979 (46)	2919 (1091)	2·978 (1·129)
6	10	2794 (322)	5577 (639)	976 (42)	2782 (829)	2·851 (0·855)
7	30	2485 (273)	4991 (578)	951 (66)	2506 (630)	2·648 (0·772)
8	6	2612 (444)	4989 (589)	1028 (42)	2377 (476)	2·322 (0·539)

* Clone 2 failed to proliferate.

Linear correlations were investigated using population data. Table 3 gives the values of the correlation coefficient and the computed probability values for comparisons between the five variables measured. If IDT were an important factor in the regulation of cell size we would expect there to be a clear correlation with final cell volume. Yet, despite the fact that both IDT and final volume show variability amongst clones (Table 1), no significant correlation between these variables was found (Table 3), nor was IDT correlated with volume increase or growth rate. IDT showed a significant correlation with initial volume ($P<0{\cdot}02$), which is accounted for by the behaviour of sister cells (see below) as shown by the lack of significant correlation between IDT and initial volume when plotted as the means of the values for sister pairs (Table 3). However, the significant correlation between growth rate and initial volume ($P<0{\cdot}005$) cannot be accounted for in this way as shown by the correlation between mean sister initial volume and mean sister growth rate (Table 3). The growth rate also shows a strong positive correlation with volume increase and final volume (Table 3). Overall size regulation in the system is reflected by the correlation between initial volume and volume increase ($P<0{\cdot}001$), which is stronger than the correlation of initial volume with either growth rate or IDT, suggesting that both factors contribute to it. However, since growth rate and volume increase are strongly correlated and there was no significant correlation between IDT and volume increase, we conclude that for these epithelial cells, under the *in vitro* conditions of cultivation employed, size control appears to operate principally through an effect on the growth rate whereby cells of lower birth volume grow more rapidly (Fig. 1).

Table 3. *Comparison of population variables by linear regression analysis*

Variables		Correlation coefficient	n	P value
IDT	Initial volume	−0·24	102	<0·02
IDT	Final volume	−0·04	102	NS*
IDT	Volume increase	0·10	102	NS
IDT	Growth rate	−0·19	102	NS
Mean sister IDT	Mean sister initial volume	−0·06	51	NS
Growth rate	Initial volume	−0·29	102	<0·005
Growth rate	Final volume	0·82	102	≪0·001
Growth rate	Volume increase	0·94	102	≪0·001
Mean sister growth rate	Mean sister initial volume	−0·32	51	<0·025
Initial volume	Volume increase	−0·36	102	<0·001
Initial volume	Final volume	0·21	102	<0·05
Volume increase	Final volume	0·83	102	≪0·001

* NS, not significant at the 5% level in a two-tailed t-test.

Sibling differences in growth rate were not correlated with differences in initial volume (Table 4) as would have been expected from the analysis of the population data. Sister cells were found to have similar growth rates (intraclass correlation coefficient = 0·52, $P<0{\cdot}001$) and this similarity was generally unaffected by inequalities in post-divisional size (Fig. 2A), which implies that growth rate is determined prior to division. In six cases the difference in growth rate between sisters was greater than $1\,\mu m^3\,min^{-1}$ and three of the most extreme examples are shown in Fig. 2B. That

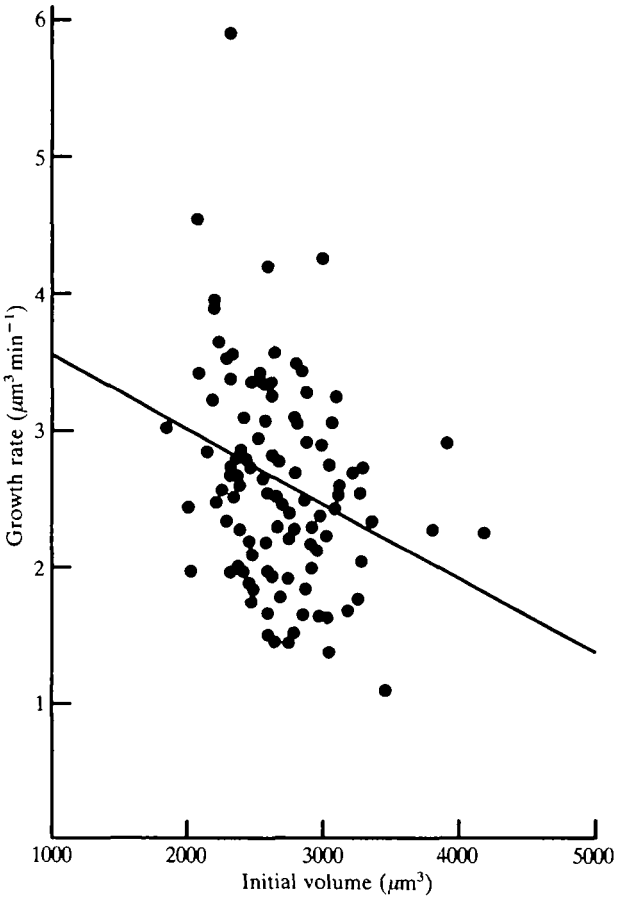

Fig. 1. Scatter plot of the average growth rate ($\mu m^3\,min^{-1}$) *versus* the initial cellular volume (μm^3). The calculated regression line for the population data is shown. $Y = 4{\cdot}03 - 0{\cdot}00053X$, 95% confidence intervals; $-0{\cdot}00088 < \beta < -0{\cdot}00018$, $P<0{\cdot}005$.

Table 4. *Comparison of differences in initial volume with differences in other variables for sister pairs by linear regression analysis*

Difference in	Difference in	Coefficient of correlation	n	P value
Initial volume	Final volume	0·09	51	NS
Initial volume	Growth rate	−0·16	51	NS
Initial volume	Volume increase	−0·31	51	<0·05
Initial volume	IDT	−0·33	51	<0·02

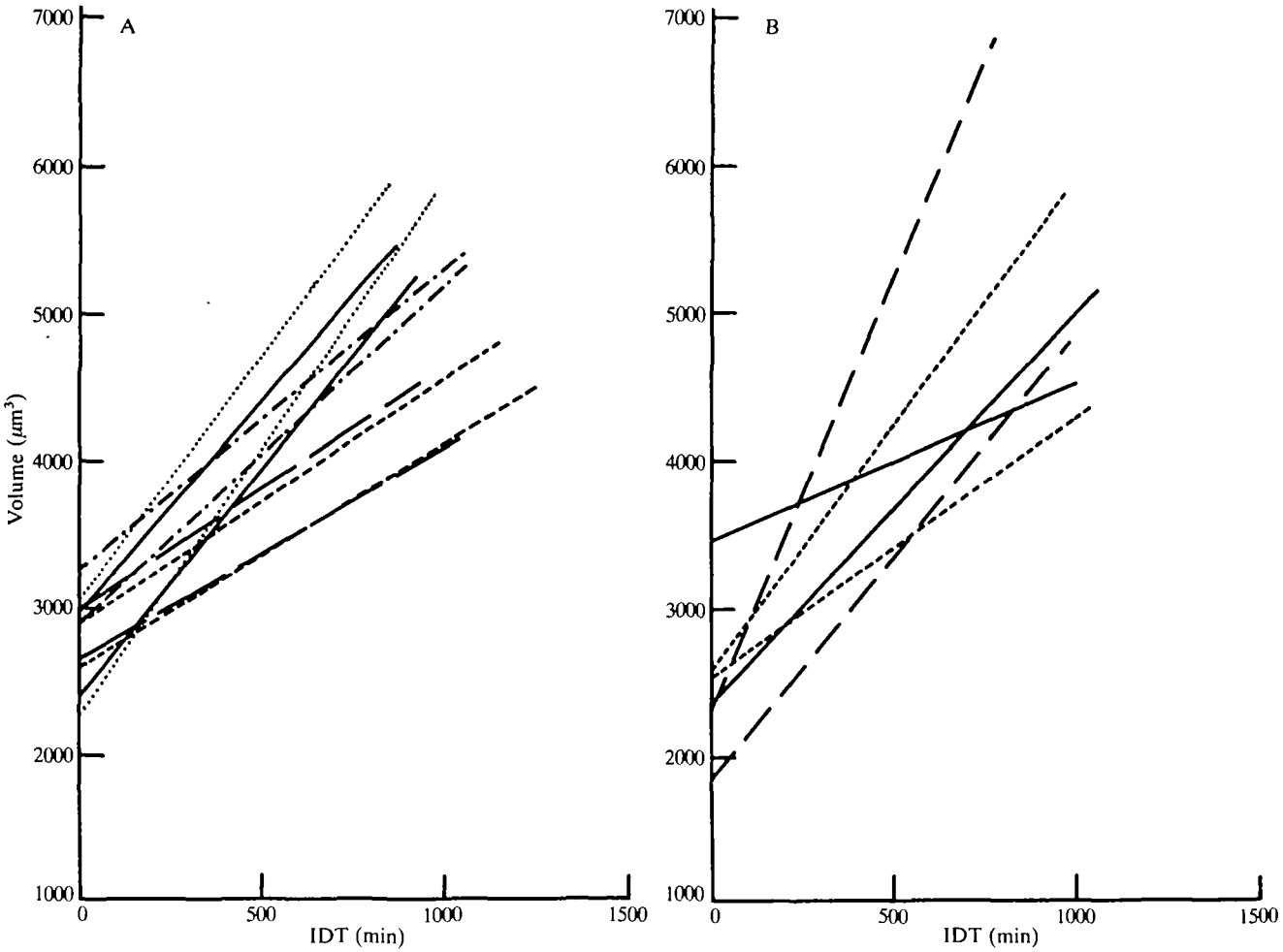

Fig. 2. A. Conservation of growth rate in sister cells of differing initial volume. The growth 'trajectories' (plotted by joining the initial and final volumes) are illustrated for sibling cells identified by pedigree number and clone (in parentheses). The sister cells are: 9 (1) and 10 (1) (——); 11 (1) and 12 (1) (– – –); 9 (4) and 10 (4) (· · · · ·); 15 (7) and 16 (7) (——); 21 (8) and 22 (8) (– · –). B. Illustration of major discrepancies between the growth rates of sister cells. The paired examples are: 5 (4) and 6 (4) (——); 27 (4) and 28 (4) (——); and 7 (8) and 8 (8) (– – –).

these large discrepancies are relatively uncommon is also indicated by the fact that the mean difference between the growth rates of sister pairs ($0{\cdot}53 \pm 0{\cdot}51$ $\mu m^3 min^{-1}$, $n = 51$) was considerably lower than the mean difference between pairs of the 102 growth rates taken in all possible combinations ($0{\cdot}83 \pm 0{\cdot}96 \mu m^3$ min^{-1}, $n = 5151$).

Sibling differences in initial volume were correlated with differences in IDT (Table 4). Thus, in the general population, the observed correlation between initial size and IDT (Table 3) is principally due to sibling differences in post-divisional size being reflected by differences in their IDTs, which are prolonged in the case of smaller sister cells (Fig. 3). The general similarity of sibling IDTs (intraclass correlation coefficient = 0·43, $P < 0{\cdot}005$) and volume increase in siblings (intraclass correlation coefficient = 0·57, $P < 0{\cdot}001$) indicates that prolongation of IDT in

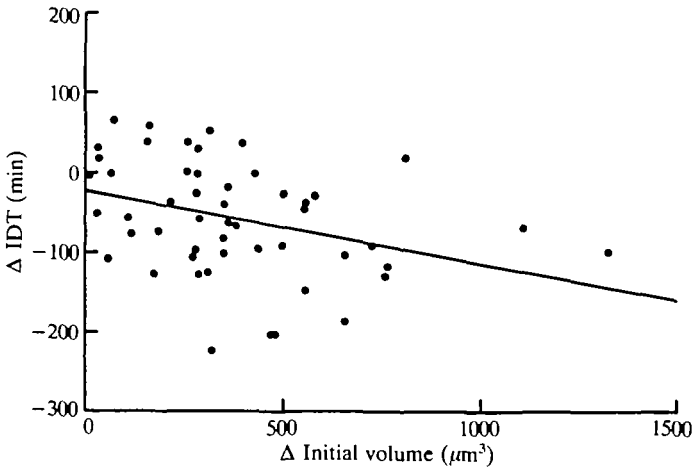

Fig. 3. Relationship between sibling differences in initial volume and differences in interdivision time. The increase in initial volume is plotted *versus* the increase or decrease in IDT. $Y = -23{\cdot}5 - 0{\cdot}09X$, 95% confidence intervals; $-0{\cdot}161 < \beta < -0{\cdot}019$, $P < 0{\cdot}02$.

Table 5. *Comparison of mean daughter growth rate and IDT with maternal cell variables by linear regression analysis*

Maternal variable	Mean daughter variable	Correlation coefficient	n	P value
Initial volume	IDT	−0·22	40	NS
IDT	IDT	0·27	40	NS
Final volume	IDT	0·01	40	NS
Growth rate	IDT	0·07	40	NS
Volume increase	IDT	0·13	40	NS
Initial volume	Growth rate	−0·01	40	NS
IDT	Growth rate	−0·34	40	<0·05
Final volume	Growth rate	−0·45	40	<0·005
Growth rate	Growth rate	−0·41	40	<0·01
Volume increase	Growth rate	−0·47	40	<0·005

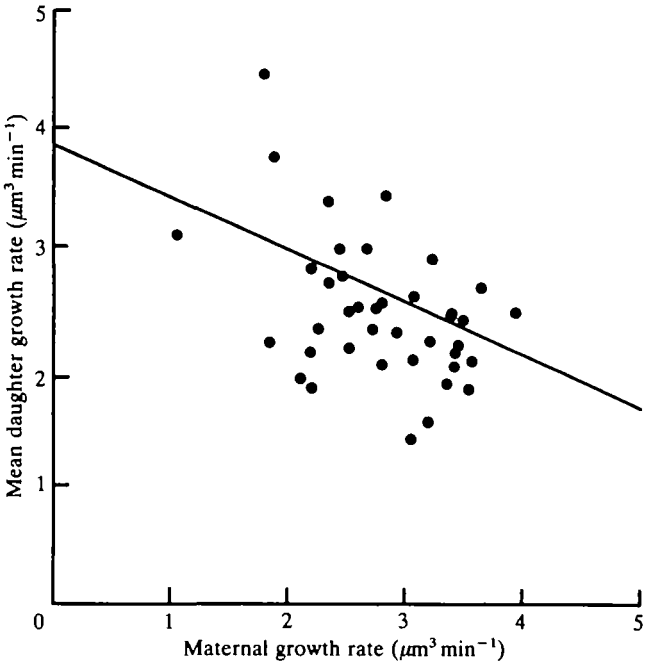

Fig. 4. Relationship between the mean daughter growth rate and the maternal growth rate. $Y = 3{\cdot}86 - 0{\cdot}44X$, 95 % confidence intervals; $-0{\cdot}73 < \beta < -0{\cdot}15$, $P < 0{\cdot}01$.

a sibling of small initial size plays a relatively minor role in the size regulation of the population.

Table 5 gives the values of the coefficient of correlation and the computed probability for comparisons between mean daughter growth rate or IDT and various maternal variables. There is a significant negative correlation between the mean growth rate of daughter cells and the growth rate of the maternal cell (Fig. 4). This is evidence against the similarity of the growth rate of sister pairs being a lineage-dependent phenomenon. The most significant relationship ($P <$ 0·005) was a negative correlation between mean daughter growth rate and maternal volume increase

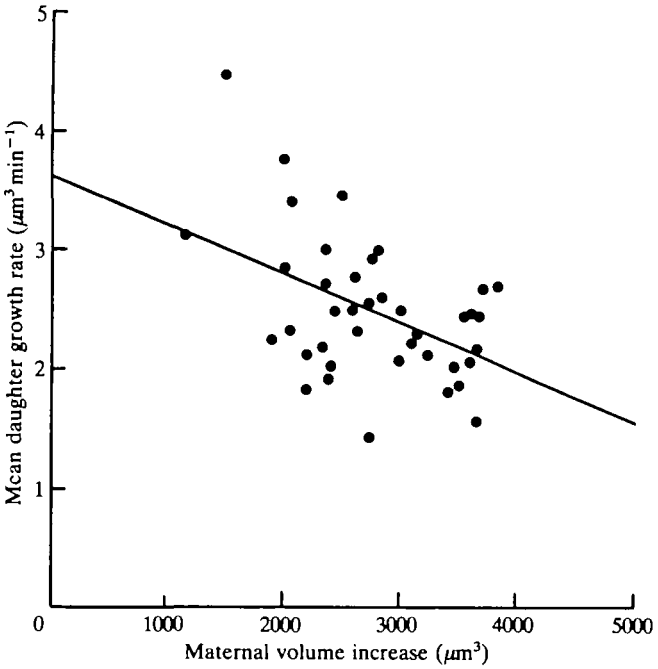

Fig. 5. Relationship between the mean daughter growth rate and the maternal volume increase. $Y = 3{\cdot}61 - 0{\cdot}0004LX$, 95 % confidence intervals; $-0{\cdot}000616 < \beta < -0{\cdot}00016$, $P < 0{\cdot}005$.

(Fig. 5), which suggests that the growth rate in daughters is determined by the amount of growth accomplished by the maternal cell. This is also reflected in the negative correlation between the final maternal volume and the mean daughter growth rate ($P < 0{\cdot}005$).

Discussion

The difference between the initial and final size of a cell is determined by the average growth rate and the duration of growth (IDT). Regulation of cellular size implies the modification of one or both of these variables. Our data indicate that, in the population of cells examined, the most significant method of size regulation is the variation of growth rate such that daughter cells produced by mother cells of low final volume exhibit an increased rate of growth. Alteration of IDT as a size regulatory mechanism appears to be significant only in relation to sister cells of differing birth volume. The evidence suggests that IDTs are, to a large extent, lineage dependent in a manner that does not appear to be related to cell volume regulation. The overall relationships may be summarized in the form of two general statements: (1) the average growth rate of daughter cells is an inverse function of the growth of the maternal cell; and (2) sibling cells have growth rates that tend to be similar, whilst differences in their initial volumes are reflected by prolongation of the IDT of the cell of smaller initial size.

No directly comparable data exist for mammalian cells although our conclusions are consistent with the observations of Shields *et al.* (1978) and Brooks & Shields (1985) on size regulation in cultures of 3T3/4A (clone 5) cells. These authors demonstrated that, in cells sorted into large and small size distributions, the average rate of increase in volume is greater for smaller cells. Moreover, their data (Shields *et al.* 1978) show that the IDTs of small cells are, on average, prolonged although to a relatively small extent, which is in agreement with our observations.

Prescott (1956) made reduced weight measurements of *Amoeba proteus* during the cell cycle and found that, in general, small post-telophase cells grew faster than large post-telophase cells. However, growth rates did not appear to vary as much as in GPK cells and smaller post-telophase cells exhibited extended interdivision times.

In most studies of fission yeasts, a negative correlation between initial length and interdivision time has been observed (James *et al.* 1975; Fantes, 1977; Miyata *et al.* 1978), although James *et al.* (1975) did find a negative correlation between initial length and growth rate for the 975h$^+$ strain of *Schizosaccharomyces pombe*. A reciprocal relationship between initial size and growth rate has not been found in bacteria (Schaechter *et al.* 1962).

The similarity of growth rates in siblings observed in this study finds a parallel in experiments with rat hepatoma cells and *Paramecium tetraurelia*, which have demonstrated that sister cells have similar G_1 periods (van Wijk *et al.* 1977; Rasmussen *et al.* 1986). This suggests that differences in sister IDTs represents variation in duration of a later phase of the cycle. The reciprocal relationship between growth in the maternal cell and growth rate in the progeny finds another parallel in the work of Rasmussen *et al.* (1986), who suggest that the length of G_1 is established during the preceding cycle in *P. tetraurelia*, as perturbations of cell mass or macronuclear gene dosage early in a cycle do not affect the length of G_1 in the same cycle but they do affect the length of G_1 in the subsequent cycle. The failure of post-divisional changes in nutrient levels to change the timing of initiation of DNA synthesis gives credence to this hypothesis (Ching & Berger, 1986), although these authors note that the timing of initiation of DNA synthesis does not appear to be established prior to division in another ciliate, *Tetrahymena* or in yeast.

The two generators of diversity in our system would appear to be: (1) factors that modify growth during a cycle and (2) inequalities in cytoplasmic distribution at cytokinesis. We believe, on the basis of our data, that perturbations in growth are regulated by changes in the growth rate of the succeeding generation. Inequalities in cytokinesis appear to be regulated in the same generation by modifications of the IDTs. In general, modifications of the IDTs appear to be of little significance except where circumstances result in a large discrepancy in the size of sister cells at cytokinesis. Such differences may arise, for example, in vertical cell divisions (piggyback divisions) (Riley & Hola, 1980*a*, 1983) and are reflected in discrepancies in IDT. These sibling differences in IDT are transient. Occasional examples of prolongation of IDT persisting in subsequent generations have been observed (Riley & Hola, 1981), suggesting a mode of origin of clonal differences in IDT, but regrettably no such instance occurred in the present study so that the relationship of such a change to growth rate is unknown.

It is not possible, on the basis of our results, to propose mechanisms to account for the observed properties. The IDT-regulating factor appears to be distributed according to volume between sister cells but not in the population as a whole. This suggests that it is of a particulate nature with a probability of distribution that varies with the apportioning of cytoplasm between sibling cells as, for example, in the manner proposed for mitochondria (Birky & Skavaril, 1984). It would appear that the growth-rate-determining factor is distributed between sister cells in a volume-independent manner, suggesting that it is concentration-dependent, although large discrepancies in sibling growth rates, whilst unusual, pose problems for such an interpretation.

References

Birky, C. W. & Skavaril, R. V. (1984). Random partitioning of cytoplasmic organelles at cell division: the effect of organelle and cell volume. *J. theor. Biol.* **106**, 441–447.

Bradbury, S. (1979). Microscopical image analysis: problems and approaches. *J. Microsc.* **115**, 137–150.

Brooks, R. F. & Shields, R. (1985). Cell growth, cell division and cell size homeostasis in Swiss 3T3 cells. *Expl Cell Res.* **156**, 1–6.

Ching, A. S.-L. & Berger, J. D. (1986). The timing of initiation of macronuclear DNA synthesis is set during the preceding cell cycle in *Paramecium tetraurelia*. Analysis of the effects of abrupt changes in nutrient level. *Expl Cell Res.* **167**, 177–190.

Collyn-D'Hooghe, M., Valleron, A.-J. & Malaise, E. P. (1977). Time-lapse cinematography studies of cell cycle and mitosis duration. *Expl Cell Res.* **106**, 405–407.

Dawson, K. B., Madoc-Jones, H. & Field, E. O. (1965). Variations in the generation times of a strain of rat sarcoma cells in culture. *Expl Cell Res.* **38**, 75–84.

Fantes, P. A. (1977). Control of cell size and cycle time in *Schizosaccharomyces pombe*. *J. Cell Sci.* **24**, 51–67.

Fisher, R. A. (1946). *Statistical Methods for Research Workers*, 10th edn. London: Oliver and Boyd.

FROESE, G. (1964). The distribution and interdependence of generation times of HeLa cells. *Expl Cell Res.* **35**, 415–419.

JAMES, T. W., HEMOND, P., CZER, G. & BOHMAN, R. (1975). Parametric analysis of volume distributions of *Schizosaccharomyces pombe* and other cells. *Expl Cell Res.* **94**, 267–276.

MIYAMOTO, H., ZEUTHEN, E. & RASMUSSEN, L. (1973). Clonal growth of mouse cells (strain L). *J. Cell Sci.* **13**, 879–888.

MIYATA, H., MIYATA, M. & ITO, M. (1978). The cell cycle in the fission yeast, *Schizosaccharomyces pombe*. I. Relationship between cell size and cycle time. *Cell Struct. Funct.* **3**, 39–46.

PRESCOTT, D. M. (1956). Relation between cell growth and cell division. II. The effect of cell size on cell growth rate and generation time in *Amoeba proteus*. *Expl Cell Res.* **11**, 86–98.

RASMUSSEN, C. D., BERGER, J. D. & CHING, A. S.-L. (1986). Effects of increased cell mass and altered gene dosage on the timing of initiation of macronuclear DNA synthesis in *Paramecium tetraurelia*. Implications for cell cycle regulation. *Expl Cell Res.* **165**, 53–62.

RILEY, P. A. & HOLA, M. (1980*a*). Variation in intermitotic interval between sister cells related to orientation at mitosis. *Eur. J. Cell Biol.* **22**, Abst. 315.

RILEY, P. A. & HOLA, M. (1980*b*). Clonal differences in generation times of GPK epithelial cells in monolayer culture. *Expl Cell Biol.* **48**, 310–320.

RILEY, P. A. & HOLA, M. (1981). Clonal variation in proliferation rate of cultures of GPK cells. *Cell Tiss. Kinet.* **14**, 489–499.

RILEY, P. A. & HOLA, M. (1983). Transient intraclonal variation in interdivision time in relation to orientation at cytokinesis of GPK cells in layer culture. *Cell Tiss. Kinet.* **16**, 189–198.

SCHAECHTER, M., WILLIAMSON, J. P., HOOD, J. R. & KOCH, A. L. (1962). Growth, cell and nuclear division in some bacteria. *J. gen. Microbiol.* **29**, 421–434.

SHIELDS, R., BROOKS, R. F., RIDDLE, P. N., CAPELLARO, D. F. & DELIA, D. (1978). Cell size, cell cycle and transition probability in mouse fibroblasts. *Cell* **15**, 469–474.

SISKEN, J. E. (1963). Analyses of variations in intermitotic time. In *Cinematography in Cell Biology* (ed. G. G. Rose), pp. 143–168. New York: Academic Press.

SISKEN, J. E. & KINOSITA, R. (1962). Variations in the mitotic cycle *in vitro*. *Expl Cell Res.* **22**, 521–525.

VAN WIJK, R. & VAN DE POLL, K. W. (1979). Variability of cell generation times in a hepatoma cell line. *Cell Tiss. Kinet.* **12**, 659–663.

VAN WIJK, R., VAN DE POLL, K. W., AMESZ, W. J. C. & GEILENKIRCHEN, W. L. M. (1977). Studies on the variations in generation times of rat hepatoma cells in culture. *Expl Cell Res.* **109**, 371–379.

WHEATLEY, D. N. & INGLIS, M. S. (1986). Protein turnover during cell growth: a re-examination of the problem of linear incorporation kinetics of radioactively labelled amino acids into protein and its relationship to growth characteristics. *Cytobios* **47**, 187–210.

YEN, A., FRIED, J., KITAHARA, T., STRIFE, A. & CLARKSON, B. D. (1975). The kinetic significance of cell size. I. Variation of cell cycle parameters with size measured at mitosis. *Expl Cell Res.* **95**, 295–302.

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