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Mitosis
Facts and Questions

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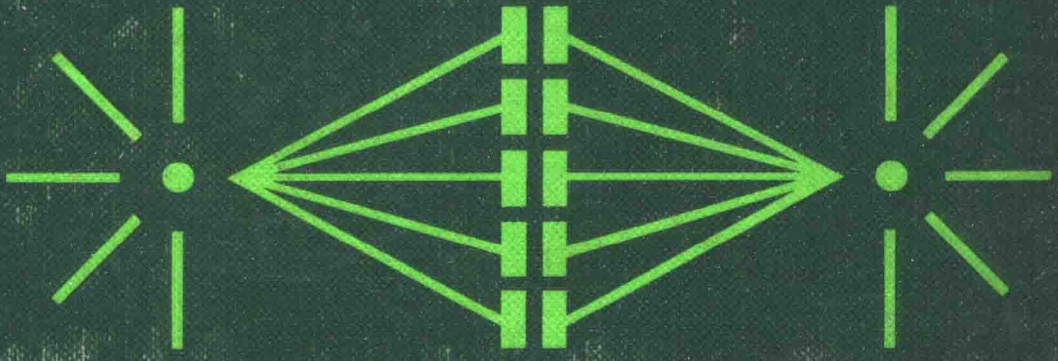

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Proceedings of a Workshop held at the
Deutsches Krebsforschungszentrum,
Heidelberg, Germany, April 25—29, 1977

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Preface

Two years ago, about twenty people gathered informally in our institute to discuss mitosis. We took this opportunity to try to separate the "hard" facts of mitosis which are accepted by most people, from the "soft" ones which are still open for discussion. Surprisingly few "hard" facts survived with their reputation still intact. This result led us to organize a similar meeting on a larger scale. The outcome was the workshop "Mitosis: Facts and Questions", which was held at the German Cancer Research Center in Heidelberg from April 25-29, 1977. An introductory lecture was given for each of nine major topics, followed by an extensive discussion of facts, questions and future experiments. Further details were provided by posters.

The proceedings of the meeting are published in this volume. We feel that many open questions and facts described here will provide stimulating ideas and a basis for further investigation of this fundamental process.

The success of the workshop would not have been possible without the help of many people. We are very grateful to the German Cancer Research Center for its interest and assistance, and for the support of the Verein zur Förderung der Krebsforschung in Deutschland represented by Prof. Dr. h.c. K.H. Bauer, the ECBO (European Cell Biology Organization) and the Deutsche Gesellschaft für Zellbiologie. Our sincere thanks are also extended to our students and technicians for their enthusiastic help, and to Mrs. Joa for typing the manuscripts.

Heidelberg, September 1977

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Contents

Session I

The Timing of Cell Cycle Events

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A. Introduction

Most of the accompanying articles are concerned with the events of mitosis and what actually happens during this intricate process of morphogenesis that completes the cell cycle. In contrast, I want to consider here not what happens but why it happens, in the sense of what signal for division is received by a growing cell. This is one of the oldest questions in cell biology with a history stretching back at least three-quarters of a century. The complete framework of the answer is unknown but many parts of it have become clearer during the last fifteen years.

Apart from mitosis and cell division, the other very well-studied event in the cell cycle is the synthesis of DNA during the S period. This is periodic and only occupies a part of the cycle in all (or nearly all) eukaryotic cells. There is no inherent reason why there should be a direct connection between this synthetic event and the morphologic events of mitosis: indeed, the immediate signals are likely to be different. Nevertheless, the controls of DNA synthesis and of division are often considered together for two reasons. The first is that there is a dependency relation between them in many systems, such that mitosis and cell division do not occur until DNA synthesis has been completed. The evidence comes from inhibitor studies in many cell systems (Mitchison, 1971) and from cell cycle mutants in yeast (Hartwell, 1974; Nurse et al., 1976). The second is the suggestion both in bacteria and in mammalian cells that the primary control point in the cell cycle is at the time of initiation of DNA synthesis and that thereafter there is a constant time until division.

I have reviewed elsewhere (Mitchison, 1977a) a number of contemporary models for the control of division and have given little space to the current work in this laboratory on division control in the fission yeast *Schizosaccharomyces pombe*. I will reverse this pattern here by considering our work on yeast in greater detail and then discussing briefly how far it is in accord with the concepts that have come from other cells.

B. Cell Cycle Events in *Schizosaccharomyces pombe*

I. The Organism

S. pombe, a fission yeast, is a cylindrical cell with rounded ends (ca. 10 μm long x 3.5 μm in diameter) that grows mainly in length during the cell cycle. It has most of the typical features of a eukaryotic cell except that its mitosis is somewhat different from that of higher eukaryotes. Nuclear division takes place at 0.75 of the cycle. At 0.85 of the cycle, a cell plate or septum appears across the middle of the cell and lasts until the end of the cycle when the two daughter cells separate physically. There is therefore an unusually large interval between mitosis and cell separation. The S period is positioned right at the end of the cycle. Earlier work suggested a G1 period of 0.2 of the cycle and an S period of 0.1 (Mitchison and

Creanor, 1971a) but recent evidence from autoradiographs after pulse labelling of DNA indicates a shorter G1 of ca. 0.1 of the cycle and a longer S of 0.2 (K.A. Nasmyth, unpublished results). With these short G1 and S periods, the cell spends most of the cycle (ca. 0.7) in G2.

Although this yeast is less well known than the budding yeast *Saccharomyces cerevisiae*, it has been used extensively for genetic work (Gutz et al., 1974) as well as for cell cycle studies (Mitchison, 1970).

II. Cell Size Homeostasis

One clue to division control comes from considering the size of a cell at division. Although this size shows a good deal of variation, the mean tends to stay constant for many micro-organisms and some higher cells that are growing exponentially under any one set of culture conditions. This suggests that there is some homeostatic mechanism that regulates cell size and maintains the constant mean value. In principle, there are two ways in which this mechanism could work. The first would be to have a constant cycle time and a variable growth rate that was inversely proportional to the size of a daughter cell at the start of the cycle. Large daughter cells would grow slowly and so reduce their size to the mean value at the end of the cycle. The second would be to have a constant growth rate and a variable cycle time that was inversely proportional to daughter size. Large daughter cells would then have short cycle times.

S. pombe is a good organism in which to explore this question since it is easy to follow the growth of single cells with time-lapse microphotographs and to get an estimate of cell size from a single measurement of cell length. These advantages have been exploited by James et al. (1975) and their results have been confirmed and extended by Fantes (1977). It is clear that size homeostasis is maintained by the second of the two mechanisms above. Large daughter cells have short cycles and small daughter cells have long cycles. The quantitative relations are such that the normal size variations can be compensated within about one cycle. It is also possible to make abnormally large cells by holding a temperature-sensitive (TS) cell cycle mutant for a period at the restrictive temperature. All the TS cell cycle mutants that have been isolated in *S. pombe* continue to grow at the restrictive temperature even though division is blocked (Nurse et al., 1976). These abnormally long cells bring out another interesting aspect of the size/cycle time relationships. Above a particular size the cycle time is scarcely shortened, however long the cell. In other words there is a minimum cycle time (about 75 % of normal in these conditions) that acts as a limit to the shortening of cycle time with long cells. As a result, it takes more than one cycle for the homeostatic mechanism to reduce the size of these very long cells to normal.

Two other results from the work of Fantes are that cell size does not seem to be "inherited" (large cells do not produce daughters that are large when they in turn divide) and that there is no correlation between the size of daughters and their average growth rate during the following cycle.

The concept that emerges from this work is that cell size is regulated, within limits, by varying the cycle time. Put in another way, which is more significant for the theme of this article, the primary trigger for mitosis and cell division comes from a mechanism that measures cell size. Bearing in mind that words suchs as primary, trigger, and size are imprecise and to some extent "loaded", how could such a mechanism work? There is no shortage of models (see the review by

Fantes et al., 1975), and a simple one for a size control mechanism, colloquially a "sizer", is to have a pulse of a mitotic inhibitor produced after division. The cytoplasmic concentration of this inhibitor is reduced by growth in volume until it reaches a low enough level to allow the next mitosis.

Alternatively, an activator is produced at a rate proportional to cell mass and triggers mitosis at a critical concentration. These models are discussed later.

A "timer" is a parallel concept to a sizer, again with a degree of imprecision. In essence, it is a mechanism that ensures a constant absolute time between two biological events under certain conditions. There could be one process that determines this time or a set of sequential processes. The relevance of timers in the cell cycle is that a sizer could operate well before division and it would then be followed by a timer, which would set a fixed time interval between the moment the sizer was triggered and the act of division. This would introduce a constant lag time for the preparations for division to be completed. As we shall see, the concept of a sizer followed by a timer is an accepted view for bacteria. One measure of the imprecision of the timer concept is that it is not a "clock" of the type envisaged in the work on circadian rhythms, which, like a real clock, is independent of temperature. Cell cycle timers in bacteria are not temperature-compensated, but they do appear to keep a fixed time with different growth rates.

Returning to *S. pombe,* the results described so far suggest a sizer control operating on division but they do not show where in the cycle this control operates and whether there is a timer involved as well as a sizer. To resolve this question, we have to examine what happens with size mutants and with nutrient shifts.

III. Size Mutants and Nutrient Shifts

While isolating conditional mutants blocked in the cell cycle, Nurse also discovered a novel and very profitable group of mutants that are altered in cell size (Nurse, 1975; Nurse and Thuriaux, 1977). These are not blocked in the cell cycle and proceed through it with the same generation time as the wild type. But they are much smaller at all stages of the cycle (about half the protein and RNA content of wild type) and because of this and of their country of origin, they have been christened *wee* mutants. So far, this small phenotype has been shown in mutants of two independent genes, *wee* 1 and *wee* 2. The first mutant isolated, *wee* 1-50 (originally named *cdc* 9-50), is TS and exhibits its mutant phenotype at the restrictive temperature of 35°C. At the permissive temperature of 25°C, it is only slightly smaller than wild type.

A TS mutation of cell size at division is a powerful tool for studying the mechanisms of size control. Let us assume that the mutation affects the sizer so that it triggers division at the *"wee"* size rather than the larger normal size. If the sizer operates near nuclear division, the effect of shifting up the temperature from the normal to the restrictive should be a rapid decrease in the size of dividing cells in an asynchronous culture and an acceleration of the larger cells through G2 and into division. This will produce a semi-synchronous burst of nuclear division. If, on the other hand, the sizer operates earlier in the cycle and is followed by a timer, there should be delay equal to the timer period before size at division changes. The results of Nurse (1975) show that the first of these alternatives is what happens and support the concept of a control operating near the

time of nuclear division. It is possible, however, that the initial assumption is incorrect and that the *wee* mutation shortens the timer rather than affecting the sizer. This alternative can be examined by the use of shifts in nutrients.

Fantes and Nurse (1977) have shown that cell size alters when the growth rate is changed by using different nutrients. In general, cell size diminishes as growth rate diminishes and cycle time increases. This also occurs in bacteria and here there is a neat explanation in terms of a sizer followed by a timer (Donachie et al., 1973). The sizer initiates DNA replication at a constant size irrespective of growth rate. During the subsequent timer period, however, cells in a poor medium grow less than cells in a rich medium and therefore divide at a smaller size. Some of the evidence comes from shift-up experiments when cells are transferred from poor to rich medium, and from the reverse shift-down situation. When these experiments are done with *S. pombe*, the results are different from those with bacteria and they are not consistent with the bacterial model. The arguments are analogous to those used for the temperature shift experiments with *wee* 1-50. After a shift-down, cells are accelerated through G2 and into nuclear division, and size at division starts to fall abruptly shortly after the change. The pattern is broadly similar to what occurs in the *wee* 1-50 shift, and in this case there is no genetic lesion. After a shift-up, there is a rapid inhibition of nuclear division followed a little later by a plateau in cell number and then subsequently by a rapid rise in number and a sharp increase in division size. This is to be expected from a sizer at nuclear division that is reset by the nutrient change to operate at a larger size.

The combined evidence from the size mutants and the nutrient shifts argues strongly for a sizer operating at the time of nuclear division in the wild type and against the existence of a timer. A necessary corollary, however, is that the size has to be modulated by the nutrient conditions, and it has to be admitted that this lacks the elegant simplicity of the bacterial model. It is also unclear what control is exercised over division in the size mutants at the restrictive temperature.

The next stage is to try to identify the components of the sizer and to understand how it triggers division. We have not got very far in this but there are some clues in the genetic analysis of the *wee* genes (Nurse and Thuriaux, unpublished results). A further search for small mutant cells has produced 37 mutants alleles (independently isolated) of the *wee* 1 gene. Only a few of these are TS, but all of them produce small cells of about the same size. This suggests that the *wee* 1 gene product is inactivated in the mutants and that its normal function in the wild type is inhibitory and restrains the sizer from operating until the normal division size has been reached. In marked contrast, only one mutant of *wee* 2 has been found. This mutant also has an interesting and significant relation to one of the cell cycle genes *cdc* 2 whose gene product is required for nuclear division (Nurse et al., 1976). *Wee* 2 maps very close to or within *cdc* 2. This suggests that the *wee* 2/*cdc* 2 gene has the complex function of both controlling division and generating a product needed for division. One simple model would be to have the *wee* 1 gene product binding reversibly to the *wee* 2/*cdc* 2 gene product and inactivating it. The *wee* 1 product would be diluted out by growth and would eventually release sufficient of the *wee* 2/*cdc* 2 product to initiate nuclear division. *Wee* 2 would be a rare mutation that would decrease the binding but still allow the product to initiate division, whereas the other mutants of *cdc* 2 (eight TS mutants have been isolated) would stop initiation. Nutrient

modulation would come in through an alteration of the binding. I must stress that this is only a very provisional model and it may have become outmoded before this article is published. It does not fit all the facts and it assumes a connection between *wee* 1 and *wee* 2 that is not yet clarified; but it is an illustration of the kinds of models that we are considering.

IV. Size and DNA Synthesis

Sizers have been considered so far in their relation to nuclear division but one can ask whether the same type of control also operates in initiating DNA synthesis. The question arose originally because of the situation in *wee* 1-50 (Nurse, 1975). Although the cycle time of the small cells of this mutant at the restrictive temperature is the same as wild type, the position of the S period is not. The S period is centered at 0.3 of the cycle, as compared to 0.0 in wild type. G1 is therefore longer than in wild type and G2 is shorter. Two alternative explanations are either that the *wee* 1 gene has a pleiotropic effect and alters the time of DNA initiation as well as cell size at division or that another control becomes operative. The evidence of Nurse and Thuriaux (1977) suggests that the latter explanation is more likely and that a size control is involved. In the small cells of *wee* 1-50 at the restrictive temperature, DNA synthesis starts when the cell size reaches a value of 6-7 pg protein/cell. If a sizer controls DNA initiation and is set to operate at this size (i.e., this is the minimum size for initiation) then it should also operate in other types of small cells that are generated in the wild type by methods that are different from those that result in the expression of the *wee* 1 mutant phenotype. This is in fact what happens. Small cells can be made by three different procedures: (1) germinating spores; (2) reinoculation after nitrogen starvation; and (3) expression of the *wee* 2 gene. In all cases, the S period takes place at 6.0-7.5 pg protein/cell.

This suggests a sizer control on DNA synthesis, but there remains the problem of the S period in the normal wild-type situation that occurs at a much larger size of about 13 pg protein/cell. Here we can invoke a second type of control. The signal from the sizer has already been given but DNA synthesis does not occur until nuclear division has been completed. This argues a dependency relation that is borne out from the study of the TS conditional cycle mutants. The presence of a G1 shows that there may be an irreducible minimum time for the preparation of the S period, but recent evidence mentioned in Section I indicates that the G1 may be very short indeed.

We are left then with two modes of control of DNA initiation. There is a sizer that operates in small cells, but this becomes cryptic in the normal wild type and the S period then takes place as soon as is possible after nuclear division.

V. Imprecision

If there were a natural variation of growth rate between individual cells and an accurate sizer triggering division, one would expect all cells to divide at the same size but after varying cycle times. There is some evidence that size is less variable than cycle time. Fantes (1977) found a coefficient of variation of 6.6-7.8 % for length at division and of 13.7-14.0 % for cycle time. Earlier measurements with different strains and growth conditions bear this out [coefficients of division length of 7.9-9.1 (Mitchison and Creanor, 1971b) and of cycle time of 9.7-17.8 (Mitchison, 1975; Faed, 1959; Gill, 1965)]. Even so, there is a marked variation of size at division and this persists during the growth of a culture, so the sizer appears to be

imprecise or sloppy. However, this statement must be a guarded one, since "size" is not itself a precise term. It should not have escaped the reader that I have loosely equated it with protein content, mass, volume, and length. For models that depend on achieving a critical concentration of an inhibitor or an activator, cytoplasmic volume is probably the best definition of size. Although cell length gives a rough measure of cytoplasmic volume in *S. pombe*, it does not give a precise one. A strict transformation of length into cytoplasmic volume would have to take into account the wall thickness, the rounded ends, the nuclear volume, and, most important, cell diameter, which does show changes during the cycle (Johnson and Lu, 1975). It is therefore conceivable, though not probable, that cytoplasmic volume at division is much more accurately controlled than length at division. Since the mechanism of the sizer is not clear, it is not worth discussing the reasons for its imprecision, though it is obvious that an inhibitor-dilution model would not be precise if only a small number of molecules were produced at each burst of synthesis (Sompayrac and Malløe, 1973).

It is not inappropriate to consider here the variation in the phases of the DNA cycle (G1, S, and G2). I suggested in Section VII that there may be a minimum length for G1, and there may also be a minimum length for G2. These two, together with S, would give the minimum cycle time described in Section II. It is, however, clear that G1 can be extended and that G2 can be both extended and shortened from its normal time, and proportion of the cycle (0.70). There is a lengthened G1 in *wee* 1-50 at the restrictive temperature (Nurse, 1975) and after temporary inhibition of DNA synthesis by deoxyadenosine (Mitchison and Creanor, 1971b). G2 is shortened in these two situations, and it can be lengthened after spore germination or after recovery from nitrogen starvation. What is not clear is how the normal variation in cycle time is distributed among the three phases, though it is unlikely to be concentrated in the short G1.

VI. Growth and Division

DNA synthesis, nuclear division, and cell division appear to be a dependent sequence of events. Each event does not occur unless the preceding event has been completed. Growth (the synthesis of most macromolecules) does not lie in this sequence since it has been known for many years that it will continue when DNA synthesis is blocked (e.g., Swann, 1957). I have formalised this in terms of two sequences, the growth cycle and the DNA-division or DD cycle, which are normally coupled but can be dissociated (Mitchison, 1971; 1975). Growth continues in *S. pombe* after the DD cycle has been blocked either by chemical inhibitors (Mitchison and Creanor, 1971b) or in TS cycle mutants (Nurse et al., 1976).

The two types of sizers that have been outlined for nuclear division and for DNA synthesis are controls that are exerted on the DD cycle by the growth cycle. Growth could be a smooth exponential increase limited by nutrients and the sizers would be sufficient to ensure that the periodic events of the DD cycle occurred at the right time. There would be no need to have any control working in the reverse direction from the DD cycle onto growth.

Growth in *S. pombe*, however, is not a smooth exponential process in many parameters, and this raises the question of whether there are periodic controls analogous to the sizers. Growth control is not strictly relevant to the theme of this Workshop, so I will only sketch the outlines of a picture that is far from clear at the moment. We thought some years ago that a number of enzymes were synthesised

periodically, as is DNA. We were misled by perturbations induced in synchronous cultures and it now seems that 18 out of 19 enzymes examined are synthesised continuously (Mitchison, 1977b). Other parameters of growth also increase continuously but careful examination shows that this increase is not exponential. Instead there is an increase at a constant rate (linear growth) until a point once per cycle where the rate doubles. This pattern is shown by total dry mass (Mitchison, 1957), three enzymes (Mitchison and Creanor, 1969), ribosomal protein and total RNA (Wain and Staatz, 1973), messenger and ribosomal RNA (Fraser and Moreno, 1976), and CO_2 evolution in minimal medium (Creanor, manuscript in preparation). One possible control mechanism is a gene-dosage control. When the genes double during the short S period, the rate of production of messenger RNA doubles. If the amount of messenger RNA is rate-limiting for protein synthesis, the rate of protein synthesis should also double after a time lag (Fraser and Moreno, 1976). This would be a control exerted by the DD cycle on the growth cycle.

Gene dosage, however, is not an adequate explanation for the results on CO_2 evolution. The linear pattern, with a rate change once a generation time, continues after the DD cycle has been blocked by inhibitors of DNA synthesis and of nuclear division. The cells do not divide but they do continue to grow and so become abnormally large. The control cannot therefore come directly from the DD cycle. Instead, and tentatively, I would suggest that there is another sizer that operates on growth or on some components of growth and causes rate doublings. This is a situation where the growth cycle would be self-regulating, though it is not impossible that one of the DD cycle sizers could also operate on growth. A single mechanism, for example, could trigger both nuclear division and a rate change in growth, and would still be effective on growth when its effect on nuclear division had been blocked by an inhibitor. This would be an "independent single timer (IST) sequence" (Mitchison, 1974).

VII. Principles

It may be helpful to illustrate the controls that have been discussed in Figure 1 and also set down a list of principles about the control of the cell cyle in *S. pombe*. I must emphasise that these are not laws or Euclidean axioms but rather a set of working hypotheses, and very much subject to change.

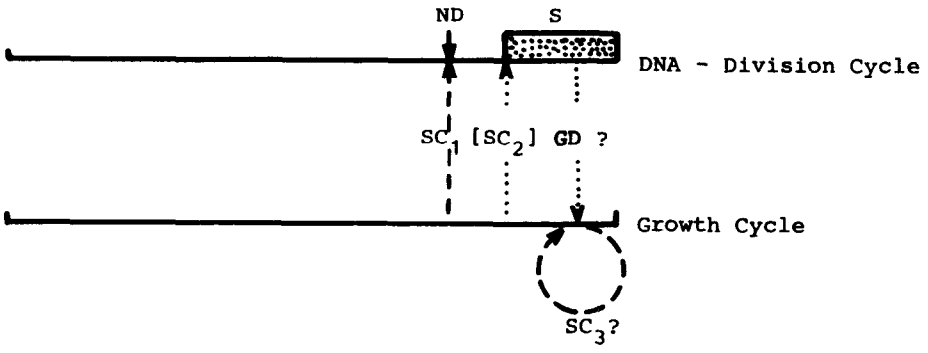

Fig. 1. Cell cycle controls in *Schizosaccharomyces pombe*. ND, nuclear division; S, period of DNA synthesis; SC_1, size control on nuclear division; SC_2, size control on DNA initiation (cryptic in normal wild-type cells); SC_3, size control on rate changes in growth; GD, gene dosage control on rate changes in growth

1. Nuclear division is initiated in normal cells when they reach a critical size.
2. The mechanism that measures cell size and controls division: (a) operates at or shortly before nuclear division; (b) is altered by nutrients; (c) is altered by mutations in two genes, *wee* 1 and 2; (d) probably involves both an inhibitor and an activator.
3. This mechanism ensures a homeostatic control of cell sizer by a control on division. Cell size is not inherited.
4. Cycle time is controlled by this mechanism, but there is a minimum cycle time that cannot be shortened.
5. A similar mechanism for measuring cell size also operates on the initiation of DNA synthesis in small cells. But it is cryptic in normal cells.
6. These mechanisms are imprecise, so cell size at division varies.
7. Neither G1 or G2 is invariant. Both can be extended and G2 can be shortened.
8. Growth may be controlled either by gene dosage or by a size-measuring mechanism.

C. Discussion

The suggestion that nuclear division is triggered by attaining a critical cell size is by no means novel. It was put forward by Hertwig (1908) at the beginning of this century and it has been discussed many times since then (e.g., Swann, 1957; Mazia, 1961). Direct experimental evidence, however, is meagre. Prescott (1956), confirming earlier work of Hartmann (1928), showed that division in *Amoeba* could be stopped for many days by periodic amputations of cytoplasm. The implication is that the cells did not divide because they were never allowed to reach the critical size. Growth continued in the amputated cells but at a slower rate than normal. Prescott also showed that small and large daughter cells (generated by experimental treatment) divided at the same final size after cycle times that were inversely proportional to size. The *Amoeba* experiments are important and would be worth repeating in greater detail. Although the amputation results suggest a sizer operating during the long G2, they do not define exactly when it operates. A timer could be running during the period of several hours before mitosis when there is no growth in size in this organism.

The regulation of size by altering cycle time has been shown in a general way in several cell systems other than *S. pombe*. Mammalian cells continue to grow after division has been blocked by DNA synthesis inhibitors ("unbalanced growth"). When released from the block, the subsequent cycle is short (Galavazi and Bootsma, 1966) and cell size reverts to normal. The same happens in *Tetrahymena* after repetitive heat shocks (Zeuthen and Rasmussen, 1972). There is an equivalent phenomenon in *Physarum* and this has been analysed in detail in two interesting recent papers by Sudbery and Grant (1975; 1976), which follow the effects in subsequent cycles after ultraviolet irradiation and inhibitor treatments. The analysis is in terms of mechanisms regulating the ratio of DNA/total protein and there is no distinction between the regulation of mitosis and of the initiation of DNA synthesis since these two events are nearly coincident in *Physarum*, in which, like *Amoeba*, there is no G1. Sudbery and Grant conclude that their data do not fit many of the models considered in the earlier paper by Fantes et al. (1975) but that they are consistent with two of them. One is an unstable inhibitor of mitosis produced at a rate proportional to the amount of DNA. There is rapid turnover, so an equilibrium amount is reached rapidly. The inhibitor concentration is reduced by growth and dilution until a level is reached that

triggers mitosis. This principle was first suggested by Yčas et al. (1965). The second model is a structural one that "counts" molecules. Sub-units are produced at a rate proportional to mass and are bound (reversibly) to sites to produce a "structure". When this structure is completed, mitosis is triggered. The units are not destroyed after mitosis but new sites are formed proportional to the amount of new DNA. This model is almost identical to one suggested earlier for *Physarum* by Sachsenmaier et al. (1972). It also has some similarities to the earlier model of "division proteins" suggested by Zeuthen and his colleagues for *Tetrahymena* (Zeuthen and Rasmussen, 1972). These two models fit the data from the experiments of Sudbery and Grant and also the important fusion experiments in *Physarum*, which show that a mitotic activator (or inhibitor) must be present in the cytoplasm (e.g., Rusch et al., 1966, Chin et al., 1972). However, as Sudbery and Grant (1975) point out, the *Amoeba* amputation experiment is much more easily explained by the unstable inhibitor model. This is also true of the experiments of Frazier (1973) in which DNA synthesis in *Stentor* is initiated prematurely by a decrease in the nuclear/cytoplasmic ratio through adding cytoplasm or removing parts of the nucleus.

The results of the *S. pombe* experiments do not provide definitive evidence for or against these models, but there are two points from our results that are relevant to the present dialogue on models: 1) The genetic evidence on the *wee* 1 locus suggests that there is at least one important inhibitory or negative control, and 2) the acceleration of cells through G2 and into nuclear division, which occurs after a temperature shift in *wee* 1-50 and after a shift-down in nutrients, argues against a structural model (Fantes and Nurse, 1977). If a mitotic structure has to be completed, not only would the rate of synthesis of sub-units have to increase but also this increase would have to be a transient one that occurred only for a short time after the shift. This is possible but unlikely.

The principle of a minimum cycle time that emerges from the results with abnormally large cells of *S. pombe* also applies to other cells in similar situations. Fantes et al. (1975) list in their Table 2 minimum cycle times for *Physarum, Saccharomyces, Amoeba,* and *Tetrahymena.* These times are between 50 and 67 % of the normal cycle time, but the authors suggest that with different growth rates the minimum cycle time is likely to be constant in time rather than being a constant proportion of the normal cycle. This is borne out in *Physarum* where Sudbery and Grant (1975) found a minimum cycle time of 6 h in a medium where the normal time was 8-9 h, and of 7 h in another poorer medium where the normal time was 16-17 h. Sudbery and Grant (1976) also found a cycle time of 7 h when there was no growth (in plasmodia that were irradiated and starved). Whatever therefore happens to growth, there is a "parallel pathway" involving the "preparations for division" that the cell has to complete before the trigger for mitosis can be pulled. These phrases, incidentally, all come from the classic work on mitosis by Mazia (1961). The minimum cycle time must include the S period and those parts of G2 (and G1 where it exists) that are incompressible. This perhaps does not say very much since we do not know what events are incompressible and, as we shall see below, the S period can be drastically shortened in early embryos. I have discussed the concept of parallel pathways elsewhere (Mitchison, 1974) and have mentioned one of the places where it applies in bacterial division (Donachie et al., 1973).

It would be cowardly to finish a discussion on size control at division without mentioning the embarrassing subject of eggs and early embryos. The cells here divide without growing, though some protein

synthesis is necessary. As a result, they halve their size at each division, and size controls of the kind that have been discussed cannot be in operation. The easiest way out is to assume that they are running on the minimum cycle time, but this raises the problem that this time is much shorter than any found in adult cells. The S period in amphibian embryos is about 100 times shorter than that in adults (Mitchison, 1971). If the S period is short in embryos, why cannot it be compressed in adult cells? There are, as yet, no solutions to these problems, and it will need careful examination of embryos to determine when, if at all, a size control starts to operate. On present evidence, it does not seem as though there is an abrupt change from one type of control to another since Graham and Morgan (1966) have shown a steady increase in G1, S, and G2 from the 4th to at least the 18th hour of development in *Xenopus* endoderm cells. There is, however, an interesting transition in the axolotl blastula, which is worth further study. Signoret and Lefresne (1971) have shown that the early cell cycles are synchronous, short and relatively constant (coefficient of variation of about 4 %). After the tenth cycle, however, the cycles become asynchronous, longer, and more variable (coefficient of variation of 12-20 %). The transition is quite sharp and it is tempting to feel that a new pattern of cycle control is appearing at that point.

Size control for DNA initiation is well established in *Escherichia coli*, though it is still in dispute whether the mechanism involves an activator or an inhibitor (Donachie, 1968; Pritchard et al., 1969). What is clear is that new rounds of replication begin when the "initiation mass" reaches a critical value. This mass is independent of growth rate. Thereafter, there is a constant time (about 1 h) until division. This single size control regulates both DNA synthesis and division size. Initiation mass is not simply total cell mass but cell mass divided by the number of chromosome origins. Some analogous size control mechanism must presumably operate in eukaryotes since cell size usually increases with the degree of ploidy (Yčas et al., 1965). Diploid cells of *S. pombe*, for example, are nearly twice the size (protein content) of the normal haploid cells.

The situation in eukaryotes is less clear. A size control for DNA synthesis in *Tetrahymena* has been suggested by Worthington et al. (1976), and, as mentioned above, for *Stentor* by Frazier (1973). *Physarum* and *Amoeba* do not normally have a G1 and it has not been possible to generate one artificially. A size control for DNA initiation cannot therefore be separated from a size control for division. Mammalian cells usually have a G1 that is more variable in length than S + G2 (Prescott, 1976). It is attractive therefore to suggest that the main control point is at DNA initiation and that there is a constant timer running thereafter, as in *E. coli*. Could this control be a sizer? The evidence in favour is that the cell mass at DNA initiation is less variable than at mitosis (Killander and Zetterberg, 1965a, b). The evidence against is that the length of G1 is not correlated with cell size in certain experimental situations (Fox and Pardee, 1970; Fournier and Pardee, 1975). This evidence has been discussed by Nurse and Thuriaux (1977), and they suggest that the conflict could be resolved if the situation in mammalian cells resembled that in *S. pombe*. If the cells were small, there would be a size control. If they were large, the size control would be cryptic and the length of G1 would be reduced to its incompressible minimum.

S. pombe appears to have two size controls that can operate on the DD cycle whereas most of the models only involve one size control. Since, however, cytoplasmic inducers are components of the models, it is sig-

nificant that there is evidence from mammalian cell fusion experiments that there are two inducers, one for DNA synthesis (Johnson and Rao, 1971) and another for mitosis (Rao et al., 1975).

The controls of both cycle time and division size are imprecise in *S. pombe*. The same is true in other cell systems. The coefficient of variation of cycle time in cultured mammalian cells ranges from 9 to 26 % with no obvious relation to the mean cycle time (data from Dawson et al., 1965; also Killander and Zetterberg, 1965a; Miyamoto et al., 1973). This range is similar to that in *S. pombe*. It is sometimes believed that the cycles of cultured mammalian cells are more variable than those of bacteria, which are thought to have a deterministic control mechanism. This is not so. Schaechter et al., (1962) found coefficients of variation of cycle time from 15 to 21 % in three bacteria. They also found, as in *S. pombe*, that the coefficients of variation of length at division were smaller - 8.5 to 13 %. The reason for these variations is not known, though those for cycle time are an essential component of one controversial theory for the control of mammalian cell cycles (Smith and Martin, 1974).

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