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REVIEW ARTICLE

Biochemistry of the cell cycle

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Cellular organization: temporal aspects

Those processes that constitute the cell cycle include all the biosynthetic mechanisms involved in growth, the provision of energy, the development of extending membranes and surface structures, the genesis of new organelles, and the mechanics of segregation of components at cell division. As well as elucidating the stages of the processes themselves, the spatial and temporal control of this developmental cycle needs to be understood. Were the synthesis of new constituents to occur continuously from one cell division to the next, then the cell would be biochemically identical at all times in its life time: individual molecular components would be always at a fixed stoichiometry. Classically, biochemistry has been studied as if this were the case. Thus most studies of growth, even those performed with micro-organisms, neglect entirely the concept of discontinuity: the growth of individual cells and increase in cell numbers are lumped together in a measurement of increased optical density or dry weight ('biomass'). Such global measurements ignore the differences between individuals: information obtained from the population gives only time-averaged values over an interval equal to the cell division time. The fine details of sequenced events are blurred and lost (Edwards, 1981; Lloyd *et al.*, 1982*b*).

Methods for study of the cell cycle

The time span of the cell division cycle may be as short as 9.8 min in the marine bacterium *Vibrio natriegens* (Eagon, 1962). It may be about 24 h in proliferating animal or plant tissues or in micro-organisms in natural ecosystems. In some cases, a quiescent stage lasting years may eventually lead to a normal cycling state or a malignant tumour growth (Baserga, 1985; Epifanova & Polunovs, 1986). The importance of polypeptide growth factors and their receptors in growth control has been reviewed recently (James & Bradshaw, 1984). Thus, even in the shortest of cell cycle times, metabolic events will have occurred by their thousands, those membrane-associated phenomena not limited by diffusion may be counted in millions, and vibrational events within proteins in hundreds of millions (Fig. 1). Although strict ordering of processes and events on a nanosecond time scale may not directly determine the course of the cell cycle, all these subsystems contribute in the hierarchy of temporal controls necessary for progression from one cell division to the next.

On a longer time scale, the cell division cycle is itself often dominated by physiological adaptation to tidal or circadian controls (Sweeney, 1982), just two examples of evolutionary matching to geophysical cycles. Until recently the biochemist's preoccupation with the structural hierarchy of living systems had sometimes led to a neglect of temporal relationships. The dynamic perspectives of sub-molecular motions (Williams, 1981), metabolism (Reich & Sel'kov, 1981), and growth processes (Lloyd *et al.*, 1982*b*) are now becoming clear. Functions and structures differ only in the time constants of their component reactions. To study the rich complexities of function and formation that occur during cellular growth and division, it is necessary to examine the processes either in single cells or in a population composed of individuals all at the same stage of cell cycle progression. The former approach is constrained by current limitations of sensitivity and applicability of non-invasive methods to single cells. Even so, many of the most fundamental questions must eventually be tackled at this level, and rapid advances (e.g. in digital scanning fluorescence microscopy techniques; Taylor *et al.*, 1986) suggest the imminent flourishing of single-cell research. The great attraction of this avenue is the emphasis that it will place on the *differences* between individual cells, and on the heterogeneity in a population even when cohorts of identical ages are taken. The extent of this natural variation in individual cells during their normal development merits investigation. In contrast, most current biochemical measurements made on a sampled population assume homogeneity; the expression of such measurements as a specific activity based on cell numbers implies a touching faith that an equivalent contribution is derived from each individual.

Apart from microscopy-based measurements there are other methods for examining the heterogeneity of cell populations. Thus, the use of flow cytometry and flow cytofluorimetry in biochemistry has become routine, especially in laboratories working with cultured mammalian cell lines (Bohmer, 1982). Most often used for cell cycle analysis by fluorimetric DNA measurement, these techniques now find varied application, especially where cellular heterogeneity can be resolved by using fluorescent monoclonal antibody staining.

The second more conventional approach to the study of the cell cycle is to prepare a synchronous culture, in which all the cells are in phase; the whole culture then behaves as a coherent population. Several practical solutions to the difficulty of synchronizing populations are available, and these methods yield sufficient amplification to allow conventional biochemical analyses. However, caution must be exercised, and it must be admitted that many of the published observations obtained by the use of synchronous cultures are controversial (Lloyd *et al.*, 1982*b*). This is because many investigators have failed to take adequate precautions to enable distinction between those phenomena which are real cell cycle events from those artifacts which result from the experimental procedures necessary to obtain synchrony. This problem is not confined to the data in the older literature which relied on induction synchrony. It is now realized that it is difficult (and perhaps not

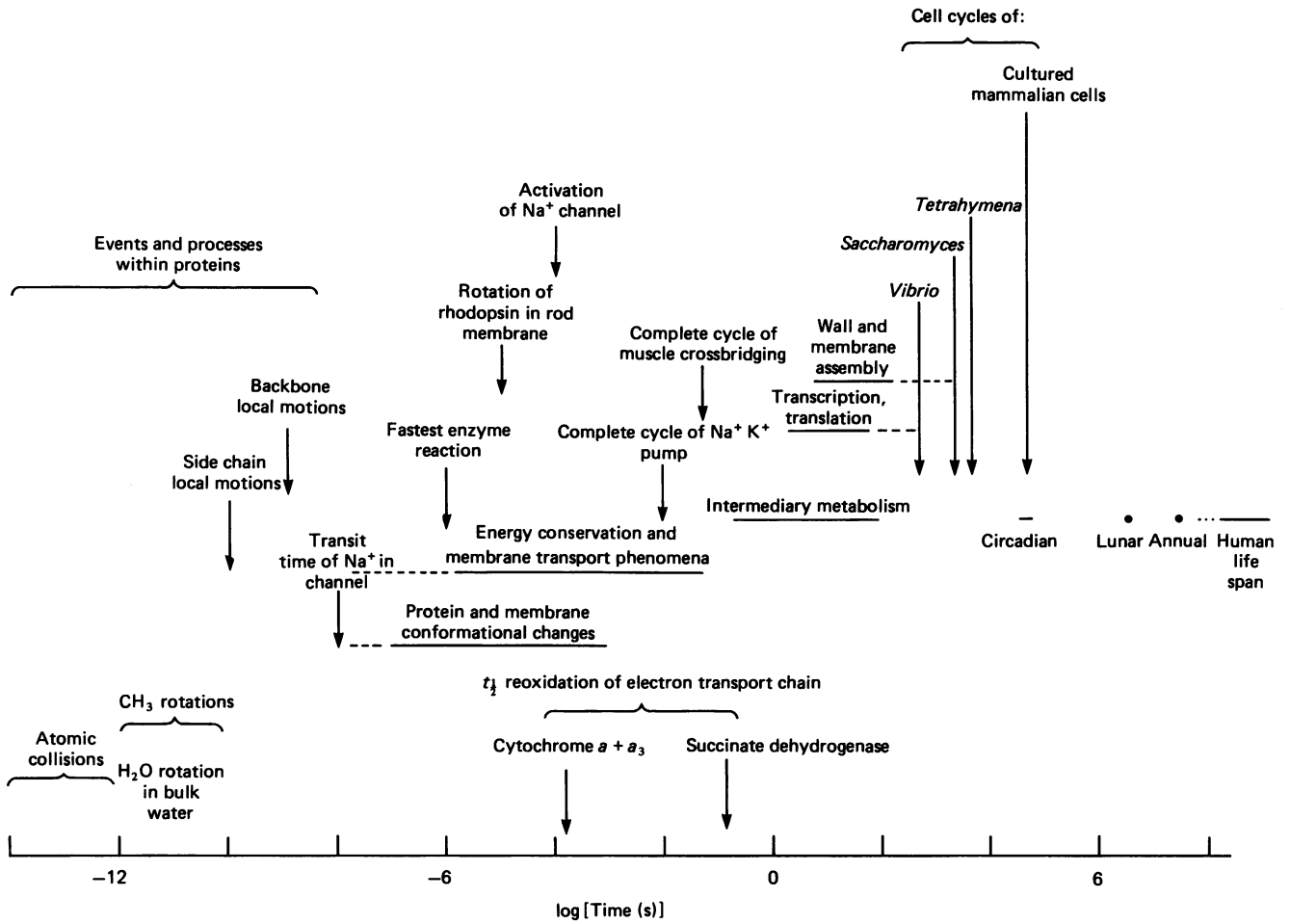

Fig. 1. The time domains of living systems

possible) to avoid perturbative influences completely; rather few methods for the preparation of synchronous cultures can claim to be minimally perturbing, and even for these it is always necessary to present as controls data from identically treated asynchronous cultures. The methods of choice involve selection of similarly-sized organisms by a short, low-speed centrifugation (e.g. $12\,g_{av.}$, 1 min) of the culture in tubes (Chagla & Griffiths, 1978) or in a continuous-flow rotor (Lloyd *et al.*, 1975; Edwards & Jones, 1977; Lloyd, 1983). These simple methods use minimal physical disturbance, are faster and simpler than others currently employed (see Lloyd *et al.*, 1982*b*, for experimental details), and have the other great advantage that organisms never lose sight of their growth medium. They thus never become transiently deprived of nutrients or O_2.

Criteria for assessment of the success of a cell synchrony procedure include the following.

(i) Measurement of a synchrony index, e.g. that of Blumenthal & Zahler (1962) given by:

$$F = (N_t/N_0 - 2^{\alpha/\beta})$$

where N_t is the number of organisms after division is completed, N_0 the number of organisms before division, α is the time taken for the organisms to divide and β is the generation time (Fig. 2). For a culture showing instantaneous and exact doubling in cell numbers, the value of F would be unity, whereas for an exponentially growing culture its value would be zero. Values greater than 0.85 suggest that induction plays a role in synchronization; those between 0.6 and 0.85 are regarded as satisfactory for selection synchrony.

(ii) The cell cycle time of the synchronous culture should be similar to the mean generation time of the exponentially growing culture from which it was derived. Deviation indicates perturbation.

(iii) Events should be repeated in successive cycles.

Often the first cell cycle is distorted by the experimental procedure, especially over the first hour or so. Synchrony in successive cycles decays, mainly because of the natural dispersion of cell cycle times. Thus, even when population growth rate is constant, individual cells have widely differing cycle times, the coefficient of variation frequently being of the order of 15–20% (Brooks, 1985).

Where feasible, it is an excellent practice to compare results from different synchronizing techniques (e.g. Knacker *et al.*, 1985). In those few cases where this has been done, large discrepancies due to perturbation effects are usually revealed when induction methods have been employed. Even light–dark synchronized cell division commonly used for studies of the cell cycles of phototrophs (Adams *et al.*, 1984) must be regarded as an induction method, and separation of the events of the cell cycle from those of light–dark adaptation processes is not straightforward.

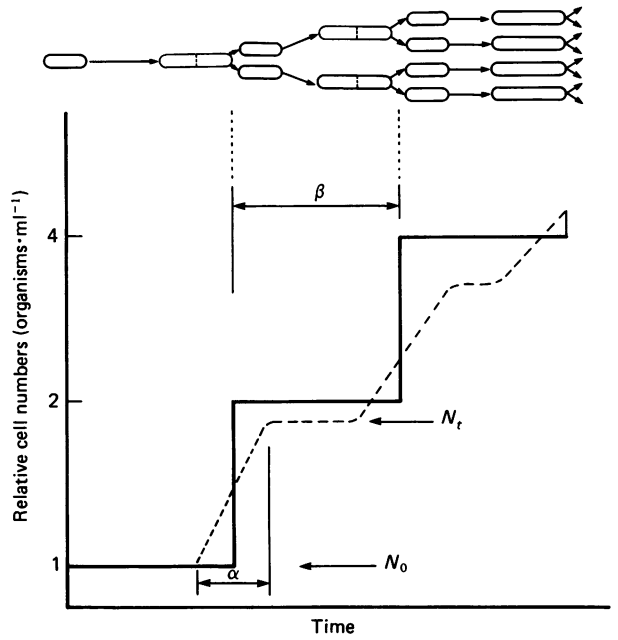

Fig. 2. Assessment of the degree of synchrony in a population of cells or organisms: the synchrony index of Blumenthal & Zahler (1962)

For explanation see the text.

Biochemical changes during the cell cycle

Table 1 shows some examples of changes in the levels of cell constituents or processes during the cell cycle. These are taken from reports of the last year. For a summary of earlier work see Lloyd *et al.* (1982*b*).

Cell cycle mutants

Isolation of cell cycle mutants and their characterization represents a major part of current endeavours; as most of these mutations are lethal they must be isolated as conditional (usually heat-sensitive) mutants. Products of thousands of genes are required for the normal growth and reproduction of cells, but the most interesting examples are those that control or execute specific cell cycle processes. Hartwell's (1974) definition which confines interest to those mutations that result in arrest of the cell cycle at a specific phase may however be unduly restrictive. There are mutants that lack a major portion of the cell cycle (Liskay, 1977) and some that arrest at two distinct stages. A very successful and complete investigation of the yeast *cdc* mutants has enabled the elucidation of the order and interdependence of cell cycle processes (Hartwell, 1978). Those cell cycle mutants which divide at a size very different from that at which the wild type divides [*whi* mutants of *Saccharomyces cerevisiae* (Sudbery *et al.*, 1980) and *wee* mutants of *Schizosaccharomyces pombe* (Nurse, 1981)] have proved valuable systems for probing 'sizer' control mechanisms.

Despite the burgeoning interest in mammalian cell cycle mutants (Table 2) few of the lesions responsible are biochemically defined. Exceptions to this are seen for ts A159, a topoisomerase II mutant (Colwill & Sheinin, 1982, 1983), and for ts 85 (Mita *et al.*, 1980; Matsumoto *et al.*, 1983). Surprisingly this latter mutant which arrests on G_2 is defective in the ubiquitin pathway. This discovery points to an unexpectedly crucial role of this pathway at a particular stage of the G_2 phase.

In the case of the yeast cell cycle, examples where specific biochemical lesions have been pinpointed are given in Table 3. It is remarkable that some of these mutations should give phenotypes falling within the definition of a cell cycle mutation, e.g. *cdc19*, which has a temperature-sensitive pyruvate kinase (Kawasaki, 1979). It may be that this enzyme, apart from its key glycolytic role in energy generation, has a second as yet unrecognized function. The application of ^{13}C-n.m.r. spectroscopy *in vivo* presents a powerful approach to the rapid and unequivocal localization of metabolic defects (Gilles & Benoit, 1983); similar studies using phosphorus or nitrogen n.m.r. offer other rapid diagnoses. Cloning of CDC genes affords another method of identification, e.g. the primary structure homology between the CDC28 gene product of *S. cerevisiae* and bovine cyclic AMP-dependent protein kinase (Lorincz & Reed, 1984).

Cell cycle mutations in several prokaryotes have been

Table 1. Constituents and processes during the cell cycle: some recent (1986) studies

Constituent process	Organism(s)	Reference
DNA replication	Eukaryotes	Campbell (1986)
DNA ligase expression	*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*	White *et al.* (1986)
Human histone gene expression	Mouse cell	Green *et al.* (1986)
Histone and tubulin gene expression	*Physarum polycephalum*	Carrino & Laffler (1986), Laffler & Carrino (1986)
α-Tubulin genes	*Schizosaccharomyces pombe*	Adachi *et al.* (1986)
Microtubule dynamics	PTK-2 cells	Debraban *et al.* (1986)
Diadenosine tetraphosphate, adenosine tetraphosphoguanosine	*Physarum polycephalum*	Garrison *et al.* (1986)
Calmodulin levels	HL-60	Yen *et al.* (1986)
Spindle pole body duplication	*Saccharomyces cerevisiae*	Baum *et al.* (1986)
Protein phosphorylation	*Saccharomyces cerevisiae*	Tripp *et al.* (1986)
Protein modification	Early mouse embryo	Howlett (1986)
Chromatophore membranes	*Rhodospirillum rubrum*	Myers & Collins (1986)

Table 2. Cell cycle mutants derived from mammalian cell lines

Organism	Cell line	Mutant	Arrest point	Reference
Chinese hamster	Wg1A	K18, K27, K33, 4/3, 4/2, 3/1	Late G_1; entry into S	Melero (1979)
	Wg1A	*ts*K/34C	G_1	Tenner *et al.* (1977), Otsuka & Scheffler (1978), Landy-Otsuka & Scheffler (1978)
	Wg1A	H3.5	G_1	Landy-Otsuka & Scheffler (1980)
	CCL39	BF113	G_1 after one cycle	Scheffler & Buttin (1973)
	CHO	MS1-1	Cytokinesis	Thompson & Lindl (1976)
	CHO	*ts*C8	DNA synthesis	McCracken (1982)
	CHO	*ts*D, *ts*224 and others	Various	Okinaka & Barnhart (1978)
	CHO*	cs^4-D3	G_0/G_1 (reversible)	Crane & Thomas (1976), Crane *et al.* (1977), Berger *et al.* (1979), Lomniczi *et al.* (1977)
	CHO*	CR^RE5	G_1	Ling (1977)
	GM75	TS11	Cytokinesis	Hatzfeld & Buttin (1975)
	D_6 (pseudo-diploid)	*ts*-1	G_1	Hori (1977)
	CCL61	1129 and 1132	Reversibly in G_1 and less reversibly in G_2	Ohlsson-Wilhelm *et al.* (1976 1979)
	CHO-K_1	11C3	Late S/G_2	Marunouchi & Nakano (1980)
	CHL-V79	G_1^+-4, G_1^+-5	G_1	Liskay & Prescott (1978)
	CHO	*ts*AMA^R-1	G_1	Ingles (1978)
Syrian hamster	BHK	NW1	Cytokinesis	Smith & Wigglesworth (1972)
	BHK	dna$^-$*ts*BN2	DNA synthesis	Eilen *et al.* (1980)
	BHK	*ts*BN75	G_2 and S	Nishimoto *et al.* (1980)
	BHK	*ts*422E	G_2 and mitosis	Mora *et al.* (1980)
	BHK21/13	*ts*Af8, *ts*11, *ts*13	Early to mid G_1	Meiss & Basilico (1972), Burstin *et al.* (1974), Burstin & Basilico (1975), Kane *et al.* (1976), Ming *et al.* (1976), Talavera *et al.* (1976), Liskay & Meiss (1977), Talavera & Basilico (1977), Chang & Baserga (1977), Moser & Meiss (1975, 1977) Floros *et al.* (1978), Rossini & Baserga (1978), Jonak & Baserga (1979), Rossini *et al.* (1979, 1980)
	BHK211	*ts*HJ4	Late G_1, early S function	Talavera & Basilico (1977)
	BHK21	*ts*BTN-1	S?	Nishimoto & Basilico (1978),
		*ts*BN-2	G_1 and S	Yanagi *et al.* (1978),
		*ts*BN75	S	Nishimoto *et al.* (1980)
	BHK21	*ts*422E	Prior to division cells have 4*n* amount of DNA and very large nuclei	Toniolo *et al.* (1973), Mora *et al.* (1980)
Hamster	HM-1	*ts*-546	Mitosis (metaphase)	Wang (1974), Wang & Yin (1976)
	HM-1	*ts*-655	Mitosis (prophase)	Wang (1976)
	HM-1	*ts*694, *ts*559	G_1	Chen & Wang (1977)
	HM-1	*ts*-550c	G_1 and G_2	Chen & Wang (1977, 1982)
	HM-1	*ts*687	Mitosis	Wissinger & Wang (1978)
Mouse	CAK	B54	Late G_1	Liskay & Meiss (1977), Liskay (1974), Farber & Liskay (1974)
	L	*ts*A1S9	S	Thompson *et al.* (1970, 1971), Sheinin (1976*a*,*b*), Setterfield *et al.* (1978), Sheinin & Gutman (1977),

Table 2. (continued)

Organism	Cell line	Mutant	Arrest point	Reference
				Sheinin *et al.* (1978), Colwill & Sheinin (1982, 1983)
	L	*ts*c1	Late S or G_2	Setterfield *et al.* (1978), Sheinin & Gutman (1977), Thompson *et al.* (1971)
	Balb/3T3	A8, A83 and 5 others	G_1	Naha *et al.* (1975), Naha (1979*a*,*b*), Naha & Sorrentino (1980)
	Balb/c 3T3	*ts*-2	S	Slater & Ozer (1976)
	FM3A	*ts*85	Late S or G_2	Mita *et al.* (1980)
	FM3A	*ts*c1.B59	Cytokinesis	Yasuda *et al.* (1981), Nakamo *et al.* (1978)
	FM3A	*ts*T244	DNA synthesis	Tsai *et al.* (1979)
	FM3A	*ts*131b	DNA synthesis	Hyodo & Suzuti (1982)
Murine leukaemic	L51787	*ts*2	Mitosis and	Shiomi & Sato (1976, 1978),
		*ts*39	cytokinesis	Sato & Hama-Inaba (1978)
Rat	3Y1	*ts*D123	G_1	Zaitsu & Kimura (1984)
African green	BSC-1	*ts*3,*ts*5,*ts*9	S	Naha (1973)

* Cold-sensitive.

described: these provide fundamental insights into mechanisms of chromosome replication, growth and cell division (e.g. in *Escherichia coli*; Donachie, 1981) and of morphological differentiation (e.g. in *Caulobacter crescentus*; Shapiro, 1976). Amongst eukaryotic cells other than yeasts (and those of mammalian origins) special mention must be made of three systems. In *Aspergillus nidulans* the mitotic mutants isolated by Morris (1980) and coworkers identify the time structure of nuclear events and the interactions of nuclear envelope, spindle pole bodies, kinetochore and microtubules during chromosome segregation. In *Chlamydomonas reinhardii* genetic dissection of the stages of organelle development was pioneered by Howell (1974). Finally, the production of a wide variety of morphologically aberrant mutants of the ciliate protozoon *Tetrahymena pyriformis* (Frankel *et al.*, 1980) provides a system suitable for the investigation of the basic mechanisms of the generation of form. The mutant protozoa provide systems suitable for testing new hypotheses, e.g. the morphogenetic field theory (Goodwin, 1980) and the participating of the ion currents observed by Jaffe (1981). Further information on methods of isolation and characteristics of cell division cycle mutants is to be found in Lloyd *et al.* (1982*b*).

The control of cell division

In rapidly growing populations, cells progress from mitosis to S-phase and on to mitosis again with a regularity that has encouraged the idea of a cycle of events. Close to this idea is that of a biochemical oscillator mechanism, and it is not surprising that many proposals for cell cycle control are based on limit cycle models. Sel'kov (1970) suggested synthesis and degradation of thiols and disulphides, oxidation and reduction providing the control variables. Gilbert (1981) developed a theory based on this type of control system, and indeed as explained by Kauffman & Wille (1975), a limit cycle mechanism, in which mitosis is triggered when an active substance reaches a critical threshold concentration, can mimic many experimental observations. For instance the

Table 3. Biochemical lesions in *cdc* mutants in yeasts

Organism	Mutant	Defective enzyme	Reference
Saccharomyces cerevisiae	*cdc7*	Protein kinase	Patterson *et al.* (1986)
	cdc8	Thymidylate kinase	Jong *et al.* (1984), Sclafani & Fangman (1984)
	cdc9	DNA ligase	Johnston & Nasmyth (1978)
	cdc19	Pyruvate kinase	Kawasaki (1979)
	cdc21	Thymidylate synthetase	Game (1976)
	cdc30	Phosphoglucose isomerase	Dickinson & Williams (1987)
	cyr1	Adenylate cyclase	Matsumoto *et al.* (1983)
	bcy1	Cyclic AMP-dependent protein kinase (regulatory subunit)	Matsumoto *et al.* (1983)
Schizosaccharomyces pombe	*cdc2+*	Protein kinase	Simanis & Nurse (1986)
	cdc17	DNA ligase	Nasmyth (1977)
	cdc22	Nucleoside diphosphokinase	Dickinson (1981)

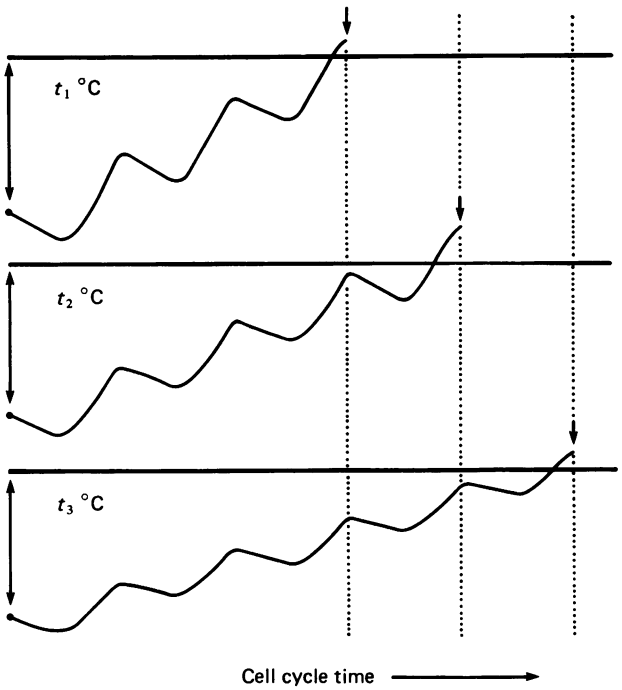

Fig. 3. Timing provided by the temperature-compensated period of the ultradian clock together with 'sizing' provided by a temperature-independent threshold concentration of some constituent may determine cell cycle time

This has discrete values; i.e., as temperature is decreased, cell cycle times increase by quantal increments (Lloyd & Kippert, 1987).

'set-backs' in cell cycle progression produced by heat shocks (Zeuthen & Scherbaum, 1954; Brewer & Rusch, 1968), or pulses of protein synthesis inhibitors, point to the assembly of a thermolabile protein-containing structure necessary for cell division. Whether the oscillator is a continuous one or is of the relaxation type is still debated (Tyson & Sachsenmaier, 1978).

Another viewpoint emphasizes the variability of cell cycles rather than their regularity (Brooks, 1985). The basis of the variability of the duration of the G_1 phase suggests that commitment to DNA replication and mitosis in mammalian cells might occur at random with constant probability per unit time (Cattaneo *et al.*, 1961; Burns & Tannock, 1970; Smith & Martin, 1973). Much of the evidence presented for this 'single transition-probability' model can be used to promote alternatives [e.g. the 'G_1 rate' model (Castor, 1980), the 'sloppy size control' model of Lord & Wheals (1984) or even the 'oscillator' model (Gilbert, 1982)].

Finally we have the 'dependent pathways' model in which one or more causal sequences finally join to give a closed loop of states. Order and interdependence of events in the cell cycle of *S. cerevisiae* clearly emerges from the work of Hartwell (1978). Identification of a key regulatory step in the G_1 phase ('start') mediated by the gene product of gene *cdc28* initiates the cell cycle provided that carbon and nitrogen sources, sulphur, phosphorus and required amino acids are all present (Hartwell, 1974; Johnston *et al.*, 1977; Shilo *et al.*, 1978) and the mating pheromone is absent (Bucking-Throm *et al.*, 1973). Nutritional shift-up experiments (Carter *et al.*, 1978) and a plethora of other data are consistent

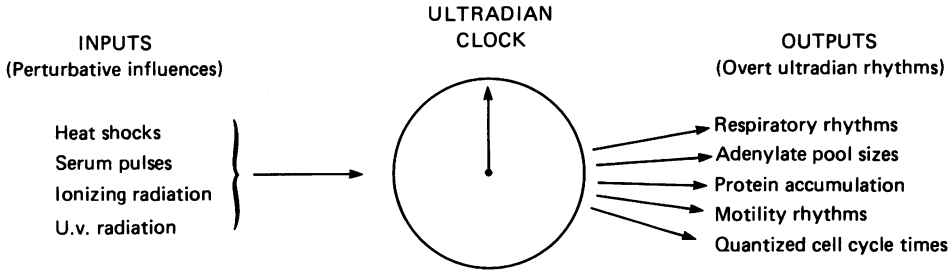

Fig. 4. Ultradian clock control of cellular processes

The period of the clock (generally within the range 0.5–4 h) differs from one organism to another.

Table 4. Some recent (1986) cell cycle studies with medical implications

Subject	Reference
Development of thermotolerance during hyperthermia	Holahan & Dewey (1986)
Factors controlling the B-cell cycle	Melchers & Andersson (1986)
Phorbol esters and interleukin-1 activation of T-cells	Davis & Lipsky (1986)
Interleukin-3 and cell cycle progression	Kelvin *et al.* (1986)
Cell cycle specific effects of anticancer drugs	Kimmel & Traganos (1986)
Bone marrow cell cycle kinetics and thymic peptides	Tucker *et al.* (1986)
Adenosine and the cell cycle of human lymphoid-cell lines	Vanderkaan *et al.* (1986)
Suppression of tumorigenicity by cell cycle control	Wille & Scott (1986)
Oestrogen stimulation and anti-oestrogen inhibition of human breast cancer cell lines	Lykkesfeldt *et al.* (1986)
Melanoma-associated antigen expression in human melanoma cell lines	Kameyama *et al.* (1986)
Regulation of epidermal growth factor receptors by glucocorticoids in HeLa cell lines	Fanger *et al.* (1986)

with the idea that initiation of the cell cycle at 'start' is followed by a constant time period before attainment of a critical size (mandatory for initiation of budding). Sizer and timer controls may involve genes (e.g. *weel* and *cdc2*) in *Schiz. pombe* in controls that monitor cell mass and determine mitotic timing (Nurse, 1981).

Attempts to reconcile these disparate models (Edmunds, 1984) of cell cycle controls may combine timing by a limit cycle oscillator with a size threshold (Shymko *et al.*, 1984). The weakness of many of the suggested mechanisms is that no definite molecular candidates are available (e.g. for the active 'mitogen'). Moreover, an oscillator is not necessarily a timer, e.g. if the period of the oscillator corresponds to the cell cycle time it will be temperature-dependent. All known timekeepers have built-in devices for temperature compensation of period. Outputs of the ultradian clock, the timer essential for internal synchronization of rapidly dividing organisms, and possibly the timing of cell division, have been observed as oscillating variables in lower eukaryotes (Edwards & Lloyd, 1978, 1980; Lloyd *et al.*, 1982*a*,*b*; Lloyd & Edwards, 1984, 1987). When combined with a temperature-independent threshold (Fig. 3), cell division timed by this clock predicts quantized cell cycle times experimentally observed in *Paramecium tetraurelia* (Lloyd & Kippert, 1987) The threshold in this model represents a critical concentration of a component necessary for the triggering of a specific cell cycle process (e.g. DNA replication, nuclear division or cell division). Many functions are coupled to the ultradian clock (Fig. 4); oscillating enzyme protein levels (Edwards *et al.*, 1982) lead to the conclusion that rates of protein turnover in rapidly growing cells have been consistently underestimated (Lloyd & Edwards, 1986).

Applications

The applied biology of the cell cycle, although outside the scope of this short Review, now sustains an extensive literature on account of its major significance in cancer chemotherapy, radiation therapy and hyperthermia. Fundamental biochemical discoveries both arise from these endeavours and contribute to them. A few representative examples taken from the many papers of the last year are given in Table 4.

REFERENCES

Adachi, Y., Toda, T., Niwa, O. & Yanagida, M. (1986) Mol. Cell Biol. **6**, 2168–2178

Adams, K. J., Weiler, C. S. & Edwards, L. N. (1984) in Cell Cycle Clocks (Edmunds, L. N., ed.), pp. 395–429, Marcel Dekker, New York

Baserga, R. (1985) The Biology of Cell Reproduction, Harvard University Press, Cambridge

Baum, P., Furlong, C. & Byers, B. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5512–5516

Berger, N. A., Petzold, S. J. & Berger, S. J. (1979) Biochim. Biophys. Acta **564**, 90–104

Blumenthal, L. K. & Zahler, S. A. (1962) Science **135**, 724

Bohmer, R. M. (1982) Prog. Histochem. **14**, 1–65

Brewer, E. N. & Rusch, H. P. (1968) Exp. Cell Res. **49**, 79–86

Brooks, R. F. (1985) in Temporal Order (Rensing, L. & Jaeger, N. I., eds.), pp. 304–314, Springer Verlag, Berlin

Bucking-Throm, E., Duntze, W., Hartwell, L. H. & Manney, T. R. (1973) Exp. Cell Res. **76**, 99–110

Burns, F. J. & Tannock, I. F. (1970) Cell Tissue Kinet. **3**, 321–334

Burstin, S. J. & Basilico, C. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 2540–2544

Burstin, S. J., Meiss, H. K. & Basilico, C. (1974) J. Gen. Physiol. **84**, 397–408

Campbell, J. L. (1986) Annu. Rev. Biochem. **55**, 733–771

Carrino, J. J. & Laffler, T. G. (1986) J. Cell Biol. **102**, 1666–1670

Carter, B. L. A., Lorincz, A. & Johnston, G. C. (1978) J. Gen. Microbiol. **106**, 221–225

Castor, L. N. (1980) Nature (London) **287**, 857–859

Cattaneo, S. M., Quastler, H. & Sherman, F. G. (1961) Nature (London) **190**, 923–924

Chagla, A. H. & Griffiths, A. J. (1978) J. Gen. Microbiol. **108**, 39–43

Chang, H. L. & Baserga, R. (1977) J. Gen. Physiol. **92**, 333–344

Chen, D. J. & Wang, R. J. (1977) J. Cell Biol. **75**, 199

Chen, D. J. & Wang, R. J. (1982) Som. Cell Genet. **8**, 653–666

Colwill, R. W. & Sheinin, R. (1982) Can. J. Biochem. **60**, 195–203

Colwill, R. W. & Sheinin, R. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 4644–4648

Crane, M. St. J., Clarke, J. B. & Thomas, D. B. (1977) Exp. Cell Res. **107**, 89–97

Crane, M. St. J. & Thomas, D. B. (1976) Nature (London) **261**, 205–208

Davis, L. & Lipsky, P. E. (1986) J. Immunol. **136**, 3588–3596

Debraban, M., Geuens, G., Nuydens, R., Willebro, R., Aerts, F., Demey, J. & Mcintosh, J. R. (1986) Int. Rev. Cytol. **101**, 215–274

Dickinson, J. R. (1985) Eur. J. Biochem. **119**, 341–345

Dickinson, J. R. & Williams, A. S. (1987) J. Gen. Microbiol. **133**, 135–140

Donachie, W. D. (1981) in The Cell Cycle (John, P. C. L., ed.), pp. 63–83, Cambridge University Press, London

Eagon, R. G. (1962) J. Bacteriol. **83**, 736–737

Edmunds, L. N. (ed.) (1984) Cell Cycle Clocks, pp. 616, Marcel Dekker, New York

Edwards, C. (1981) The Microbial Cell Cycle, Nelson, Surrey

Edwards, C. & Jones, C. W. (1977) J. Gen. Microbiol. **99**, 383–388

Edwards, S. W. & Lloyd, D. (1978) J. Gen. Microbiol. **108**, 197–204

Edwards, S. W. & Lloyd, D. (1980) FEBS Lett. **109**, 21–26

Edwards, S. W., Evans, J. B., Williams, J. L. & Lloyd, D. (1982) Biochem. J. **202**, 453–458

Eilen, E., Hand, R. & Basilico, C. (1980) J. Cell Physiol. **105**, 259–266

Epifanova, O. I. & Polunovs, V. A. (1986) J. Theor. Biol. **120**, 467–477

Fanger, B. O., Currie, R. A. & Cidlowski, J. A. (1986) Arch. Biol. **249**, 116–125

Farber, R. A. & Liskay, R. M. (1974) Cytogenet. Cell Genet. **13**, 384–396

Floros, J., Ashihara, T. & Baserga, R. (1978) Cell Biol. Int. Rep. **2**, 259–269

Frankel, J., Mohler, J. & Frankel, A. K. (1980) J. Cell Sci. **43**, 75–91

Game, J. C. (1976) Mol. Gen. Genet. **146**, 313–315

Garrison, P. N., Mathis, S. A. & Barnes, L. D. (1986) Mol. Cell Biol. **6**, 1179–1186

Gilbert, D. A. (1981) S. Afr. J. Sci. **77**, 541–546

Gilbert, D. A. (1982) Biosystems **15**, 317–339

Gilles, R. J. & Benoit, A. G. (1983) Biochim. Biophys. Acta **762**, 466–470

Goodwin, B. C. (1980) Symp. Soc. Gen. Microbiol. **30**, 377–403

Green, L., Schlaffe, I., Wright, K., Moreno, M. L., Berand, D., Hager, G., Stein, J. & Stein, G. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2315–2319

Hartwell, L. H. (1974) Bacteriol. Rev. **38**, 164–198

Hartwell, L. H. (1978) J. Cell Biol. **77**, 627–637

Hatzfeld, J. & Buttin, G. (1975) Cell **5**, 123–129

Holahan, P. K. & Dewey, W. C. (1986) Radiat. Res. **106**, 111–121

Hori, T. (1977) Jpn. J. Genet. **52**, 53–64
Howell, S. H. (1974) in Cell Cycle Controls (Padilla, G. H., Cameron, I. L. & Zimmerman, A., eds.), pp. 235–249, Academic Press, New York
Howlett, S. K. (1986) Cell **45**, 387–396
Hyodo, M. & Suzuki, K. (1982) Exp. Cell Res. **137**, 31–38
Ide, T., Ninomiya, J. & Ishibashi, S. (1984) Exp. Cell Res. **150**, 60–67
Ingles, C. J. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 405–409
Jaffe, L. F. (1981) Proc. R. Soc. London Ser. B **295**, 553–566
James, R. & Bradshaw, A. A. (1984) Annu. Rev. Biochem. **53**, 259–292
Johnston, G. C., Pringle, J. R. & Hartwell, L. H. (1977) Exp. Cell Res. **105**, 79–98
Johnston, L. A. & Nasmyth, K. A. (1978) Nature (London) **274**, 891–893
Jonak, G. J. & Baserga, R. (1979) Cell **18**, 117–123
Jong, A. Y. S., Kuo, C. L. & Campbell, J. L. (1984) J. Biol. Chem. **259**, 1052–1059
Kameyama, K., Takezaki, S., Kanzaki, T. & Nishiyam, S. (1986) J. Invest. Dermatol. **87**, 313–318
Kane, A. Basilico, C. & Baserga, R. (1976) Exp. Cell Res. **99**, 165–173
Kauffman, S. A. & Wille, J. J. (1975) J. Theor. Biol. **55**, 47–93
Kawasaki, G. H. (1979) in The Molecular Biology of Yeast, Cold Spring Harbor Meeting, p. 35 (abstr.), Cold Spring Harbor Laboratory, New York
Kelvin, D. J., Chance, S., Shreeve, M., Axelrad, A. A., Connolly, J. A. & Mcleod, D. (1986) J. Cell Physiol. **127**, 403–409
Kimmel, M. & Traganos, F. (1986) Math. Biosci. **80**, 187–208
Knacker, T., Harwood, J. L., Hunter, C. N. & Russell, N. J. (1985) Biochem. J. **229**, 701–710
Laffler, T. G. & Carrino, J. J. (1986) Bioessays **5**, 62–65
Landy-Otsuka, F. & Scheffler, I. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 5001–5005
Landy-Otsuka, F. & Scheffler, I. (1980) J. Cell Physiol. **105**, 209–220
Ling, V. (1977) J. Cell Physiol. **91**, 209–224
Liskay, R. M. (1974) J. Cell Physiol. **84**, 49–56
Liskay, R. M. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 1622–1625
Liskay, R. M. & Meiss, M. K. (1977) Somat. Cell Genet. **3**, 343–347
Liskay, R. M. & Prescott, D. M. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 2873–2877
Lloyd, D. (1983) in Fungal Differentiation (Smith, J. E., ed.), pp. 107–141, Marcel Dekker, New York
Lloyd, D. & Edwards, S. W. (1984) in Cell Cycle Clocks (Edmunds, L. N., ed.), pp. 27–46, Marcel Dekker, New York
Lloyd, D. & Edwards, S. W. (1986) J. Interdiscip. Cycle Res. **17**, in the press
Lloyd, D. & Edwards, S. W. (1987) Adv. Chronobiol. (Pauly, J. E. & Scheving, L. E., eds.), Alan R. Liss, New York, in the press
Lloyd, D. & Kippert, F. (1987) Symp. Soc. Exp. Biol., in the press
Lloyd, D., John, L., Edwards, C. & Chagla, A. H. (1975) J. Gen. Microbiol. **88**, 153–158
Lloyd, D., Edwards, S. W. & Fry, J. C. (1982*a*) Proc. Natl. Acad. Sci. U.S.A., **79**, 3785–3788
Lloyd, D., Poole, R. K. & Edwards, S. W. (1982*b*) The Cell Division Cycle, pp. 523, Academic Press, London
Lomniczi, B., Bosch, F. X., Hay, A. J. & Skehel, J. J. (1977) J. Gen. Virol. **35**, 187–190
Lord, P. G. & Wheals, R. E. (1984) J. Gen. Microbiol. **130**, 2803–2808
Lorincz, A. T. & Reed, S. I. (1984) Nature (London) **307**, 183–185
Lykkesfeldt, A. E., Larsen, J. K. & Christensen, I. J. (1986) Breast Cancer **7**, 83–90
McCracken, A. (1982) Somat. Cell Genet. **8**, 179–195
Marunouchi, T. & Nakano, M. M. (1980) Cell Struct. Funct. **5**, 53–67
Matsumoto, K., Uno, I. & Ishikawa, T. (1983) Exp. Cell Res. **146**, 151–161
Matsumoto, Y., Yasuda, H., Marunouchi, T. & Yamada, M. (1983) FEBS Lett. **151**, 139–142
Meiss, H. K. & Basilico, C. (1972) Nature (London) New Biol. **239**, 66–68
Melero, J. A. (1979) J. Cell Physiol. **98**, 17–30
Melchers, F. & Andersson, J. (1986) Annu. Rev. Immunol. **4**, 13–36
Ming, P.-M. L., Chang H. L. & Baserga, R. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 2052–2055
Mita, S., Yasuda, H., Marunouchi, T., Ishiko, S. & Yamada, M. (1980) Exp. Cell Res. **126**, 407–416
Mora, M., Darzynkiewicz, Z. & Baserga, R. (1980) Exp. Cell Res. **125**, 241–249
Morris, R. N. (1980) Symp. Soc. Gen. Microbiol. **30**, 41–76
Moser, G. C. & Meiss, H. R. (1975) J. Cell Biol. **67**, 297A
Moser, G. C. & Meiss, H. R. (1977) Somat. Cell Genet. **3**, 449–456
Myers, C. R. & Collins, M. L. P. (1986) J. Bacteriol. **166**, 818–823
Naha, P. M. (1973) Nature (London) New Biol. **241**, 13–14
Naha, P. M. (1979*a*) J. Cell Sci. **35**, 53–58
Naha, P. M. (1979*b*) J. Cell Sci. **40**, 33–42
Naha, P. M. & Sorrentino, R. (1980) Cell Biol. Int. Rep. **4**, 365–378
Naha, P. M., Meyer, A. & Hewitt, K. (1975) Nature (London) **258**, 49–53
Nakamo, M. M., Selciguchi, T. & Yamada, M. (1978) Somat. Cell Genet. **4**, 169–178
Nasmyth, K. A. (1977) Cell **12**, 1109–1120
Nishimoto, T. & Basilico, C. (1978) Somat. Cell Genet. **4**, 323–340
Nishimito, T., Takahashi, T. & Basilico, C. (1980) Somat. Cell Genet. **6**, 465–476
Nurse, P. (1981) Symp. Soc. Gen. Microbiol. **31**, 292–315
Ohlsson-Wilhelm, B. M., Freed, J. J. & Perry, R. P. (1976) J. Cell Physiol. **89**, 77–88
Ohlsson-Wilhelm, B. M., Leary, J. & Pacilio, M. (1979) J. Cell Biol. **83**, 4A
Okinaka, R. T. & Barnhart, B. J. (1978) J. Cell Biol. **79**, 383A
Otsuka, F. & Scheffler, I. E. (1978) J. Supramol. Struct. **S2**, 334
Patterson, M., Sclafani, R. A., Fangman, W. L. & Rosamond, J. (1986) Mol. Cell Biol. **6**, 1590–1598
Reich, J. G. & Sel'kov, E. E. (1981) Energy Metabolism of the Cell: A Theoretical Treatise, pp. 346, Academic Press, London
Rossini, M. & Baserga, R. (1978) Biochemistry **17**, 858–863
Rossini, M., Baserga, S., Huang, C. H., Ingles, C. J. & Baserga, R. (1980) J. Cell Physiol. **103**, 97–103
Rossini, M., Weinmann, R. & Baserga, R. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4441–4445
Sato, K. & Hama-Inaba, H. (1978) Exp. Cell Res. **14**, 484–486
Scheffler, L. E. & Buttin, G. (1973) J. Cell Physiol. **81**, 199–216
Sclafani, R. A. & Fangman, W. L. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 5821–5825
Sel'kov, E. E. (1970) Biophysika **15**, 1065–1073
Setterfield, G., Sheinin, R., Dardick, I., Kiss, G. & Dubsky, M. (1978) J. Cell Biol. **77**, 246–263
Shapiro, L. (1976) Annu. Rev. Microbiol. **30**, 377–407
Sheinin, R. (1976*a*) J. Virol. **17**, 692–704
Sheinin, R. (1976*b*) Cell **7**, 49–57
Sheinin, R. & Gutman, S. (1977) Biochim. Biophys. Acta **479**, 105–118
Sheinin, R., Humbert, J. & Pearlman, R. E. (1978) Annu. Rev. Biochem. **47**, 277–316
Shilo, B., Shimchen, G. & Pardee, A. B. (1978) J. Cell Physiol. **97**, 177–188
Shiomi, T. & Sato, K. (1976) Exp. Cell Res. **100**, 297–302
Shiomi, T. & Sato, K. (1978) Cell Struct. Funct. **3**, 95–102

Shymko, R. M., Klevecz, R. R. & Kauffman, S. A. (1984) in Cell Cycle Clocks (Edmunds, L. N., ed.), pp. 273–294, Marcel Dekker, New York
Simanis, V. & Nurse, P. (1986) Cell **45**, 261–268
Slater, M. L. & Ozer, H. L. (1976) Cell **7**, 289–295
Smith, J. A. & Martin, L. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 1263–1267
Smith, B. J. & Wigglesworth, N. M. (1972) J. Cell Physiol. **80**, 253–260
Sudbery, P. E., Goodey, A. R. & Carter, B. L. A. (1980) Nature (London) **288**, 401–404
Sweeney, B. M. (1982) Plant Physiol. **70**, 272–276
Talavera, A. & Basilico, C. (1977) J. Cell Physiol. **92**, 425–436
Talavera, A., Nishimoto, T. & Basilico, C. (1976) J. Cell Biol. **70**, 341A
Taylor, D. L., Waggoner, A. S., Murphy, R. F., Lanni, F. & Birge, R. R. (eds.) (1986) Applications of Fluorescence in the Biomedical Sciences, pp. 639, Alan R. Liss, New York
Tenner, A., Zeig, J. & Scheffler, I. E. (1977) J. Cell Physiol. **90**, 145–160
Thompson, L. H. & Lindl, P. A. (1976) Somat. Cell Genet. **2**, 387–450
Thompson, L. H., Mankovitz, R., Baker, R. A., Till, J. E., Siminovitch, L. & Whitmore, G. F. (1970) Proc. Natl. Acad. Sci. U.S.A. **66**, 377–384
Thompson, L. H., Mankovitz, R., Baker, R. M., Wright, J. A., Till, J. E., Siminovitch, L. & Whitmore, G. F. (1971) J. Cell Physiol. **78**, 431–440
Toniolo, D., Meiss, H. K. & Basilico, C. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 1273–1277
Tripp, M. L., Pinon, R., Meisenhe, J. & Hunter, T. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5973–5977
Tsai, Y., Hanaoka, F., Nakano, M. M. & Yamada, M. (1979) Biochem. Biophys. Res. Commun. **91**, 1190–1195
Tucker, A. M., Hong, L., Boorman, G. A. & Luster, M. I. (1986) Thymus **8**, 121–127
Tyson, J. & Sachsenmaier, W. (1978) J. Theoret. Biol. **73**, 723–728
Vanderkraan, P. M., Vanzandv, P. M., Deabreu, R. A., Bakkeren, J. A., Vanlaarh, J. P. & Debruyn, C. H. M. (1986) Adv. Exp. Med. **195**, 553–559
Wang, R. J. (1974) Nature (London) **248**, 76–78
Wang, R. J. (1976) Cell **8**, 257–261
Wang, R. J. & Yin, L. (1976) Exp. Cell Res. **101**, 331–336
White, J. H. M., Barker, D. G., Nurse, P. & Johnston, L. H. (1986) EMBO J. **5**, 1705–1709
Wille, J. J. & Scott, R. E. (1986) Int. J. Cancer **37**, 875–881
Williams, R. J. P. (1981) Biochem. Soc. Symp. **46**, 57–72
Wissinger, W. & Wang, R. J. (1978) Exp. Cell Res. **112**, 82–94
Yanagi, K., Talavara, A., Nishimoto, T. & Rush, M. G. (1978) J. Virol. **25**, 42–50
Yasuda, H., Matsumoto, Y., Mita, S., Marunouchi, T. & Yamada, M. (1981) Biochemistry **20**, 4414–4419
Yen, A., Freeman, L., Powers, V., Vansant, R. & Fishbaug, J. (1986) Exp. Cell Res. **165**, 139–151
Zaitsu, H. & Kimura, G. (1984) J. Cell Physiol. **119**, 82–88
Zeuthen, E. & Scherbaum, O. H. (1954) Colston Pap. **1**, 141–155