BioSystems, 27 (1992) 17–24
Elsevier Scientific Publishers Ireland Ltd.

The cell division cycle: a physiologically plausible dynamic model can exhibit chaotic solutions

David Lloyd[a], Alun L. Lloyd[b] and Lars Folke Olsen[c]

[a]*Microbiology Group (PABIO), University of Wales College of Cardiff, P.O. Box 915, Cardiff CF1 3TL, Wales (UK)* [b]*Trinity College, Cambridge CB2 1TQ (UK) and* [c]*Biokemisk Institut, Odense Universitet, Campusvej 55, 5230 Odense M (Denmark)*

(Received February 14th, 1992)
(Revision received April 28th, 1992)

A mitotic oscillator with one slowly increasing variable (τ_L of the order of hours) and one rapidly increasing variable (τ_R of the order of minutes) modulated by a timer (ultradian clock) gives an auto-oscillating solution: cells divide when this relaxation oscillator reaches a critical threshold to initiate a rapid phase of the limit cycle. Increasing values of the velocity constant in the slow equation give quasiperiodic, chaotic and periodic solutions. Thus dispersed and quantized cell cycle times are consequences of a chaotic trajectory and have a purely deterministic basis. This model of the dispersion of cell cycle times contrasts with many previous ones in which cell cycle variability is a consequence of stochastic properties inherent in a sequence of many thousands of reactions or the random nature of a key transition step.

Keywords: Cell cycle dispersion; Chaos; Limit cycle; Quantal cell cycle; Ultradian clock

1. Introduction

In rapidly growing populations of lower eukaryotic organisms or cultured metazoan cells the progress of cells from S phase to mitosis and then to S phase again can occur repeatedly (Lloyd et al., 1982a). This cycle of processes and events has often been discussed as if it is a clock (Edmunds, 1988), or more correctly, as if it is partially controlled by a clock (Nurse, 1990). Several limit-cycle models for the mitotic oscillator have been proposed (Sel'kov, 1970; Gilbert, 1974, 1981; Kauffman and Wille, 1975). A special characteristic of all timekeepers (which distinguishes them from all those oscillating systems not contributing to biological clock function) is their temperature-compensated outputs and yet the cell cycle is as temperature-dependent as most other typical biochemical reactions and biological processes. Neither does the cell division cycle show the precision typical of other clock-controlled rhythms (Pittendrigh and Bruce, 1957). Thus, individual organisms have widely differing cycle times; the coefficient of variation sometimes reaches 15–25% (Brooks, 1981). In the mammalian cell growing rapidly in culture under optimum conditions, the interval between the start of S phase and mitosis varies only a little, and almost all the dispersion of cell cycle times arises in G_1 (Prescott, 1976). There have been several different suggestions that the timekeeper may be a biochemical oscillator. For limit cycle models, cell cycle variability is modelled by inclusion of a 'noise term' to take account of the inherent variability of a process that represents the sum total of several thousands of steps (Gilbert, 1981; Mustafin and Volkov, 1977). Alternatively it has been suggested that a random event characterizes the

Correspondence to: David Lloyd, Microbiology Group (PABIO), University of Wales College of Cardiff, P.O. Box 915, Cardiff CF1 3TL, Wales, UK.

0303-2647/92/$05.00

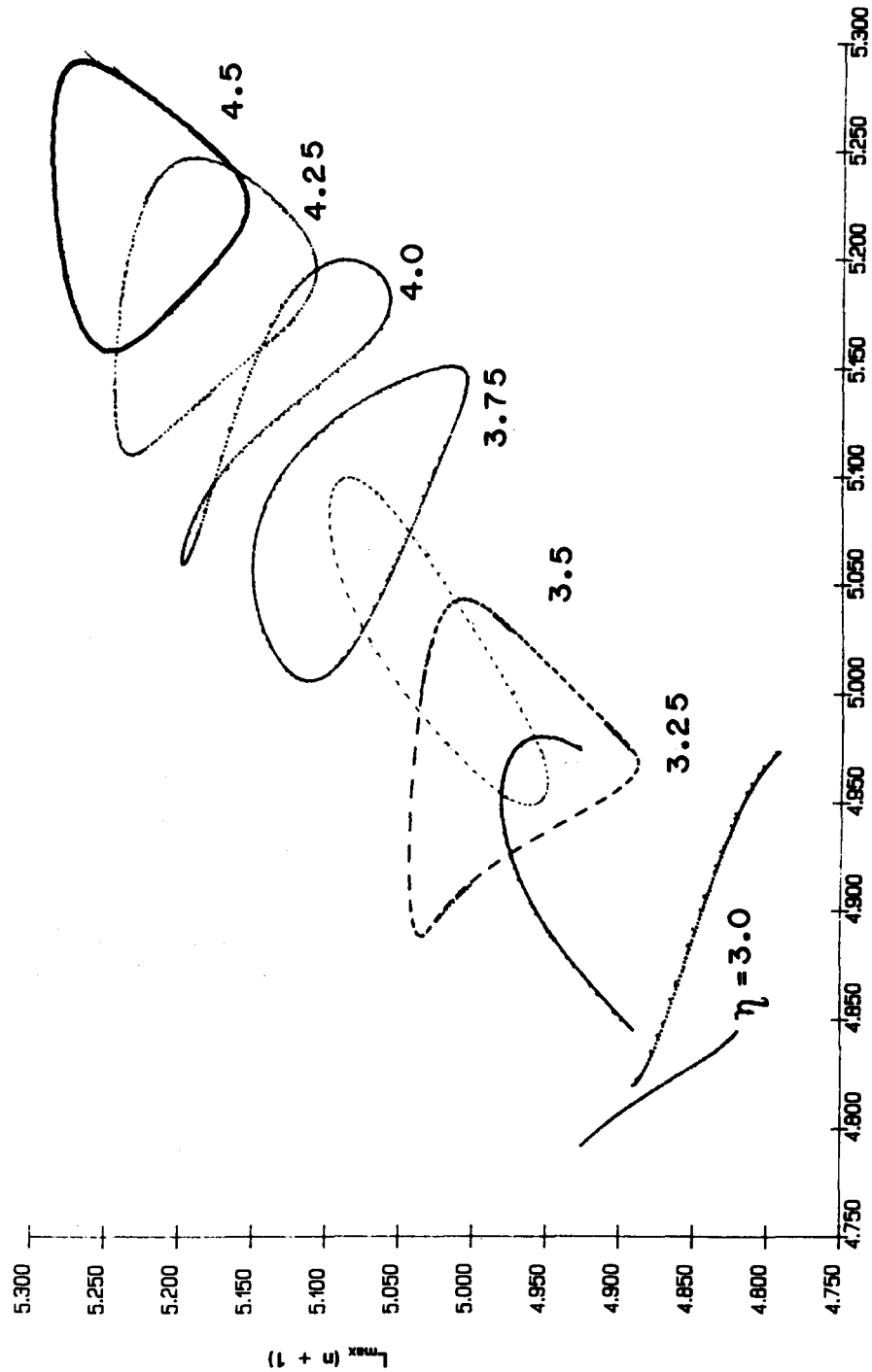

Fig. 1. Plot of approximately 600 maxima (including transients) for equations (2) with $\Omega = 7.0$ and for various values of η. Other parameters set as in Fig. 2. A modified Adams-Bashforth algorithm was employed; accuracy values used in the corrector iteration were 10^{-6} and the step length was 0.1.

transition to S (the Transition Probability Model) (Burns and Tannock, 1970; Smith and Martin, 1973). In this paper we show that it is not essential to invoke stochastic events to explain the variability of cell cycle times: a relaxation oscillator interacting with ultradian clock pulses (Lloyd and Kippert, 1987) can show chaotic behaviour. This provides a deterministic mechanism which may have advantages over a noisy one for evolutionary survival (Conrad, 1986). Extensive evidence for the existence of an ultradian clock with species specific period (of the order of an hour) comes from observations made on several different lower eukaryotes (yeasts and protozoa, Lloyd, 1992).

2. The Model

The model is based on earlier proposals of Chernavskii et al. (1982) and Lloyd and Volkov (1990, 1991) in which a mitotic or cell division cycle oscillator with one slow variable (τ_L of the order of hours) and one fast variable (τ_R of the order of minutes) may be described by the following system of equations:

$$\tau_L \frac{dL}{dt} = \eta - 2LR - DL$$

$$\tau_R \frac{dR}{dt} = \mathcal{K} + LR - R^2 - \frac{\gamma R}{(R + \delta)} \qquad (1)$$

where L and R are concentration terms, τ_L and τ_R are their characteristic times and η, D, $\mathcal{K}$, γ and δ are velocity constants. Both the slow and fast components oscillate with the same period but with very distinctive waveforms. The non-symmetrical time dependence of the slow variable becomes more pronounced as the system approaches the bifurcation point (i.e. the non-proliferating state). As in the Sel'kov-Gilbert hypothesis, these coupled differential equations reflect the operation of a control system, as the rates of change in the levels of the two variables, L and R, are each dependent on the levels of the other component (Sel'kov, 1970; Gilbert, 1974).

A variety of biochemical identities have been proposed for appropriately paired state variables; current ideas of cell cycle control focus on p34-cyclin oscillations (Hyver and Le Guyaders 1990). Sel'kov (1970) proposed a redox (thiol-disulphide) dependent limit cycle, whereas Chernavskii et al. (1977) favoured a lipid dependent mechanism. Modulation by an output of the endogenous temperature-compensated ultradian clock (Lloyd et al., 1982a) (period T_{UR}) is represented as a first approximation in the simplest form possible (i.e. as having a single defined frequency). The sinusoid output of this clock is simulated by the introduction of a harmonic term as a forcing function into the slow equation, which then becomes:-

$$\tau_L \frac{dL}{dt} = \eta - 2LR - DL + C \sin \Omega t \qquad (2)$$

where

$$T_{UR} = \frac{2\pi}{\Omega} \ll T_{\text{cell cycle}}$$

The auto-oscillating solution considers that cells divide when L(t) reaches a threshold to initiate a rapid phase of the limit cycle. Here, we consider only a fixed-threshold model; in a more complex scenario this threshold (and also other characteristics of the model) could have temperature dependencies.

3. Computer Simulations

The dynamic structure of the equations was studied numerically. Whereas previously we have also included a noise term to imitate the dispersion of generation times shown by real systems, solutions of between 3.00 and 4.50 in equation [2] show quasiperiodicity (circular or toroidal next-amplitude maxima plots) (Fig. 1) without any such stochastic term (L_{max} (n) is the value attained with L at its n'th maximum).

The time-dependent behaviour of the cellular concentrations of L and R (Fig. 2), show that different temporal characteristics of cell division behaviour can be obtained by setting the critical threshold at different levels; presumably it is only higher concentration maxima that could be

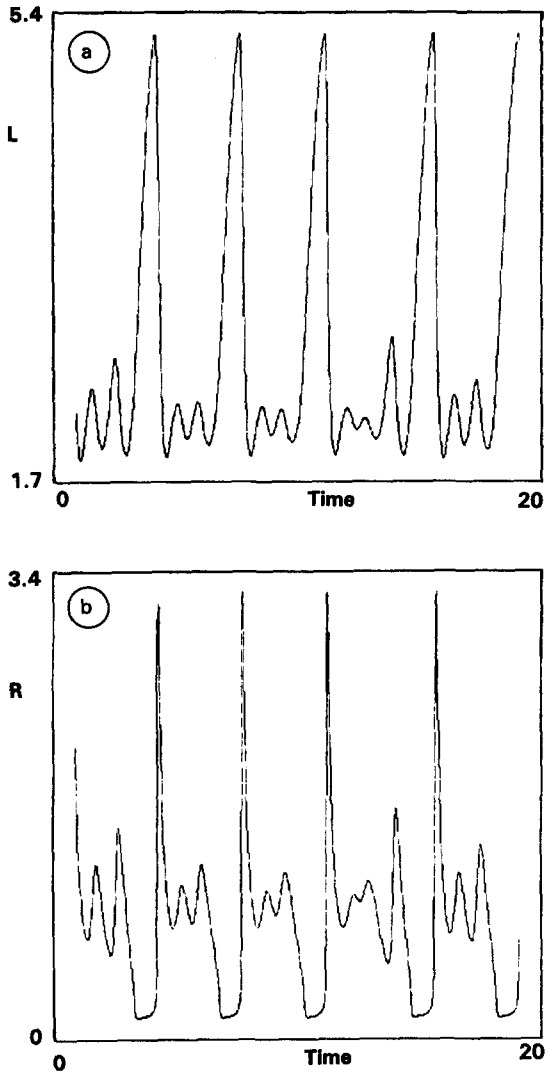

Fig. 2. The solution of the model described in equations (1) and (2) with $\eta = 4.95$, D = 0.4, $\mathcal{K} = 0.15$, $\gamma = 1.5$, $\delta = 0.15$, C = 0.45, $\Omega = 5.0$, $\tau_L = 1$ and $\tau_R = 0.1$. Time dependence of the slow (L) and fast (R) variables, (a) and (b), respectively.

used in the real system to trigger mitosis or cell division.

Further exploration of higher values for η (e.g. $\eta = 4.95$) indicates chaotic dynamics as illustrated by the phase portrait of R vs. L in Fig. 3a and the stroboscopic plots of Figs. 3b and 3c. The latter represent plots of R vs. L at time intervals of T_{UR} ('time one maps'). These plots would show the presence of a rhythm with period T_{UR} or multiples or fractions of this period, if present, as a finite set of points. We note that the time-one map in Fig. 3a does not exhibit this property and its dispersion indicates that the period is not a multiple of T_{UR}. Figure 3b has an intricate structure which can be revealed by magnification of a small subsection of the map (Fig. 3c). Magnifications beyond that in Fig. 3c did not show additional structure. Thus we may conclude that either the phase portrait in Fig. 3a corresponds to (i) a complex torus with an integer dimension or to (ii) a chaotic attractor with an almost integer fractal dimension. Calculation of the correlation dimension, D_2, by the method of Grassberger and Procaccia (1983) gave a value of 1.95, thus confirming an almost integer dimension. We also used the method of Wolf et al. (1985) to compute the spectrum of Lyapunov exponents. This method assumes that none of the differential equations contain time explicitly (i.e. are autonomous). To eliminate t on the right-hand side of equation [2], we replaced the sine term with the term:

C sin X

and added an additional differential equation:

$$\frac{dX}{dt} = \Omega \qquad (3)$$

This gives a set of differential equations equivalent to [1] and [2].

The Lyapunov exponents are in bit per time unit: $\lambda_1 = 0.21$, $\lambda_2 = 0$ and $\lambda_3 = -6.32$. From the Lyapunov exponents we calculate the Lyapunov dimension $D_L = 2.03$ (Farmer et al., 1983). D_L is a much better approximation to the fractal dimension than D_2. A positive λ_1 and a non-integer D_L confirm that the dynamics are chaotic. The bifurcation diagram (L_{max} vs. η, Fig. 3d) shows that the route to chaos for increasing η is via a quasiperiodic regime. The chaotic region begins at $\eta = 4.65$ and gives way to a simple periodic oscillation at $\eta = 5.0$.

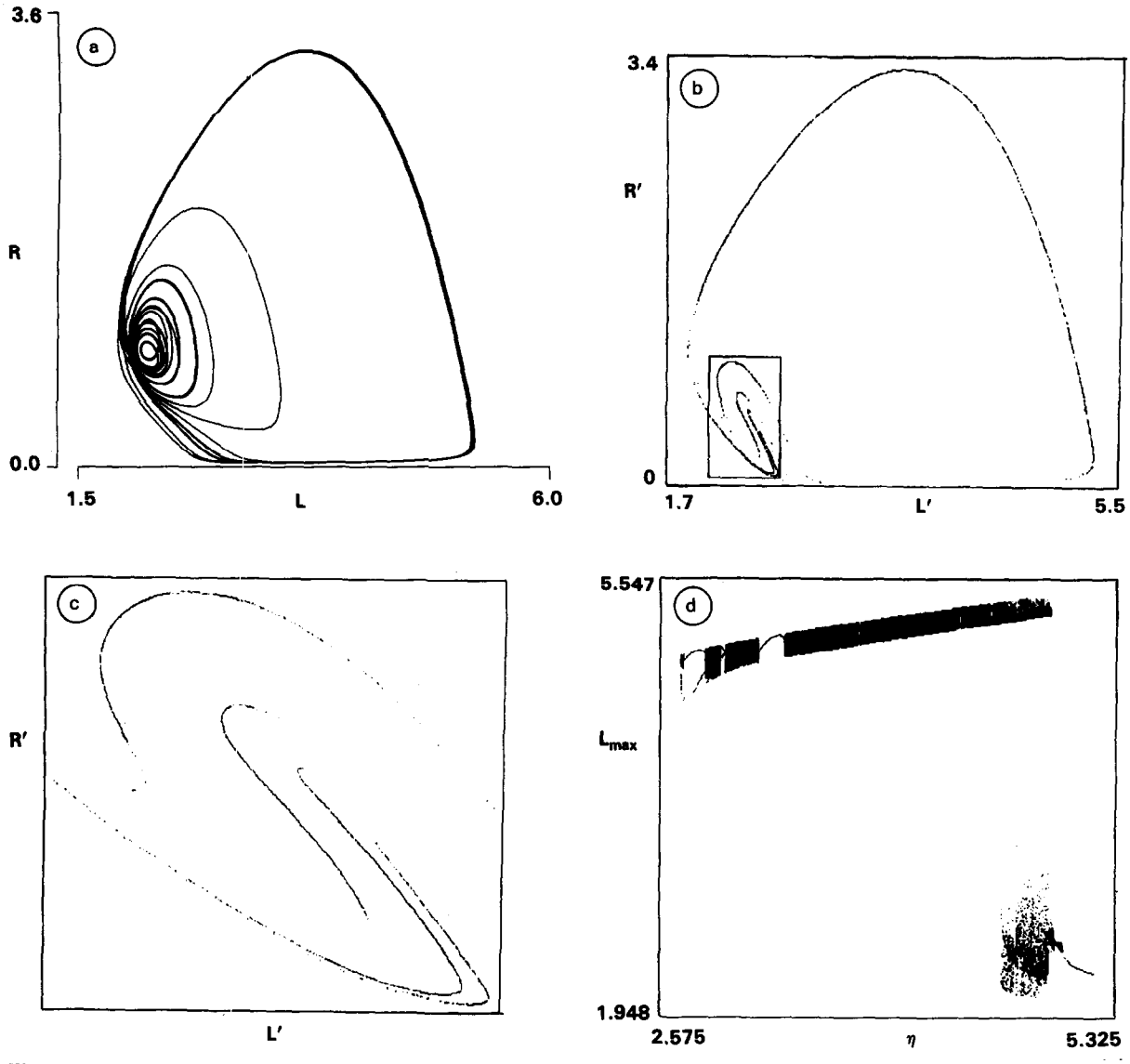

Fig. 3. (a) Phase portrait of the system described in equations (1) and (2) for parameter values shown in Fig. 2. (b) Time-one map of the simulation in (a): 50 000 corresponding values of R and L are plotted at intervals of $T_{UR} = 2\pi/\Omega$. (c) Magnification of the boxed region in (b). (d) Bifurcation diagram for n varying from 2.5 to 5.2. For each value of η 100 maxima of L are plotted following a transient of 100 time units. Other parameters as in (a).

A relative frequency plot of cell cycle times for $\eta = 4.95$ shows a trimodal distribution (Fig. 4) with negatively skewed dispersions. The bifurcation diagram (Fig. 3d) indicates that this is just one possible outcome: other values for η will give different consequences (e.g. in the quasi-periodic regime a dispersed unimodal distribution of cell cycle times will result). Quantized cell cycle times have been predicted from experiments with synchronous cultures of protozoa (Lloyd et al., 1982a) and shown for mammalian cells (Klevecz, 1976); precise measurements

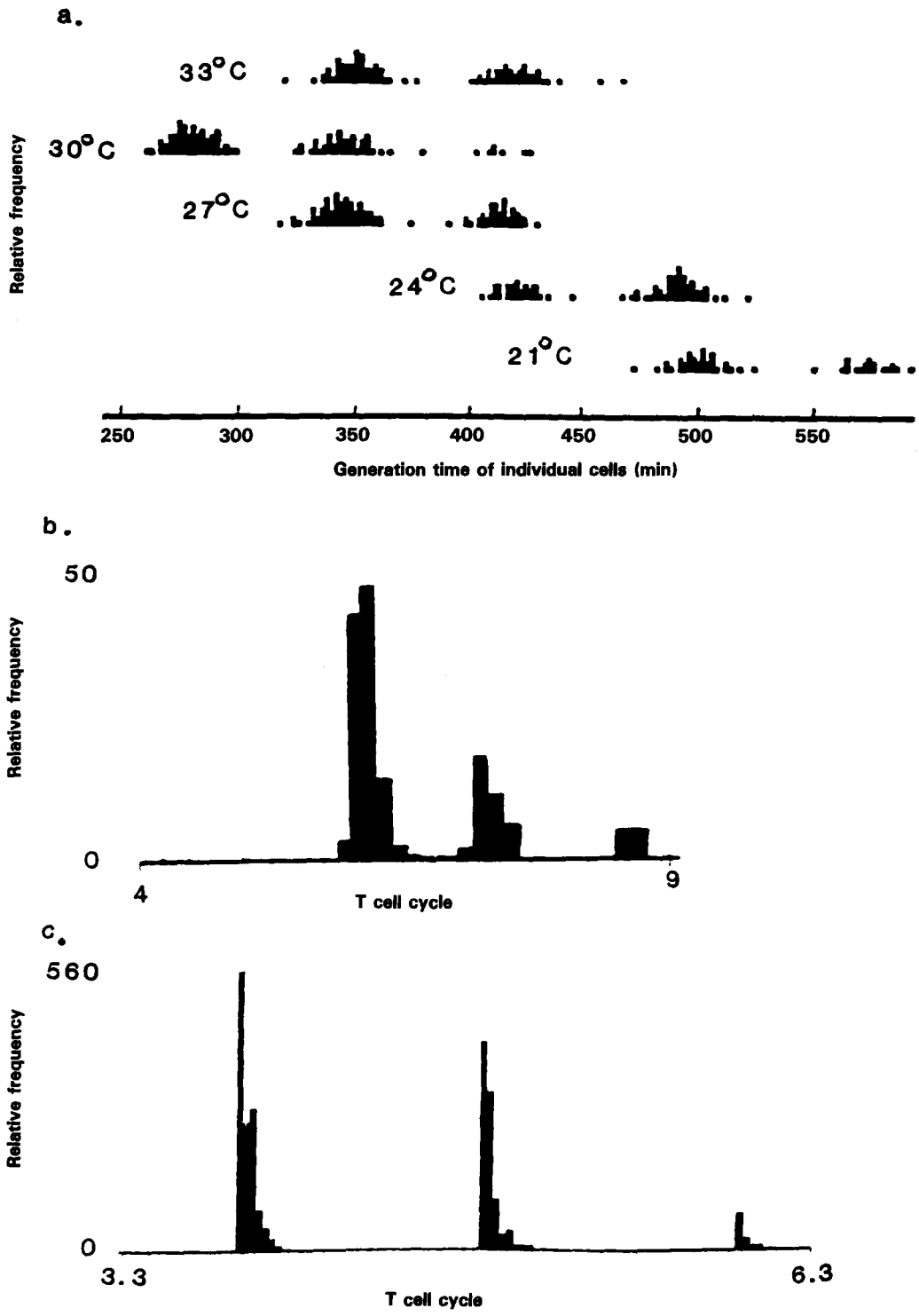

Fig. 4. Quantized cell cycle times. (a) Experimentally observed distributions of generation times of individual *Paramecium tetraurelia* cells at various steady-state growth temperatures. Each square represents timing of division of an isolated organism. (Reproduced with permission from Lloyd and Kippert, 1987). (b) Generation time distributions from the model system incorporating noise (Lloyd and Volkov, 1990, Eqn. 3) when $\tau_L = 1$; $\tau_R = 0.1$; $\mathcal{C} = 0.15$; D = 0.4; $\gamma = 1.5$; $\delta = 0.15$; $\Delta\eta = 0.3$; ξ = random numbers, (0,1); C = 0.45 $T_{UR} = 1.25$ and $\eta = 5.55$. (Reproduced with permission from Lloyd and Volkov, 1990). (c) Frequency distribution of cell cycle times ($T_{cell\ cycle}$) for parameter values as follows: $\eta = 4.95$, D = 0.4, $\mathcal{C} = 0.15$, $\gamma = 1.5$, $\delta = 0.15$, C = 0.45, $\Omega = 5.0$, $\tau_L = 1$ and $\tau_R = 0.1$. For Eqn. 2 (in the absence of a noise term).

made on interdivision times of individual *Paramecium tetraurelia* demonstrate this phenomenon most clearly (Lloyd and Kippert, 1987). Circadian clock control of the cell cycle in other organisms often reveals itself as 24-h quantal increments in division times (Sweeney, 1982; Edmunds, 1988).

4. Conclusions

In the model considered here, chaotic dynamics can be a consequence of the interaction of two oscillators (the action of the ultradian clock on the cell cycle oscillator). In the chaotic mode (i.e. for a special set of parameter values) this gives rise to dispersion of cell cycle times and multimodality (quantized cell cycle times) (Lloyd et al., 1982b; Lloyd and Kippert, 1987). It differs from the earlier model of Lloyd and Volkov (1990, 1991) in that complex dynamic structure is generated in the absence of external noise. The system described here has analogies with the periodically-perturbed Hodgkin-Huxley oscillator (Hodgkin and Huxley, 1952; Fitzhugh, 1961; Rose and Hindmarsh, 1985; Aihard and Matsumoto, 1987). But the organism is an ensemble of many potentially oscillating systems operating on a broad range of timescales (Lloyd, 1987). In a multi-oscillator system of weakly-coupled units it would be surprising not to be able to find that chaotic regimes and chaotic systems are generally more robust to perturbations than their periodic counterparts (Schaffer et al., 1986). The exploitation of the benefits that accrue to an organism able to utilise these dynamic characteristics ensure the evolutionary survival of any useful mechanism. Although the benefits of dispersed and quantized cell cycle times have not been investigated, we conjecture that the generation of diversity and the maintenance of the functional independence of the individual (e.g. by the avoidance of entrainment) might enhance survival of the population. Too narrow an age structure could be a severe limitation (Conrad, 1986). Some systems may achieve this by a chaotic mechanism, whereas others may use random fluctuations or a superposition of noise on chaos.

In the Transition Probability Model a stochastic event is the key regulatory step that controls cell proliferation rates. In the present model, variability of cell cycle times is a consequence of a chaotic trajectory with a purely deterministic basis. Although we have assumed throughout that the critical threshold is achieved at cell division, similar arguments hold for the control of triggering of the S phase or of mitosis, as do the salient features of the model. We do not imply that the consequences of these alternatives are equivalent; indeed that mitosis and division can be dissociated (Baserga, 1985) clearly indicates that this is not so.

The existence of a cell cycle oscillator with a strange attractor has previously been considered (Engleberg, 1968; Mackey, 1985; Grasman, 1990) but possible consequences were not investigated numerically. Further developments of the model should include consideration of the relatedness of sister cell cycle times and effects of period and waveform of the forcing function on distribution of cell cycle times.

Acknowledgments

Supported by the Danish Natural Science Council. The authors wish to thank an anonymous referee for invaluable comments leading to clarification of several issues.

References

Aihara, K. and Matsumoto, G., 1987, Forced oscillations and routes to chaos in the Hodgkin-Huxley axons and squid giant axons. in: Chaos in Biological Systems, H. Degn, A.V. Holden, and L.F. Olsen (eds.) (Plenum, New York) pp. 121–131.

Baserga, R., 1985, The Biology of Cell Reproduction (Harvard University Press) pp. 91–102.

Brooks, R.F., 1981, Variability in the cell cycle and the control of proliferation. in: The Cell Cycle, P.C.L. John (ed.) (Cambridge University Press) p. 35.

Burns, F.J. and Tannock, I.F., 1970, On the existence of a G_0-phase in cell cycle. Cell Tissue Kinet. 3, 321–334.

Chernavskii, D.S., Palamarchuk, E.K., Polezhaev, A.A., Solyanik, G.I. and Burlakova, E.B., 1982, Mathematical model of periodic processes in membranes with applications to cell regulation. BioSystems 9, 187–193.

Conrad, M., 1986, What is the use of chaos? in: Chaos, A.V. Holden (ed.) (Manchester University Press) pp. 3–14.

Edmunds, L.N. Jr., 1988, Cellular and Molecular Bases of Biological Clocks (Springer–Verlag, New York).

Engleberg, J., 1968, On deterministic origins of mitotic variability. J. Theor. Biol. 20, 249–259.

Farmer, J.D., Ott, E. and Yorke, J.A., 1983, The dimension of chaotic attractors. Physica 7D, 153–180.

Fitzhugh, R., 1961, Impulses and physiological states in theoretical models of nerve membranes. Biophys. J. 1, 445–466.

Gilbert, D.A., 1974, The nature of the cell cycle and the control of cell replication. BioSystems 5, 197–206.

Gilbert, D.A., 1981, The cell cycle 1981. One or more limit cycle oscillations? South Afr. J. Sci. 77, 541–546.

Grasman, J., 1990, A deterministic model of the cell cycle. Bull. Math. Biol. 52, 535–547.

Grassberger, P. and Procaccia, I., 1983, Measuring the strangeness of strange attractors. Physica 9D, 189–208.

Hodgkin, A.L. and Huxley, A.F., 1952, A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol., London 349, 227–247.

Hyver, C. and Le Guyader, H., 1990, MPF and cyclin: modelling of the cell cycle minimum oscillator. BioSystems 24, 85–90.

Kauffman, S. and Wille, J.J., 1975, The mitotic oscillator in *Physarum polycephalum*. J. Theor. Biol. 55, 47–93.

Klevecz, R.R., 1976, Quantized generation ties in mammalian cells as an expression of the cellular clock. Proc. Natl. Acad. Sci. U.S.A. 73, 4012–4016.

Lloyd, D., 1987, Biochemistry of the cell-cycle. Biochem J. 242, 313–321.

Lloyd, D., 1992, Intra cellular time keeping: epigenetic oscillations reveal the functions of an ultradian clock. in: Ultradian Rhythms in Biological Systems, D. Lloyd, and E.L. Rossi, (eds.) (Springer, London), pp., in press.

Lloyd, D. and Kippert, F., 1987, A temperature-compensated ultradian clock explains temperature-dependent quantal cell cycle times in: Temperature and Animal Cells, K. Bowler, and B.J. Fuller, (eds.) (Society of Experimental Biologists, Cambridge) pp. 135–155.

Lloyd, D. and Volkov, E.I., 1990, Quantized cell cycle times: interaction between a relaxation oscillator and ultradian clock pulses. BioSystems 23, 305–310.

Lloyd, D. and Volkov, E.I., 1991, The ultradian clock: timekeeping for intracellular dynamics. in: Complex Dynamics and Biological Evolution, E. Mosekilde, (ed.) (Plenum, New York) pp. 51–60.

Lloyd, D., Edwards, S.W. and Fry, J.C., 1982a, Temperature-controlled oscillations in respiration and cellular protein content in synchronous cultures of *Acanthamoeba castellanii*. Proc. Natl. Acad. Sci. U.S.A. 79, 3785–3788.

Lloyd, D., Poole, R.K. and Edwards, S.W., 1982b, The cell division cycle: temporal control of cellular growth and reproduction. in: The Cell Division Cycle: Temporal Control of Cellular Growth and Reproduction (Academic, London).

Mackey, M.C., 1985, A deterministic cell cycle model with transition probability-like behaviour. in: Temporal Order, L. Rensing and N.I. Jaeger (eds.) (Springer–Verlag, Berlin) pp. 315–320.

Mustafin, A.T. and Volkov, E.I., 1977, On the distribution of cell cycle generation times. BioSystems 15, 111–126.

Nurse, P., 1990, Universal control mechanism regulating onset of M-phase. Nature 344, 503–508.

Pittendrigh, C.S. and Bruce, V.G., 1957, An oscillator model for biological clocks. Rhythmic and synthetic processes, in: Growth, L. Rudnick (ed.) (Princeton University Press) p. 75.

Prescott, D.M., 1976, Reproduction of Eukaryotic Cells (Academic, New York).

Rose, R.M. and Hindmarsh, J.L., 1985, A model of a thalamic neuron. Proc. R. Soc. London B225, 161–193.

Schaffer, W.M., Ellner, S. and Kot, M., 1986, Effects of noise on some dynamical models in ecology. J. Math. Biol. 24, 479–523.

Sel'kov, E.E., 1970, Two alternate self-oscillating stationary states in thiol metabolism—two alternate types of cell division normal and malignant ones. Biophysika 15, 1065–1073.

Smith, J.A. and Martin, L., 1973, Do cells cycle? Proc. Natl. Acad. Sci. U.S.A. 70, 1263–1267

Sweeney, B.M., 1982, Interaction of the circadian cycle with the cell cycle in *Pyrocystis fusiformis*. Plant Physiol. 70, 272–276.

Wolf, A., Swift, J.B., Swinney, H.L. and Vastano, J.A., 1985, Determining Lyapunov exponents from a time series. Physica 16D, 285–318.