Observ. Spec. Rep. 244 (1966); *J. Geophys. Res.* **73**, 1373 (1968); C. Sagan, J. Pollack, R. M. Goldstein, *Astron. J.* **72**, 20 (1967).
26. G. Colombo, *Nature* **208**, 575 (1965); ——— and I. I. Shapiro, *Astrophys. J.* **145**, 296 (1966).
27. H.-S. Liu, *Science* **159**, 306 (1968).
28. C. C. Counselman and I. I. Shapiro, *ibid.* **161**, 602 (1968).
29. P. Goldreich and S. J. Peale, *Astron. J.* **71**, 425 (1966); *Annu. Rev. Astron. Astrophys.* **6**, 287 (1968).
30. J. D. Anderson, L. Efron, G. E. Pease, *Astron. J.* **73**, S162 (1968).
31. P. M. Muller and W. L. Sjogren, *Science* **161**, 680 (1968).
32. B. T. O'Leary, *Nature* **220**, 1309 (1968).
33. The proportionality of the maximum spacecraft accelerations with the mascon masses would arise from Newton's law of gravitation for similar mascon depths and distributions beneath each circular mare.
34. J. Lorell and W. L. Sjogren, *Science* **159**, 625 (1968).
35. E. W. Hobson, *The Theory of Spherical and Ellipsoidal Harmonics* (Cambridge Univ. Press, London, 1931), p. 151.
36. M. J. Campbell, B. T. O'Leary, C. Sagan, *Science* **164**, 1273 (1969).
37. R. B. Baldwin, *Astron. J.*, in press.
38. S. T. Habibullin, *Sov. Astron. AJ Engl. Transl.* **2**, 622 (1958).
39. A. A. Yavokin, *Trans. Int. Astron. Union* **8**, 231 (1952).
40. J. E. Conel and G. B. Holstrom, *Science* **162**, 1403 (1968).
41. E. M. Shoemaker, R. J. Hackman, R. E. Eggleton, *Interplanetary Correlation of Geologic Time* (U.S. Geological Survey, Washington, D.C., 1961); R. B. Baldwin, *The Measure of the Moon* (Univ. of Chicago Press, Chicago, 1962).
42. F. Press, personal communication.
43. We also point out that uncompensated escarpments surrounding the circular maria may contribute significantly to the distribution of mass. For example, Muller and Sjogren's map (*31*) shows a positive gravity anomaly centered at the Altai escarpment.
44. J. G. Stipe, *Science* **162**, 1402 (1968); J. A. O'Keefe, *ibid.*, p. 1405; R. B. Baldwin, *ibid.*, p. 1407; H. C. Urey, *ibid.*, p. 1408.
44a. J. J. Gilvarry, *Nature* **221**, 732 (1969).
45. C. Oesterwinter, *Space Res.* **6**, 999 (1966). Newspaper reports by D. Kirkman (Scripps-Howard News Service, 26 December 1968) indicate that the lunar orbits of Apollo 8 were perturbed by mascons. The spacecraft was "dragged . . . almost one-third of a mile (toward the moon's surface) per revolution" and the extent of this acceleration was greater than NASA had anticipated, leading to the "suspicion that undetected (mascons) may be buried under the Moon's hidden side." Thus, the Apollo 8 orbital data seems to support our conclusion made when this paper was submitted for publication (1 month prior to the Apollo 8 mission). We await a detailed analysis of the orbits for a more accurate description of the accelerations.
46. The circularities, locations, and sizes of Martian bright areas (Table 2) are deduced from the following maps of Mars: a Roth globe (obtained from F. R. Engineering and prepared under the guidance of G. de Vaucouleurs), the Mars Planning Chart (Army Map Service), the MEC-1 prototype map (U.S. Air Force Aeronautical Chart and Information Center under the guidance of E. C. Slipher), the North American Aviation map of Mars (prepared under the guidance of A. Dollfus), and the International Astronomical Union map of Mars. Many basins that we have selected are only approximately circular—as is indeed the case for the "circular" maria on the moon.
47. D. G. Rea, *Nature* **200**, 114 (1963); *ibid.* **201**, 1014 (1964); R. A. Wells, *ibid.* **207**, 735 (1965); B. T. O'Leary and D. G. Rea, *Science* **155**, 317 (1967).
48. C. R. Chapman, J. G. Pollack, C. Sagan, *Smithsonian Astrophys. Observ. Spec. Rep. 268* (1968).
49. C. R. Chapman, *Earth Planet. Sci. Lett.* **3**, 381 (1967); D. P. Cruikshank and C. R. Chapman, *Sky Telescope* **34**, 24 (1967).
50. H. Camichel and A. Dollfus, in *Findings on Planetary Surfaces and Atmospheres from a Study of the Photographic Collections of the IAU Planetary Data Center at Meudon*, part I (Final Report of U.S. Air Force contract AF-61(052)-873, Observatoire de Meudon, France, 1967).
51. There is a possible categorization of mascon models into those in which there is a net gain of matter during the impact event, and those in which there is a net loss of matter. In a recent study by A. H. Marcus (*Icarus*, in press) it is suggested that impacts into the moon by objects with a velocity at great distances from the moon of 1 or 2 km/sec led to a net gain of matter; higher velocities led to a net loss. The value of the critical velocity depends on the bonding of the lunar surface material. The above numbers assume unbonded quartz sand. For better-bonded material, the critical velocities are considerably higher. It seems likely that if the lunar circular basins were produced by objects in roughly the same orbit as the moon—for example, by the final impacts in the accretion of the moon—then a net gain of matter occurred. If the basins were produced by higher velocity impacts—for example, at the present position of the moon but by asteroidal debris—then a net loss is likely to have occurred.
52. We are grateful to J. Winters and Y. S. Yang for performing calculations; to S. Soter, W. Sjogren, G. Pease, R. Tolson, H. Masursky, F. Press, T. Gold, R. Wells, S. Peale, and P. Gottlieb for helpful discussions. Supported in part by NASA grants NGL-33-010-005 and NGR-33-010-082.

Enzyme Synthesis in Synchronous Cultures

The patterns of enzyme synthesis suggest an ordered sequence of transcription throughout the cell cycle.

J. M. Mitchison

Some of the most important information on the cell cycle in recent years has come from studies of enzyme synthesis in synchronous cultures. They have thrown a new light on gene regulation in growing cells, and they have forced us to regard the cycle as a series of ordered chemical changes and not as a period of steady uniform growth between one cell division and the next. Most of the work has been done on bacteria and yeast, but there is some information on cells from higher forms, and certainly there will be more in the future. The whole of this field has not been reviewed before, but there is a good review article by Donachie and Masters (*1*) which considers the situation in bacteria.

The patterns of enzyme synthesis can be classified into two broad groups, depending on whether or not synthesis is continuous during the cycle, and each of these groups can be subdivided into two categories (Fig. 1). Most of the enzymes that have been examined (69 out of 84) are synthesized discontinuously at a particular stage of the cycle which is characteristic for each enzyme. If the enzyme is stable, the pattern is a "step" which resembles the pattern of DNA synthesis in higher cells. Each "step enzyme" therefore has its own G1, S, and G2 phase (to use the terminology for mammalian cells). A "peak enzyme" is also synthesized at one point in the cycle, but it is unstable and the activity falls off because of inactivation or breakdown of the molecule. The other, rarer group is that of enzymes which are formed continuously, and the simplest pattern is an exponential curve. Finally, a "linear enzyme" is one synthesized at a constant rate until a characteristic point in the cycle is reached, where the rate suddenly doubles. The results from most of the recent work have been divided into these categories and are listed in Tables 1 and 2.

This classification, like many others, is somewhat artificial and does not fit all cases. Unstable enzymes, for in-

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Table 1. Patterns of enzyme synthesis in synchronous cultures of growing cells (prokaryotes); S, step enzymes; P, peak enzymes; C(E), continuous exponential enzymes; C(L), continuous linear enzymes; (FR), fully repressed; (FD), fully derepressed or induced.

Strain	Enzyme*	Pattern	Reference
	Escherichia coli		
K12 Hfr CS-101-G-1	β-Galactosidase (FR)	C(L)	(5)
K12 Hfr CS-101-G-1	Alkaline phosphatase (FR)	C(L)	(5)
K12 Hfr CS-101-G-1	Aspartate transcarbamylase	S	(5)
K12 Hfr CS-101-G-1	Dihydroorotase	S	(5)
K12 Hfr CS-101-G-1	Histidinol dehydrogenase	S	(5)
K12 Hfr C(met⁻)	Glycyl-glycine dipeptidase	P	(34)
K12 Hfr H	Glycyl-glycine dipeptidase	S	(34)
K12 E64 (F^-, B_1^-)	Glycyl-glycine dipeptidase	P	(34)
ML 308	Glycyl-glycine dipeptidase	S (or P?)	(35)
ML 308	Leucine aminopeptidase	S (or P?)	(35)
ML 308	Protease	S (or P?)	(35)
B/r	β-Galactosidase (FD)	C(E)	(4)
B/r	β-Galactosidase (FD)	C(L)	(1)
B	β-Galactosidase (FD)	C(E)	(36)
	Bacillus subtilis		
W23	Aspartate transcarbamylase	S	(6, 16, 37)
W23	Ornithine transcarbamylase	S	(6, 16)
W23	Dehydroquinase	S	(6)
W23	Histidase	S	(6, 37)
W23	Alkaline phosphatase (FR)	C(L)	(16)
W23	Sucrase	S	(17)
W23	Sucrase (FR)	C(E)	(17)
	Rhodopseudomonas spheroides		
	Succinyl CoA thiokinase	S	(30)
	Aminolevulinic acid synthetase	S	(30)
	Aminolevulinic acid dehydrase	S	(30)
	Alkaline phosphatase	S	(30)
	Ornithine transcarbamylase	S?	(30)
	Ornithine transcarbamylase (FR)	C?	(30)

* The names of the enzymes in this and the other tables are those used in the papers cited and are not always those recommended by the Enzyme Commission.

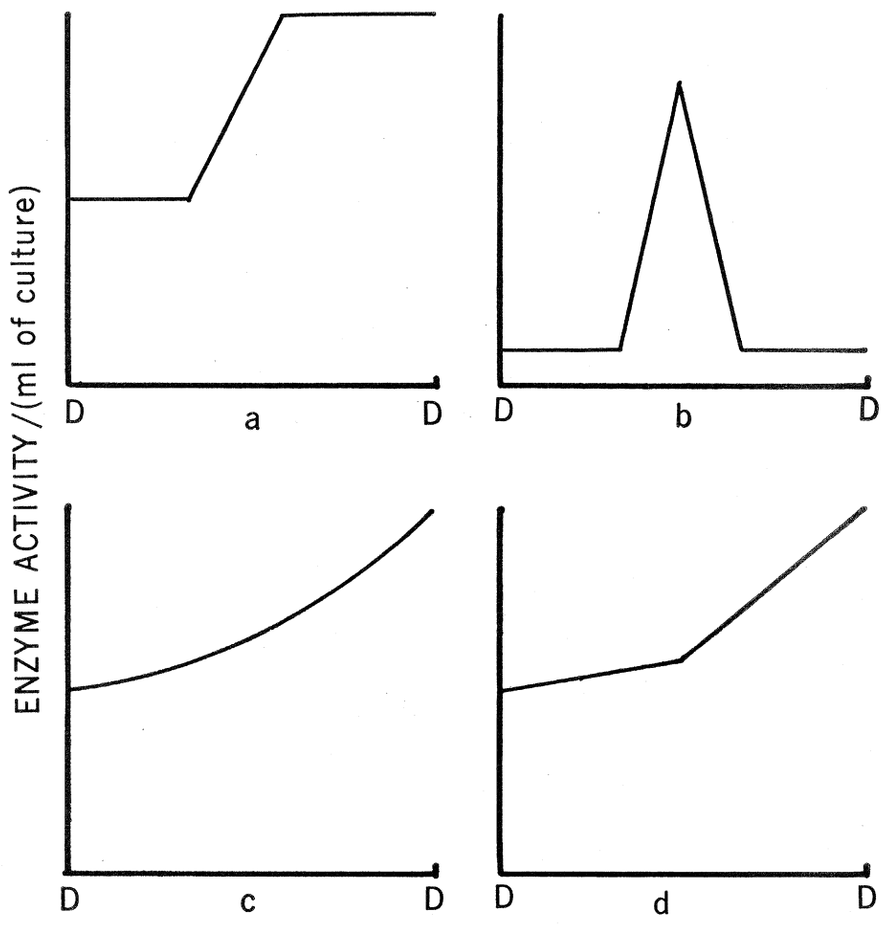

Fig. 1. Patterns of enzyme synthesis in synchronous culture during one cell cycle: (a) step; (b) peak; (c) continuous exponential; (d) continuous linear; *D*, cell division.

stance, could give a step pattern—a point that is discussed below. Also, continuous enzymes may follow a pattern different from either an exponential curve or linear segments (see, for example, *2*). It is difficult to show conclusively that the linear pattern exists, since there is a maximum difference of only 3 percent between an exponential curve that doubles in rate over a cycle and the straight line of best fit. The enzyme assays must be very accurate, and even then it is better to make a statistical analysis of the results and to run controls (*3*). The effort may be worth while, since there is an interesting interpretation that can be made of the linear pattern (as discussed below) and it may be that a number of the exponential enzymes are, in fact, linear ones. Fully induced β-galactosidase in *Escherichia coli* B/r is a case in point. It was originally reported to be a continuous enzyme (*4*), but, on closer examination, it seems to be linear (*1*).

Changes in Potential

Let us leave the possible explanations of these patterns for the time being and turn to another way of using enzyme assays with synchronous cultures. Samples can be removed from a culture and challenged to synthesize an enzyme by induction or derepression. The rate at which the enzyme is then made may be called the inducibility, or the "potential" (*5*), for that enzyme at that stage of the cell cycle. In bacteria, in the majority of cases the pattern of change in potential through the cycle (Table 3) is very similar to the pattern of synthesis for a step enzyme. The potential doubles sharply at a particular point in the cycle and then stays constant until the same point is reached in the next cycle.

There is good reason for supposing that this doubling in potential in bacteria is a manifestation of the doubling of the structural gene for the enzyme in question at that particular point in the cycle. The most direct evidence for this is the observation that the sucrase potential in *Bacillus subtilis* doubles at the same time that the sucrase-transforming ability of the DNA does (*6*). We would expect that the points of potential doubling for a series of enzymes would occur during the cycle in the same order and with the same spacing as the order and spacing of appropriate structural genes on the chromosome, provided DNA replication took the

whole cycle, and provided there was only one replicating fork. This has been shown to be approximately true for six enzymes in *Escherichia coli* B/r (*1*), and shown to be accurately true for three of them by means of an elegant technique in which induction precedes synchronization (*7*; see also *8*). We would also expect that there should be no increase in potential if DNA synthesis was blocked and growth continued. This has been shown to be the case for D-serine deaminase in *E. coli* 15 T$^-$ deprived of thymine (*9*) and for β-galactosidase in *E. coli* B/r after treatment with fluorodeoxyuridine or nalidixic acid (*8*). Finally, there is the general supporting evidence of gene-dosage effects on enzyme concentrations in *Neurospora* (*10*) and *E. coli* (*11*): the concentration of an enzyme increases as the number of copies of the appropriate gene increases.

All the bacterial data on the cell cycle agree with this scheme except for the work of Nishi and Horiuchi (*12*). They found that an F$^-$ strain of *Escherichia coli* K12 showed a continuous rise in potential, although the Hfr H strain had the usual step pattern. They suggested that this indicated a random origin of replication in the F$^-$ strain. Donachie and Masters (*1*) have suggested another explanation—that there may be an asynchrony of replication with respect to cell division which is produced by the conditions of synchronization. They have evidence that this can occur in the K12 strain. The fact that there is a stepwise increase in the potential of β-galactosidase in the K12 strain having the F factor carrying that gene, and that there is a continuous increase in the potential of D-serine dehydratase (whose gene is chromosomal), can be explained by assuming synchronous replication of the episome and asynchronous replication of the chromosome. The explanation, however, is a little forced, and this interesting situation needs further exploration.

The other bacterial situation that is perhaps relevant here is the enzyme potential in germinating spores of *Bacillus cereus* (*13*). Both α-glucosidase and histidase are inducible, but only at particular times during spore outgrowth. This restriction of potential is quite different from the continuous potential that is characteristic of the bacterial cycle. It remains to be seen whether or not the events of spore outgrowth are under the same controls as the cell cycle.

Apart, then, from these two cases, the position in prokaryotes seems to be straightforward—the potential is not restricted during the cycle and doubles when the gene doubles. Does the same thing hold true for eukaryotes? The answer here can only be a preliminary one because there is evidence from only two systems (see Table 4), but so far it is equivocal. In the fission yeast *Schizosaccharomyces pombe*, sucrase potential follows just the same stepwise pattern that it does in bacteria, but the time of doubling does not occur during DNA synthesis (*3*). In *Chlorella*, the potential for three enzymes (nitrite reductase, acid phosphatase, and alkaline phosphatase) follows a periodic pattern. The curve for potential rises to a peak in mid-cycle and then falls off (*14, 15*). Some of the fall may be due to the genome's becoming inaccessible during mitosis, but, whatever the reason, the situation is conspicuously different from that in prokaryotes, where there is no restriction of potential.

Linear Enzymes

Returning now to the normal patterns of enzyme synthesis set out in Tables 1 and 2, we can find a connection between linear enzymes and changes in potential. In bacteria the linear pattern has been found in repressed alkaline phosphatase in *Bacillus subtilis* (*16*), in fully induced β-galactosidase in *Escherichia coli* (*1*), and in repressed β-galactosidase and alkaline phosphatase in *E. coli* (*5*). It has been interpreted in the same way that the potential curves have been: the rate of enzyme synthesis doubles when the appropriate gene doubles. There is no reason to doubt the validity of this interpretation, but it rests on evidence which is more slender than that for the changes in potential. There is the general argument about gene dosage, given above, and there is also the fact that the rate-change points for two enzymes in *E. coli* are at the same place in the cycle

Table 2. Patterns of enzyme synthesis in synchronous cultures of growing cells (eucaryotes); S, step enzymes (numerals after S indicate more than one step per cycle); P, peak enzymes (numerals after P indicate more than one peak per cycle); C(L), continuous linear enzymes.

Enzyme	Pattern	Reference
Saccharomyces cerevisiae		
Protease	P	(*53*)
Peptidase	P	(*53*)
α-Glucosidase	S	(*20, 23, 26*)
α-Glucosidase	S (2 and 3)	(*26*)
Sucrase	S(2)	(*23*)
Alkaline phosphatase	S(2)	(*23*)
Histidinol dehydrogenase	S	(*20*)
Orotidine-5′-phosphate decarboxylase	S	(*20*)
Aspartokinase	S	(*20*)
Phosphoribosyl-ATP-pyrophosphorylase	S	(*20*)
Threonine deaminase	S	(*20*)
Argininosuccinase	S	(*20*)
Saccharopine dehydrogenase	S	(*20*)
Saccharopine reductase	S	(*20*)
Alcohol dehydrogenase	S	(*38*)
Hexokinase	S	(*38*)
Glyceraldehyde-3-phosphate dehydrogenase	S	(*38*)
DNA polymerase	P	(*39*)
Saccharomyces dobzhanskii		
β-Glucosidase	S	(*23*)
Saccharomyces dobzhanskii × *fragilis*		
α-Glucosidase	S	(*26*)
β-Glucosidase	S(2)	(*26*)
Alkaline phosphatase	S(2)	(*26*)
Schizosaccharomyces pombe		
Aspartate transcarbamylase	S	(*18*)
Ornithine transcarbamylase	S	(*18*)
Tryptophane synthetase	S	(*19*)
Alcohol dehydrogenase	S	(*19*)
Homoserine dehydrogenase	S	(*19*)
Alkaline phosphatase	C(L)	(*3*)
Acid phosphatase	C(L)	(*3*)
Sucrase	C(L)	(*3*)
Maltase	C(?)	(*18*)
Chlorella pyrenoidosa		
Aspartate transcarbamylase	C(?)	(*2*)
Deoxythymidine monophosphate kinase	P(?)	(*40*)
Deoxycytidine monophosphate deaminase	S(?)	(*41*)
Alkaline phosphatase	S	(*15*)
Acid phosphatase	S	(*15*)
Chlamydomonas reinhardtii		
Aspartate carbamoyltransferase (aspartate transcarbamylase)	S	(*42*)
Ornithine carbamolytransferase (ornithine transcarbamylase)	S	(*42*)
Phosphoenolpyruvate carboxylase	S	(*42*)
Alanine dehydrogenase	S	(*42*)
Glutamate dehydrogenase	S	(*42*)
Physarum polycephalum		
Thymidine kinase	P	(*43*)
Glucose-6-phosphate dehydrogenase	C(?)	(*43*)
Mouse L cells		
Thymidine kinase	S	(*44*)
DNA polymerase	P	(*49*)
Ribonucleotide reductase	P	(*50*)
DNA polymerase	C(?)	(*50*)
Thymidine kinase	P	(*51*)
Deoxycytidine monophosphate deaminase	P	(*51*)
Chinese hamster Don C cells		
Thymidine kinase	P	(*45*)
Glucose-6-phosphate dehydrogenase	P(3)	(*46*)
Lactate dehydrogenase	P(3)	(*46*)
Human HeLa cells		
Thymidine kinase	P	(*47*)
Thymidylate kinase	P	(*47*)
Alkaline phosphatase	P	(*48*)
DNA polymerase	P(?)	(*52*)
Human Henle cells		
Alkaline phosphatase	P	(*48*)

Table 3. Patterns of change in potential (inducibility) of enzymes in synchronous cultures (prokaryotes); S, step pattern; C, continuous increase.

Strain	Enzyme	Pattern	Reference
	Escherichia coli		
K12 Hfr CS-101-G-1	Aspartate transcarbamylase	S	(*5*)
K12 Hfr CS-101-G-1	Alkaline phosphatase	S	(*5*)
K12 Hfr CS-101-G-1	Tryptophanase	S	(*5*)
K12 58-161(F^-)	Tryptophanase	S	(*9*)
K12 58-161(F^-)	β-Galactosidase	S	(*9*)
K12 58-161 ($F'Lac^+/Lac^+$)	Tryptophanase	S	(*9*)
K12 58-161 ($F'Lac^+/Lac^+$)	β-Galactosidase	S(2)	(*9*)
K12 HfrH	β-Galactosidase	S	(*12*)
K12 HfrH	D-Serine dehydratase	S	(*12*)
K12 E52 + F_{18}	β-Galactosidase	S	(*12*)
K12 E52 + F_{18}	D-Serine dehydratase	C	(*12*)
K12 E64 (F^-)	β-Galactosidase	C	(*12*)
K12 E64 (F^-)	D-Serine dehydratase	C	(*12*)
$15T^-$	D-Serine deaminase	S	(*9*)
B/r	β-Galactosidase	S	(*1, 7–9*)
B/r	Tryptophanase	S	(*1, 7–9*)
B/r	D-Serine deaminase	S	(*1, 7, 8*)
B/r	Aspartate transcarbamylase	S	(*1*)
B/r	Dihydroorotase	S	(*1*)
B/r	Orotidine monophosphate pyrophosphorylase	S	(*1*)
	Bacillus subtilis		
W23	Sucrase	S	(*6*)

as the points of potential doubling (*5*).

Linear enzymes have been examined in only one eukaryotic cell, the yeast *Schizosaccharomyces pombe* (*3*). Three enzymes (sucrase, acid phosphatase, and alkaline phosphatase) have a linear pattern, and all of them double at a point in the cycle which is called the "critical point." This is also the point where the sucrase potential doubles. It occurs, however, during the G2 phase, a third of a cycle later than the very restricted period of DNA synthesis. This is the first evidence of a delay between the formation of a new genome and the time when it comes into use at the critical point—in other words, a delay between chemical replication and "functional" replication. Other physiological events take place at the critical point, and this suggests that what may be taking place is a change at the chromosomal level. Whatever may be the final explanation of the critical point, it does raise an interesting question for other eukaryotes. We know that an extra genome is made during the S period. When is it first used—at once (as in prokaryotes), or during the G2 phase (as in *S. pombe*), or only after it has separated as a new nucleus after mitosis?

Step and Peak Enzymes

The patterns of linear enzymes and of changes in potential appear to be closely associated with the replication of the genome. But several lines of evidence show that this is not the case with the step and peak enzymes, which make up the great majority of the enzymes of Tables 1 and 2. Steps of ornithine transcarbamylase in *Bacillus subtilis* continue after DNA replication has been blocked with fluorodeoxyuridine (*17*). The steps in aspartate transcarbamylase in *Escherichia coli* occur at a point in the cycle different from the point for potential doubling (*5*). In *Schizosaccharomyces pombe*, there are steps in aspartate transcarbamylase and ornithine transcarbamylase (*18*) and in alcohol dehydrogenase and homoserine dehydrogenase (*19*) which are spread through the cycle and do not occur at the time of DNA synthesis. Also, the nine enzymes whose steps have been mapped in budding yeast (*20*) are spread through three-quarters of the cycle, while DNA synthesis occupies only one-third (*21*).

This work shows that the discontinuous synthesis which is assumed to be responsible for the steps and peaks is not closely and causally related to DNA synthesis, and it raises the question of what other regulatory controls are involved. Here we meet two rival theories.

Oscillatory repression. The first theory, which may be called "oscillatory repression," has been developed primarily for prokaryotes, in a number of papers (*5, 6, 17, 22*); it is well expounded in the recent review by Donachie and Masters (*1*). In essence, the idea is simple and persuasive. A system where the product of an enzyme can repress the synthesis of that enzyme has negative feedback and, with a suitable choice of constants, will produce stable oscillations. When the pool of end product is high, the synthesis of the enzyme will be repressed; when the pool is low, the enzyme will be synthesized in a burst. The system generates its own steps, so they have been called "autogenous" (*5*). There is no obvious reason why these oscillations should have the same frequency that the cell cycle has, so that an enzyme step (or more than one, as in some eukaryotes) occurs at the same place in successive cycles. This objection has been circumvented by supposing that the oscillations are entrained by an event which is dependent on the cycle—for instance, a pulse of messenger RNA produced near the time of gene replication (*22*). Although the steps for different enzymes could come at varying and relatively long intervals after the entraining pulse, there is no reason to suppose that the steps bear any relation to the order of the controlling genes on the genetic map—a point I return to below.

The oscillations should occur when the enzyme is being controlled by end-product repression and is partially derepressed. If the enzyme is at basal level (complete repression) or is fully induced or fully derepressed, the oscillations should cease and the enzyme should be synthesized continuously, with synthesis probably following the linear pattern. The potential for induction or derepression should also be present throughout the cycle, and, in the simplest model, there should be no periods of restricted inducibility.

There is no doubt that oscillatory repression is an attractive theory for prokaryotes. It fits all the bacterial data of Table 1, and it provides a neat explanation of the observation that sucrase and alkaline phosphatase in *Bacillus subtilis* are linear (or exponential) enzymes when repressed and step enzymes when derepressed (*16, 17*). There are fewer opportunities to test it in eukaryotes, but in two cases at least it does not fit the data. It does not fit either the restricted inducibility in *Chlorella* (*14, 15*) or the observation that induced α- and β-glucosidases in yeast have step patterns rather than continuous ones, with the steps at the same point in the cycle as the steps in uninduced cultures (*23*). These steps, however, might be controlled by catabolite repression, which is less specific than the end-product repression of a biosynthetic enzyme.

Linear reading. The second theory of the control of step enzymes is one which has been developed by Halvorson

and his colleagues (*20, 23–26*) from their work on budding yeast, and which may be called "linear reading." This theory suggests that genes are transcribable for only a restricted part of the cycle, that they are transcribed in order, and that this order is the same as their linear sequence in the genome. As a result, the sequence of enzyme steps should be the same as the sequence of the appropriate genes on the chromosomes.

Several lines of evidence support this theory. *Saccharomyces dobzhanskii* has one β-glucosidase step per cycle. Its hybrid with *S. fragilis* produces two species of this enzyme which are immunologically distinct but subject to the same regulatory system. With oscillatory repression, one would expect one step per cycle. But, instead, there are two, suggesting that there are two nonallelic genes for the enzyme which are transcribed at different times in the cycle (see *23* and *24*; for a criticism, see *27*). This question of gene dosage has been followed up in a study of the multiple *M* genes for α-glucosidase in *S. cerevisiae* (*26*). The homozygote M_1M_1 and the heterozygote M_1m_1 both produce a single enzyme step per cycle, and always at the same place in the cycle, showing that an increase in gene dosage at a particular locus does not alter the step pattern. But the introduction of other nonallelic structural genes (M_2 and M_3) produces additional steps —two per cycle for two nonallelic genes and three per cycle for three genes. In a recent paper (*20*), the step timings for nine enzymes in *S. cerevisiae* are correlated with the position of their genes in the genetic map. The timings are consistent with linear transcription of the chromosomes from end to end, though not with transcription starting at the centromere and moving simultaneously along each arm.

As it stands, the linear reading theory is not easily applicable to prokaryotes—nor do Halvorson and his colleagues suggest that it is. It does not allow for continuous synthesis, or for potential being present throughout the cycle. A more telling criticism is that *Schizosaccharomyces pombe*, a fairly close relative of budding yeast, not only has three linear enzymes (as well as five step enzymes) but also has an unrestricted sucrase potential throughout the cycle (*3*). It has been suggested (*26*) that the continuous enzyme pattern might be in reality a number of steps caused by the presence of several nonallelic genes and not resolvable in the experimental curves. This, however, would not explain either the rate changes in the linear pattern or the changes in potential.

Table 4. Patterns of change in potential (inducibility) of enzymes in synchronous cultures (eukaryotes); S, step pattern; P, peak of potential in the cell cycle.

Enzyme	Pattern	Reference
Schizosaccharomyces pombe		
Sucrase	S	(*3*)
Chlorella pyrenoidosa		
Nitrite reductase	P	(*14*)
Acid phosphatase	P	(*15*)
Alkaline phosphatase	P	(*15*)

Discussion

Both of the theories for step enzymes are stimulating, and linear reading is particularly exciting since it suggests that there is a reason for the order of genes on the chromosomes—an order which is otherwise mysterious. But neither of them fit all the facts in prokaryotes and eukaryotes. Can we therefore devise crucial experiments to test the validity of the theories?

Linear reading is the easier theory to test. It predicts an invariant order for the enzyme steps, so it can be disproved by finding a case where the order is altered when the cycle is changed or distorted—for example, by changes in medium or temperature. Specific derepression can produce a step at an abnormal time in bacteria (*17*)—an observation which tells against the theory—and it will be important to see whether this is also true in the case of eukaryotes. For such a test, it would be better if the genes for the altered enzyme steps were on the same chromosome, since otherwise it could be argued that the changes in the cycle altered the rate of reading on different chromosomes. Another elegant test would be to alter the genome by an inversion and see whether the steps controlled by the inverted region were themselves inverted (*28*).

The most difficult problem here is to find a good synchronous system in a eukaryote that (i) has its genome well mapped for enzyme markers and (ii) has a small number of chromosomes. Things are much simpler in the case of prokaryotes, since *Escherichia coli* (especially strain K12) satisfies these criteria very well. This raises the problem of an important piece of work not yet discussed. Using sucrase potential as a marker for aligning the enzyme step map with the genetic map of *Bacillus subtilis*, Masters and Pardee (*6*) found that the order and timing of the steps of three enzymes (aspartate transcarbamylase, ornithine transcarbamylase, and dehydroquinase) during the cell cycle were the same as the order and spacing of the relevant genes on the genetic map. This result is mildly embarrassing for both theories. It is just what would be expected from linear reading, yet it is obtained in a prokaryote which has other properties (such as potential throughout the cycle) which do not agree with the linear reading theory. With oscillatory repression, on the other hand, there is no reason to postulate any identity between step order and genetic order (unless the time between the replication of a gene and its step is constant, in which case the steps in yeasts are difficult to explain). It would certainly be worth while to follow up this work and see whether the identity was a matter of chance or whether it holds good with a larger number of genes and enzymes.

The oscillatory repression theory is much more difficult to test (*1*). If the end-product pool was found to fluctuate, this would fit in with the theory but it would not prove that the pool fluctuations caused the enzyme fluctuations, since the converse might be equally true. Enzyme synthesis might be switched on and off by another mechanism, and this would produce pool oscillations. If the pool was found not to fluctuate, then it might be argued either that this pool was not the controlling one or that this pool was partitioned spatially in the cell and the measurements could not resolve the controlling fluctuations within one of the compartments. This does not mean that pool measurements are worthless, since they provide vital data about the cycle, but they cannot give the crucial test for this theory. The outworks of the theory are easier to attack. It will be important to see whether other eukaryotes show the same combination of step enzymes with restricted potential that has been found in *Chlorella* (*14, 15*). It will also be important to find if enzymes in eukaryotes change from a continuous pattern to a step pattern and then back again as the degree of repression decreases. The practical difficulty with such tests, of course, is to find repressible enzymes

in many of the eukaryotic systems. Another interesting test in either prokaryotes or eukaryotes would be to investigate those enzymes on a pathway which are repressed by the same end product. With oscillatory repression, their steps should be simultaneous, or nearly so.

It is much too early to say whether either theory is correct or to say that both are correct and that there are quite different methods of regulation in the prokaryotes and the eukaryotes. I find it difficult to believe that the mechanisms of regulation are totally different, and I am inclined to take a compromise position which admits some of both theories. Oscillatory repression could occur in situations where the gene was not transcribable for certains parts of the cycle (as must be the case during mitosis in higher cells). It could also occur in what appeared to be a constitutive enzyme in the sense that its concentration could not be changed by agents applied to the cell. Linear reading might take place in normal cycles even though some of the genes involved could, with the appropriate stimulus, be transcribed at other points of the cycle. It is also possible, as Tauro, Halvorson, and Epstein (*20*) have suggested, that linear reading might take place over restricted regions of the genome rather than running sequentially along the whole chromosome. In any case, these two methods of regulation may not be the only ones. Neither of them seems very suitable as an explanation either of the single, widely spaced synthetic events that are likely to be the chemical basis of development in higher organisms or of the periods of synthesis following stimulation that are characteristic of specialized cells that are outside the cell cycle (for example, cells in digestive glands).

One final point should be made about the control of step enzymes. In the theories it is assumed that the step pattern is produced by stable enzymes, and the peak pattern by unstable enzymes. But there is some evidence that step enzymes are, or at least can be, unstable (*1, 16*). Steps could be produced by the continuous production of an unstable enzyme with a doubling in the rate of production at the time of the step. This doubling would change the enzyme concentration from one steady state to another, higher one (*16*). As in the case of the linear pattern, this doubling in rate would be expected to occur at the time of doubling of the structural gene. The prediction would be that this type of step, unlike the step of a stable enzyme, would be stopped if DNA synthesis were inhibited. This prediction has not, however, been tested.

The data of Tables 1 to 4 may seem impressive, but the fact is that we need a lot more information if this field is to be clarified. The most obvious general gap is in our knowledge about enzyme patterns in eukaryotic cells other than yeast. There is some information, but it is still fragmentary, with a definite bias toward the enzymes concerned with DNA synthesis.

Another large gap, at all cellular levels, is our ignorance about the balance of these patterns of synthesis in giving the curve of increase of total cell protein. Is the step pattern the dominant one, with the steps spread through the cycle to give a continuous increase of total protein? Or is the linear pattern the dominant one, giving a pattern for total protein with a rate change at a particular point in the cycle which is related to the time of DNA synthesis (*29*)? Or are there quite different patterns for structural proteins or ribosomal proteins which outweigh any of the enzyme patterns? Also, we know little about the patterns of different categories of enzymes. Is there, for instance, a tendency for biosynthetic enzymes in eukaryotes to follow a step pattern, and for catabolic enzymes to be continuous?

Throughout this article I have been discussing enzyme synthesis, but what has been measured is enzyme activity. No one in this field has yet shown that the increase in activity at a stage in the cycle is directly due to a net synthesis of enzyme protein. We cannot, in practice, expect this to be shown frequently, since the work involved in isolating and measuring the amount of an enzyme is very much greater than that involved in a simple measurement of activity. But it should be realized that substantial errors, especially in timing, may come from equating activity with synthesis. A case in point is alkaline phosphatase in *Schizosaccharomyces pombe* (*3*). Experiments with cycloheximide indicate that there is a delay of about half an hour (20 percent of the cycle) between synthesis of the enzyme precursor and its final activation. Alkaline phosphatase is a linear enzyme; thus, at a particular point in the cycle, there is a change in the rate of increase of its activity. The "precursor delay," however, means that the rate of synthesis changes half an hour before this point in the cycle.

Two features characteristic of most of the published results on step enzymes are variability in the time of the step and imprecise doubling of the enzyme activity at the step (*6, 18, 30*). Some—perhaps all—of this variability may be due to experimental errors, but it would be useful to know whether there is a genuine variability between different synchronous cultures and between successive cycles. On the basis of the linear reading theory we should not expect such variation, but oscillatory repression involves a relatively loose coupling between enzyme steps and the cell cycle, and variation in timing would not be surprising. But we should remember that what is being measured in a synchronous culture is the average behavior of many millions of cells, and so variations which might be expected at the single-cell level would not be manifest in the whole culture. I might point out that, from the standpoint of the cell, doubling the amount of a protein is a very different problem, at the chemical level, from doubling the amount of DNA. The mechanism of DNA replication allows a precise doubling, but this elegant molecular machinery does not apply to protein. On the other hand, whether or not enzymes are exactly doubled may be unimportant to a cell.

The presence of step enzymes is in some ways surprising. A cell, during interphase, grows without any visible signs of differentiation (*31*), and the simplest model of the chemical system within it would be a collection of enzymes and other components which increased in total bulk but did not change their relative proportions. Instead, we now find a system in which the enzymes suddenly double at different points in the cycle. This would seem to be an excellent way of unbalancing the whole process of growth, yet the cell's growth, in terms of gross parameters such as total protein or volume, is a smooth, continuous process. Clearly we do not know enough to resolve this apparent paradox. Steps in a planned order may be the cell's best way of achieving its objective of doubling itself, or they may be a necessity of regulation which the cell has to put up with and adapt to, or they may be the markers of some fundamental timing mechanism.

In many cells the only markers which can be used to chart the progress through the cell cycle are mitosis and the beginning and end of DNA synthesis—hence the division into the phases G1, S, G2, and M. The discovery of step enzymes means that we are now in a position to provide many more markers. I have discussed the possibilities of marker analysis elsewhere (*32*), and it is sufficient here to give two examples. Linear reading being assumed, the question of whether all the cells in a synchronous culture stop together at the same stage of the cycle on reaching stationary phase can be answered with precision by finding the last enzyme step that is expressed. If enzyme markers could be detected in cells in tissues, it would be possible to learn whether the "G2 cells" described by Gelfant (*33*) are stationary in the G2 phase or are passing slowly through it and thus showing a series of enzyme steps.

The most important general point about the enzyme patterns is that they lead to a new concept of the cell cycle. Until recently it has been possible to regard the cycle as a period of steady growth in which all the components increase at the same rate and in which there is no change in the chemical composition except one of increasing bulk. The main exceptions to this concept were the discontinuous synthesis of DNA in many cells and the morphological changes at the end of the cycle, when the cell entered mitosis. The new cell cycle is different and much more complicated, since the enzyme patterns imply a chemical composition that is continually changing. Cell growth is not a smooth, continuous process but rather a prescribed sequence of discontinuous events. The parallel with embryology now becomes very close. Both the growing cell and the growing embryo show an ordered pattern of chemical syntheses. Both show morphogenesis, that of the cell occurring not only in mitosis but also in the growth of the organelles. In both, the same pattern of events recurs in the next generation, and, in both, the fundamental problem is that of gene regulation and expression. In a very real sense those who work on the cell cycle are studying the developmental biology of the cell.

Summary

Most enzymes are synthesized discontinuously at periods in the cell cycle which are characteristic for each enzyme. If the enzyme is stable, this produces a "step" pattern similar to that for DNA in higher cells. If the enzyme is unstable, a "peak" pattern is produced.

Two theories have been put forward to explain this periodic ordered pattern of synthesis. "Ocillatory repression," primarily developed for prokaryotes, suggests that the periods of synthesis are due to oscillations in the negative-feedback system of an enzyme with end-product repression, and that these oscillations are entrained in phase with the cell cycle. "Linear reading," primarily developed for eukaryotes, suggests that the genes are transcribed in a sequence which corresponds to their order on the chromosomes. Both theories fit some but not all of the facts, and it is too early to decide between them.

A few enzymes are synthesized continuously, the pattern of synthesis being linear, with a doubling in rate at a particular point in the cycle. In the majority of systems there is also a doubling in the rate of inducibility (or "potential") of an enzyme at a particular point in the cycle. The points of rate doubling correspond with the functional replication of the appropriate genes. In bacteria, the functional replication occurs at the same time as the chemical replication; in yeast, it occurs later.

These patterns imply that the cell cycle (and cell growth) is an ordered sequence of syntheses, with continuous change in the chemical composition of the cell.

References

1. W. D. Donachie and M. Masters, in *The Cell Cycle*, G. M. Padilla, G. L. Whitson, I. L. Cameron, Eds. (Academic Press, New York, 1969).
2. F. E. Cole and R. R. Schmidt, *Biochim. Biophys. Acta* **90**, 616 (1964).
3. J. M. Mitchison and J. Creanor, *J. Cell. Sci.*, in press.
4. D. J. Cummings, *Biochim. Biophys. Acta* **85**, 341 (1965).
5. P. L. Kuempel, M. Masters, A. B. Pardee, *Biochem. Biophys. Res. Commun.* **18**, 858 (1965).
6. M. Masters and A. B. Pardee, *Proc. Nat. Acad. Sci. U.S.* **54**, 64 (1965).
7. C. E. Helmstetter, *J. Bacteriol.* **95**, 1634 (1968).
8. M. L. Pato and D. A. Glaser, *Proc. Nat. Acad. Sci. U.S.* **60**, 1268 (1968).
9. W. D. Donachie and M. Masters, *Genet. Res.* **8**, 119 (1966).
10. W. D. Donachie, *Biochim. Biophys. Acta* **82**, 293 (1964).
11. J. Pittard and T. Ramakrishnan, *J. Bacteriol.* **88**, 367 (1964).
12. A. Nishi and T. Horiuchi, *J. Biochem. (Tokyo)* **60**, 338 (1966).
13. W. Steinberg and H. O. Halvorson, *J. Bacteriol.* **95**, 469 (1968); *ibid.*, p. 479.
14. G. Knutsen, *Biochim. Biophys. Acta* **103**, 495 (1965).
15. ———, *ibid.* **161**, 205 (1968).
16. W. D. Donachie, *Nature* **205**, 1084 (1965).
17. M. Masters and W. D. Donachie, *ibid.* **209**, 476 (1966).
18. C. J. Bostock, W. D. Donachie, M. Masters, J. M. Mitchison, *ibid.* **210**, 808 (1966).
19. A. A. Robinson, unpublished results.
20. P. Tauro, H. O. Halvorson, R. L. Epstein, *Proc. Nat. Acad. Sci. U.S.* **59**, 277 (1968).
21. D. H. Williamson, in *Cell Synchrony*, I. L. Cameron and G. M. Padilla, Eds. (Academic Press, New York, 1966).
22. B. C. Goodwin, *Nature* **209**, 479 (1966).
23. H. O. Halvorson, R. M. Bock, P. Tauro, R. Epstein, M. LaBerge, in *Cell Synchrony*, I. L. Cameron and G. M. Padilla, Eds. (Academic Press, New York, 1966).
24. J. Gorman, P. Tauro, M. LaBerge, H. Halvorson, *Biochem. Biophys. Res. Commun.* **15**, 43 (1964).
25. H. O. Halvorson, J. Gorman, P. Tauro, R. Epstein, M. Laberge, *Fed. Proc.* **23**, 1003 (1964).
26. P. Tauro and H. O. Halvorson, *J. Bacteriol.* **92**, 652 (1966).
27. L. W. Fleming and J. D. Duerksen, *ibid.* **93**, 142 (1967).
28. H. O. Halvorson, personal communication.
29. D. M. Prescott, *J. Cell Biol.* **31**, 1 (1966);
30. E. Stubblefield, R. R. Klevecz, L. Deaven, *J. Cell Physiol.* **69**, 345 (1967).
31. J. J. Ferretti and E. D. Gray, *J. Bacteriol.* **95**, 1400 (1968).
32. E. Robbins and M. Scharff, in *Cell Synchrony*, I. L. Cameron and G. M. Padilla, Eds. (Academic Press, New York, 1966).
33. J. M. Mitchison, in *The Cell Cycle*, G. M. Padilla, G. L. Whitson, I. L. Cameron, Eds. (Academic Press, New York, 1969).
34. S. Gelfant, *Exp. Cell Res.* **26**, 395 (1962).
35. A. Nishi and S. Hirose, *J. Gen. Appl. Microbiol. (Tokyo)* **12**, 293 (1966).
36. T. Kogoma and A. Nishi, *ibid.* **11**, 321 (1965).
37. F. E. Abbo and A. B. Pardee, *Biochim. Biophys. Acta* **39**, 478 (1960).
38. M. Masters, P. L. Kuempel, A. B. Pardee, *Biochem. Biophys. Res. Commun.* **15**, 38 (1964).
39. H. Eckstein, V. Paduch, H. Hilz, *Biochem. Z.* **344**, 435 (1966).
40. ———, *European J. Biochem.* **3**, 224 (1967).
41. R. A. Johnson and R. R. Schmidt, *Biochim. Biophys. Acta* **129**, 140 (1966).
42. S. R. Shen and R. R. Schmidt, *Arch. Biochem. Biophys.* **115**, 13 (1966).
43. J. R. Kates and R. F. Jones, *Biochim. Biophys. Acta* **145**, 153 (1967).
44. W. Sachsenmaier and D. H. Ives, *Biochem. Z.* **343**, 399 (1965).
45. J. W. Littlefield, *Biochim. Biophys. Acta* **114**, 398 (1966).
46. E. Stubblefield and S. Murphree, *Exp. Cell Res.* **48**, 652 (1967).
47. R. R. Klevecz and F. H. Ruddle, *Science* **159**, 634 (1968).
48. T. P. Brent, J. A. V. Butler, A. R. Crathorn, *Nature* **207**, 176 (1965).
49. G. Melnykovych, C. F. Bishop, M. A. B. Swayze, *J. Cell Physiol.* **70**, 231 (1967).
50. M. Gold and C. W. Helleiner, *Biochim. Biophys. Acta* **80**, 193 (1964).
51. M. K. Turner, R. Abrams, I. Lieberman, *J. Biol. Chem.* **243**, 3725 (1968).
52. C. Mittermayer, R. Bosselmann, V. Bremerskov, *European J. Biochem.* **4**, 487 (1968).
53. D. L. Friedman and G. C. Mueller, *Biochim. Biophys. Acta* **161**, 455 (1968).
54. B. Sylvén, C. A. Tobias, H. Malmgren, R. Ottoson, B. Thorell, *Exp. Cell Res.* **16**, 75 (1959).