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Discontinuous Variability, in the Form of a Geometric Progression, of Albumin Production in Hepatoma and Hybrid Cells

(reiterated genes/control of gene expression and differentiation)

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ABSTRACT A clonal rat hepatoma cell line (Fu5) produces rat serum albumin at a constant rate over at least 3 months of continuous cultivation. Ten hybrid cell clones derived from the fusion of Fu5 cells and mouse fibroblasts, as well as 14 hepatoma subclones of Fu5 cells, all produce albumin but at different rates, ranging from about 0.09 to 36.7 μg/mg of protein per 72 hr. Despite this variability in albumin production, the distribution of clones is not random but discontinuous, with both hepatoma and hybrid clones clustering around discrete values that can be fitted to the geometric progression: a, $a(\sqrt{2})^1$, $a(\sqrt{2})^2$..... $a(\sqrt{2})^n$. The values of the majority of clones fall into alternate members of this geometric progression, which differ by a factor of 2. Hepatoma subclones with indistinguishable karyotypes differ in level of albumin production by as much as 4-fold. In contrast to hepatoma clones, albumin production in hybrid clones decreases with increasing cell generations. A survey of 28 enzymes of different hepatomas reveals a large variability in enzyme levels which, for most enzymes, can be arranged into classes that form a geometric progression. The apparent widespread nature of this discontinuous phenotypic variability suggests that it may reflect a basic mechanism of control of gene expression in animal cells.

Phenotypic changes occur in animal cells in culture at a much higher frequency than would be expected as a result of mutation, i.e., a change in nucleotide sequence of the structural gene. The rate of mutation in bacteria is about 10^{-9}–10^{-6} per cell per generation, while studies with mouse myeloma cells reveal a higher rate of phenotypic change (10^{-3} per cell per generation) (1). Studies of the effect of ploidy on rate of "mutation" in hamster (2) and frog (3) cells are also incompatible with changes in nucleotide sequences being involved.

Phenotypic variability is also observed in neoplasia. Not only do different tumors arising from the same tissue differ in their degree of malignancy, but they also have different morphological, biochemical, and growth properties (4). Since many phenotypic changes observed in both tumors and cell lines are apparently not genetic, they may reflect a perturbation of normal cellular mechanisms controlling cell differentiation, for differentiation is generally presumed to involve selective gene expression rather than irreversible changes in genetic information. Thus, the study of phenotypic variability could help to elucidate the control of differentiation and neoplasia.

My studies on the production of serum albumin in rat hepatoma cells and rat hepatoma–mouse fibroblast hybrids reveal two novel examples of phenotypic variability. One is the induction of mouse albumin production in rat hepatoma–mouse fibroblast hybrids (5) and the other is the subject of this paper. Since the mouse fibroblasts did not produce mouse albumin, its production by the hybrids must result from the activation of a gene(s) that was repressed during differentiation. These results have been confirmed in other interspecific hybrid cells (6, 7). The reappearance in hybrids of enzymes that were lost during cultivation *in vitro* of one of the parental cell lines has also been observed (8, 9).

The present paper deals with the other example of phenotypic variability in hepatoma and hybrid cells, namely a variation in constitutive level of albumin production.

MATERIALS AND METHODS

Cells were cultured in a modified Ham's F12 medium supplemented with 5% fetal-calf serum, as described (5).

The origins of cell lines Fu5 and 3T3 and the isolation of the hybrids used in these studies have been described (10). All other hepatoma clones are subclones of Fu5.

To assay for the production of rat serum albumin (RSA), 2 × 10^6 hepatoma, 1.5 × 10^6 3T3, or 1 × 10^6 hybrid cells were seeded in 250-ml Falcon plastic tissue-culture bottles containing 15 ml of medium. For each cell type, the number of cells seeded was such that they just approached confluency after 72 hr of growth. After an incubation period of 72 hr, the medium was removed. The cells were detached from the bottle with trypsin, collected, and counted, and an aliquot was washed once with phosphate-buffered saline (11) and then frozen for protein assay at a later date by the method of Lowry (12).

Rat albumin was assayed by microcomplement (C′) fixation (13) with a total reaction volume of 7 ml. All reactants were diluted in the standard C′ fixation buffer (10 mM Tris·HCl, pH 7.4; 0.15 M NaCl; 0.15 mM $CaCl_2$; 0.5 mM $MgSO_4$; and 0.1% gelatin w/v). Rabbit antiserum against rat albumin was prepared from rabbits immunized with purified rat albumin as described (5). One antiserum from a single bleeding that had good complement fixation activity was used throughout. Maximum C′ fixation was obtained with 0.085 μg of purified rat albumin per reaction tube (Fig. 1) and this was used as a standard for estimating albumin content in growth media. This purified rat albumin is shown to be identical to that in freshly prepared rat serum since the two C′ fixation curves superimpose completely (14). The rat serum dilution that gives maximum C′ fixation is 1:400,000, equivalent to 0.085 μg of albumin. Samples of medium to be assayed were centrifuged at 500 × g for 5 min, heated at 60° for 20 min, dialyzed against two changes of 100 times the sample volume of C′ fixation buffer without gelatin, centrifuged at 27,000 × g for 30 min, and then frozen until assayed. Dialysis of the medium samples allowed addition of up to 4 ml of sample to the reaction mixture rather than 1 ml as in the original technique, thus increasing the sensitivity of the assay 4-fold.

Control experiments with purified rat albumin diluted in dialyzed growth medium gave a peak of C′ fixation at the same albumin concentration as when it was diluted in C′ fixa-

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Abbreviations: C′, complement; RSA, rat-serum albumin.

TABLE 1. *Rat albumin production in hepatoma–fibroblast hybrids*

Class	Hybrid clone	µg/mg of protein per 72 hr†		P*	No. of generations
8	3F12	2.95		<0.01	25
	3F11	2.90 (3.04,2.76)		<0.01	33
		2.9 ± 0.08‡	[2.5]		
6	3F13	1.33		<0.02	27
	3F14	1.28 (1.17,1.4)		<0.02	26
		1.3 ± 0.07‡	[1.25]		
4	3F15	0.85 (0.74,0.94)		n.s.	26
	3F16	0.66 (0.64,0.68)		<0.05	26
	3F10	0.78		<0.05	52
		0.76 ± 0.05‡	[0.625]		
2	3F1a	0.37 (0.38,0.37)	[0.31]	<0.05	42
0	3FCa§	~0.12			85
	3F75m§	~0.09			—

* Probability that the rate of production is the same as the average rate of the closest adjacent class.

† Values in *brackets* are of a theoretical geometric progression (see *Results*).

‡ Mean of class ± SEM.

§ Amount of albumin produced is at lower limit of assay.

n.s. = not significant.

tion buffer. Standard deviation for repeated assays of a single medium collection and for assays of media collected on different days from a given clone were similar, between 10 and 16% when expressed per mg of cell protein. Samples frozen for as long as 6 months showed no significant change in content of rat albumin detectable by C′ fixation.

RESULTS

Ten hybrid cell clones derived from the fusion of rat hepatoma cells (clone Fu5) with 3T3 mouse fibroblasts secrete rat-serum albumin (RSA) into their growth medium. For Fu5 cells, the production of RSA represents 3% of protein synthesis, while 3T3 fibroblasts do not produce albumin (5). The identity of the RSA produced by the hybrid cells has been demonstrated by immunodiffusion (5) and by micro-C′ fixation. Similar curves of C′ fixation activity are obtained with media from hepatoma and hybrid cells (Fig. 1*b*). This similarity demonstrates the identity of the antigens (15). Media from 3T3 cells show no C′ fixation activity (Fig. 1*b*). The slightly lower height of the peak (10–20%) seen for media from both hepatoma and hybrid cells (Fig. 1*b*) as compared to the standard of purified RSA (Fig. 1*a*) is assumed to be due to a small amount of crossreaction of the antibodies against RSA with components of fetal-calf serum in the growth medium that bind a small fraction of the antibodies against RSA but do not fix complement (16), rather than to any difference in the albumin molecules. This is shown by the fact that a similar reduction in the maximum C′ fixation is seen when purified RSA is diluted in growth medium rather than C′ fixation buffer (Fig. 1*c* and *d*). Medium alone does not fix complement (Fig. 1*d*). None of Fu5–3T3 hybrids produces mouse albumin (5).

Notwithstanding that all 3T3–Fu5 hybrids produce RSA, they produce less than Fu5 and differ drastically from one another in amount. The difference between the lowest and

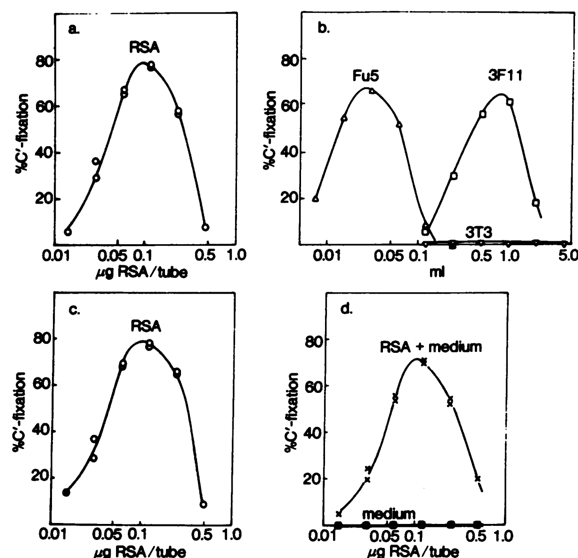

FIG. 1. C′ fixation curves obtained with antibody against rat albumin, diluted 1:1800; and rat albumin (RSA) (○); (*b*) medium from hepatoma (△), hybrid (□), or fibroblast (▽) cells; (*d*) rat albumin diluted in growth medium (×); or growth medium alone (■). (*a* and *c*): standard curves for the two indedependent assays shown in (*b*) and (*d*), respectively.

highest producing clones is more than 20-fold; however, the hybrids are not distributed randomly but cluster around discrete values, which can be fitted to a geometric progression where successive group values differ by a factor of 2 (Table 1).

Since there is some loss of chromosomes in all of the hybrid clones (7–16%) (10), it is possible that the respective levels simply represent the number of chromosomes carrying the albumin gene present in the particular hybrid. This is an unlikely interpretation since it would not explain the hybrid classes forming a geometric progression. Moreover, there is no correlation between the gross karyotype (10) of the hybrids and the level of albumin they produce.

The observation that the hybrids that have gone through the greatest number of generations produce the least albumin (Table 1) suggests that albumin production in these hybrids is unstable. If it is unstable, then the existence of discrete classes suggests that the loss of the ability to produce albumin occurs discontinuously. It is also possible that clone Fu5 contains subpopulations that produce albumin at different rates and that these gave rise to the different hybrid clones.

In order to investigate the stability of albumin production, hepatoma clone Fu5 and one hybrid clone 3F11 were grown with weekly transfers for 73 and 54 generations, respectively, and albumin production was monitored periodically. The rate of production of the hepatoma clone remained constant (13.8 ± 0.5 µg/mg of protein per 72 hr) throughout (100 days). In contrast, production by the hybrid clone decreased rapidly during the first month of cultivation from an initial rate of 2.9, and then leveled off during the next month and a half at about 1.0. Therefore, albumin production may drop discontinuously in this hybrid clone. The greater instability of the hybrid clone is also seen in its karyotype. There is no significant change in the karyotype of the hepatoma cells during the cultivation period, while the chromosome number decreases in the hybrid. From an initial total chromosome number of 113.0 (107–118) at day 0, after 32 days the total dropped to 102.1 (93–107) and than did not change significantly for the following 45 days.

TABLE 2. *Rat albumin production in hepatoma clone Fu5 and subclones*

Class	Hepatoma clone	μg/mg of protein per 72 hr*		Statistically significant A†	Statistically significant B‡
16	Fu5-8	36.7	(1)	<0.02	<0.01
	Fu5-2	36.0	(1)	<0.02	<0.01
		36.3¶	[40.0]		
14	Fu5	22.7 ± 1.23	(4)	<0.001	<0.001
	Fu5-8	21.3 ± 1.42	(3)	<0.01	<0.02
	Fu5-7	20.8 ± 1.13	(3)	<0.001	<0.001
	2sFu5-cl.1E	25.6	(1)	<0.05	<0.01
	967.3	23.7	(1)	<0.05	<0.02
		22.8 ± 0.86¶	[20.0]		
13	Fu5-2	15.8 ± 0.57	(7)	<0.001	<0.001
	967.4	14.0	(1)	<0.02	<0.01
	Fu5	13.8 ± 0.50	(5)	<0.001	<0.001
		14.5 ± 0.64¶	[14.1]		
12	967	9.2 ± 0.41	(4)	n.s. (<0.001)§	0.1-0.05
	967.4	9.0 (9.1,8.9)	(2)	n.s.	n.s.
	967.5	9.8	(1)	n.s.	n.s.
	967.7	9.6	(1)	n.s.	n.s.
	967.8	11.0	(1)	n.s.	n.s.
	963	10.3	(1)	n.s.	n.s.
		9.8 ± 0.30¶	[10.0]		
11	967.6	7.93 ± 0.76	(3)	n.s.	<0.05
	967.9	7.87 ± 0.61	(3)	n.s.	<0.05
		7.9 ± 0.75¶	[7.1]		
10	Fu5-5	5.9 ± 0.13	[5.0]	<0.02	<0.02

* Values in *brackets* are of a theoretical geometric progression (see *results*) and integers in *parentheses* are numbers of assays of media collected on different days.

† Probability that the rate of production is the same as that of the closest clone in the adjacent class.

‡ Probability that the rate of production is the same as the average rate of the closest adjacent class.

§ Significantly different from clone Fu5.

¶ Mean of class ±SEM.

n.s. = not significant.

The possibility that hepatoma clone Fu5 contains subpopulations that produce albumin at different rates was tested by subcloning. The 14 subclones of Fu5 differ among each other in rate of albumin production (Table 2); but, like the hybrid clones, they fall into discrete classes. In Table 2 albumin production is expressed per mg of protein; however, when expressed per 10^6 cells, the same classes are observed.

The assigning of clones to classes was justified statistically. Each member of a class was compared both to the closest member of the adjacent class by the Student's *t*-test either by difference from a mean or difference between two means (column *A* in Table 2), and to the mean value of the closest adjacent class taken as a group (column *B* in Table 2). By this type of analysis, the arrangement of classes is justified in a highly significant manner for all of the hepatoma clones except for 967.6 and 967.9, which are not significantly different from clone 967 in the class immediately above. However, they are significantly different from the mean of the closest adjacent class and, therefore, have been assigned to a separate class.

As was the case for hybrid clones, the classes of hepatoma clones can be arranged in a geometric progression. The majority fall into classes that continue up the geometric series observed with the hybrids where successive classes in the series increase by a factor of 2. (These have been arbitrarily assigned even class numbers in Table 1 and 2.) The remaining five hepatoma clones fall into two classes (class 11 and 13 in Table 2) that do not fit the above geometric series. However, in a series where successive members differ by a factor of $\sqrt{2}$, all of the groups of hepatoma and hybrid clones will fit; with the majority in alternate members of the series, differing by a factor of 2. That they fit such a geometric series has been demonstrated by regression analysis where the experimental data is compared to the values (shown in brackets in Tables 1 and 2) of the theoretical geometric progression: a, $a(\sqrt{2})^1$, $a(\sqrt{2})^2$, $a(\sqrt{2})^3$, ... $a(\sqrt{2})^n$ (a = constant). The resulting correlation coefficient is +0.994, which, with 8 degrees of freedom, is highly significant.

TABLE 3. *Chromosome numbers of five hepatoma clones**

Hepatoma clone	μg of albumin produced	Total	A	B	C	D
Fu5	22.7	51.7(50-53)	26.8	22.5	0.45	1.9
Fu5-8	21.3	53.3(51-55)	28.5	22.1	0.9	1.9
Fu5-2	15.8	49.3(47-52)	27.3	19.9	0.2	1.9
967	9.2	51.8(50-55)	26.0	22.9	1.0	2.0
Fu5-5	5.9	52.0(51-53)	26.1	22.6	1.0	2.0

* A, B, C, and D are telocentric, small metacentric, large metacentric, and large submetacentric chromosomes, respectively.

The dramatic difference in level of albumin production in the different hepatoma clones, as well as hybrid clones, cannot be explained by karyotypic changes or by differences in rate of export of albumin. Clones that differ by as much as a factor of 4 have indistinguishable karyotypes (Table 3). The percentage of synthesized albumin remaining inside the cells after 72 hr is so low—less than 0.0015% for both hepatoma and hybrid clones—that it would not influence the results.

The family tree of hepatoma clones (Fig. 2) shows that even after three successive clonal isolations, subclones are isolated that produce albumin at different levels. One possible interpretation for this is that each clonal population is made up of a majority of cells that produce albumin at a given level, but minority subpopulations produce albumin at different levels. The frequency at which a shift to another level occurs is high enough that variants can be isolated simply by subcloning. Moreover, if the frequency at which a shift occurs to a higher or lower level is the same, then the subpopulations would not effect the population level. From the two successive subclonings of clone Fu5-5 it appears that a shift up in level or return toward the level of the progenitor clone Fu5 is most frequent. This could explain why the level of Fu5-5 is slightly higher than that expected from the theoretical geometric progression. That the majority of a population produces albumin at a given level is suggested by the results from subcloning 967. In this case, contrary to subcloning of Fu5 and Fu5-5, the subclones were picked randomly, and the majority of suclones produce albumin at the same level at 967.

Each hepatoma clone produces albumin at a characteristic level; however, upon one occasion each with progenitor clone Fu5 and three subclones (Fu5-8, Fu5-2, and 967.4), quite unexpectedly, they produced albumin at another level. In all four cases the shift was to another level in the geometric progression (Table 2 and Fig. 2). Considering that the standard deviation in the C′ fixation assay was no more than ±16%, the new levels were significantly different. Preliminary results suggest that the shifts occurred as a result of an unexplained consequence of thawing a frozen aliquot of cells. Hepatoma or hybrid clones were maintained frozen in aliquots at −90°.

TABLE 4. *Hepatoma enzymes and geometric progression**

Enzyme	Geom. prog.	Ref.
Adenylate kinase (EC 2.7.4.3)	yes	21
Thymidine kinase (EC 2.7.1.21)	yes	19
Uridine kinase (EC 2.7.1.48)	yes	19
Uracil reductase (EC 1.3.1.2)	yes?	19
Arginase (EC 3.5.3.1)	yes?	23
Aspartate carbamoyltransferase (EC 2.1.3.2)	yes	19
Tryptophan pyrrolase (EC 1.13.11.11)	yes	19
Glutamine synthetase (EC 6.3.1.2)	yes	20
Glutamine aminotransferase (EC 2.6.1.15)	yes	20
Ornithine carbamoyltransferase (EC 2.1.3.3)	yes	19
Argininosuccinate synthetase (EC 6.3.4.5)	yes	23
Tyrosine α-ketoglutarate aminotransferase (EC 2.6.1.5)	yes	19, 24
Enolase (EC 4.2.1.11)	yes	25
Glucokinase (EC 2.7.1.2)	yes	25
Citrate cleavage enzyme (EC 4.1.3.8)	yes?	24
Catalase (EC 1.11.1.6)	yes?	4
Serine-threonine dehydrase (EC 4.2.1.16)	yes?	19
Pyruvate kinase (EC 2.7.1.40)	yes?	25
Glycerolphosphate hydrogenase (EC 1.1.1.8)	yes?	25
Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)	yes?	25
Phosphofructokinase (EC 2.7.1.11)	yes?	25
β-Glucuronidase (EC 3.2.1.31)	yes?	22
Fructose 1,6-diphosphatase (EC 3.1.3.11)	no	25
Phosphoglycerate kinase (EC 2.7.2.3)	no	25
Glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12)	yes?	25
Aldolase (EC 4.1.2.7)	no	25
Lactate dehydrogenase (EC 1.1.1.27)	no	25
Transglutaminase	no	20

* For enzymes indicated with a question mark, the reduced number of hepatomas assayed limited the significance of the fit.

For an albumin assay, one aliquot was thawed and the cells were grown for at least a week. In the case of clone Fu5, upon two independent thawings 7 months apart of the same initially frozen lot, the level of the albumin production was 24.6 and 22.7 μg/mg of protein per 72 hr. After 8 more months, another aliquot of the same lot was thawed and a lower level of 13.8 ± 0.50 was maintained for 3 months of continuous cultivation with weekly transfers. When a new aliquot was unfrozen and tested 2 months later, its level of production was 19.3 and 24.6 in two independent assays. It is doubtful that this shift in level of albumin production is due to experimental error since it occurred in four different hepatoma clones. No correlation was noted between the shift in level and a change in a lot of serum or media. Despite the unexplained nature of this shift in level of albumin production, the shift was always to a value that fits the geometric progression.

DISCUSSION

The high frequency at which hepatoma and hybrid clones that produce different levels of albumin arise argues against the possibility that mutations in the classical sense are responsible. Phenotypic variability in neoplastic cells is not an unprecedented observation; isolation of variants by subcloning of heteroploid lines has been reported (17, 18). However, a discontinuous pattern of variability in the form of a geometric progression has not been remarked upon. Because of this, I undertook a literature survey of enzyme studies in hepatomas.

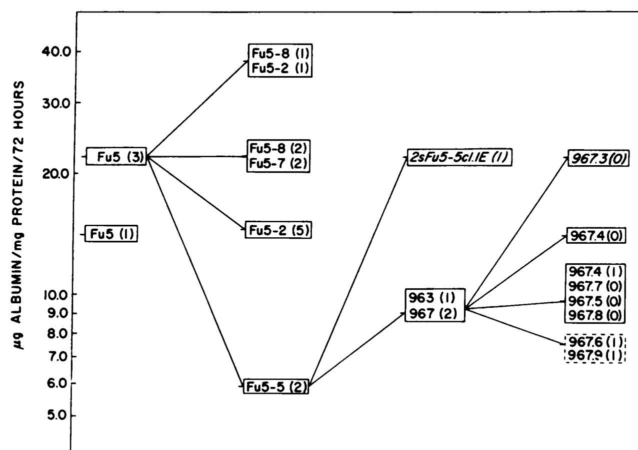

FIG. 2. Family tree of progenitor hepatoma clone Fu5 and subclones ranked according to level of albumin production. *Lines connecting boxes* represent subclonings. *Numbers in parentheses* indicate the number of independent thawings that gave the corresponding level of albumin production.

For the majority of 28 different enzymes surveyed (listed in Table 4), which were assayed in a significant number of different hepatomas (at least 5 and in most cases more than 10), the variation from hepatoma to hepatoma in enzyme level was between 4- and 100-fold, with a maximum of 700-fold. Moreover, for 23 of the 28 enzymes, the hepatomas cluster around discrete values that fit the same geometric progression as albumin production (Table 4). For most enzymes, the different hepatomas fall into alternate classes in the geometric series, thus increasing stepwise by a factor of 2. Two enzymes for which the pattern can be seen most readily are thymidine kinase and glutamine synthetase (19, 20). For the five enzymes that do not fit the geometric progression, there is less variation among hepatomas (less than 10-fold). Considering the many possible levels of control of protein synthesis (transcription, translation, enzyme and messenger RNA degradation, and messenger RNA processing and transport), it is significant that a common pattern of variation is seen at all.

Discontinuous phenotypic variation in the form of a geometric progression is also observed in normal tissues. Paigen and Felton have assayed β-galactosidase activity in the brain, liver, kidney, and spleen of 45 strains of mice (26). They found a variation from tissue to tissue and from strain to strain, but the activities clustered around distinct values that formed a geometric progression with a factor of $\sqrt{2}$ difference between successive classes. Eleftheriou and Bailey (27) have measured the resting level of circulating corticosterone in mice of two unrelated inbred strains, C57BL/6By and BALB/cBy, and seven of their derived recombinant-inbred strains as well as their F_1 hybrid and backcross generations. C57BL/6By and BALB/cBy have levels (μg/100 ml of plasma) of 3.9 ± 0.5 and 11.3 ± 0.3, respectively, whereas the various derived strains can be arranged into four groups, two of which have levels similar to the progenitor strains, one an intermediate level, and one a group beyond the progenitor strain levels. The average values of these groups are 4.4, 8.7, 11.5, and 15.9. These values are surprisingly close to a geometric series where each successive member of the series increases by a factor of $\sqrt{2}$:4.0 (5.64), 8.0, 11.3, and 16.0. The authors interpret their results to support a genetic model involving at least two genetic loci, with epistatic gene action;

however, as they assert, their limited number of tests for this model leaves the results open to other interpretations.

The widespread occurrence of this discontinuous pattern of variability in the form of a geometric progression suggests that it reflects some basic mechanism of control of gene expression. Paigen and Felton interpreted their results by suggesting that the levels of β-galactosidase reflect the presence of extra copies of the structural gene arising from excess duplications occurring after fertilization and that the number varies from one tissue to another. The $\sqrt{2}$-fold interval between adjacent classes is explained by assuming that either the two alleles do not replicate simultaneously or if they do, the geometric series would arise if degradation of messenger RNA is second order (26). The fact that genes for ribosomal RNA (28) and for histones (29) are reiterated supports the hypothesis that the geometric pattern of phenotypic variation could reflect different degrees of structural gene duplication. However, recent evidence that there is no selective reiteration of the genes for hemoglobin (30), ovalbumin (31), and silk fibroin (32) argues against this hypothesis. An alternative hypothesis is that the levels reflect different degrees of duplication of the regulatory sequences rather than the structural gene. That much of the DNA of eukaryotic cells is moderately repetitive (10 to 200 copies per genome) has been well established, and its possible role in regulation has been discussed extensively (33). Moreover, recent evidence indicates that the sequence next to the hemoglobin structural gene is moderately repetitive (34). The reiteration frequency or expression of "regulatory sequences" could directly affect the rate of transcription or messenger RNA processing or transport.

Direct evidence that changes in DNA content of specific regions of chromosomes do occur in the form of a geometric progression, presumably resulting from consecutive duplications, comes from studies both in amphibia and insects. Comparison of the number of ribosomal genes in 22 species of amphibia has demonstrated that each species falls into one of four different classes that have, respectively, averages of about 1000, 2000, 4000, and 8000 ribosomal genes per diploid somatic cell (35). Studies of salivary gland chromosomes in different subspecies of *Chironomus thummi* have revealed that when an increase in amount of DNA occurs in homologous bands, it is always by a factor of 2, 4, 8, or 16 (36). Finally, the increase in DNA content in "DNA-puffs" of *Sciara coprophila* occurs by stepwise doublings (37).

If the different levels of albumin production and activities of different enzymes of hepatomas actually reflects the number of reiterations of some genetic unit, the metastable nature of the levels would mean that the degree of reiterations must be metastable. The magnification of ribosomal genes in "bobbed" mutants of Drosophila is an example of such a genetic instability (38) where an actual increase in the number of ribosomal genes occurs. Interestingly enough, the reduction in number of ribosomal genes in "bobbed" mutants is usually of the order of 50% or 25% of the wild type (35).

Whatever the molecular basis for phenotypic variability in the form of a geometric progression, its widespread occurrence suggests its biological importance in regulation of phenotypic expression and differentiation in eukaryotic cells.

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