Clonal Heterogeneity in Populations of Normal Cells and Tumor Cells

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I. ABSTRACT

A review of published literature indicates that heterogeneity within populations of mammalian cells has been repeatedly observed and frequently modeled. However, heterogeneity is not well enough understood to ameliorate its consequences in populations of tumor cells. Our own experimental observations, using time lapse photography, indicate that when murine erythroleukemia cells are induced to terminally differentiate the distribution of life times of unrelated cells becomes more heterogeneous and that sister cells become more similar. Time lapse photography of NIH3T3 murine fibroblasts reveals that the human ras oncogene decreases the similarity of life times of sister cells and increases the similarity of mother-daughter cells. Observations of colony sizes also detect heterogeneity of cell life times within a population. Inspite of this heterogeneity, cell growth rates apparently persist when primary colonies are recloned and allowed to form secondary colonies since the sizes of secondary colonies resemble each other and the primary colony from which they were derived. The ras oncogene weakens the persistence of cell growth rates since it increases the variance of sizes between related secondary colonies. These observations have lead to the development of a new Divided-Colony Assay for detecting the response of heterogeneous tumor cell populations to chemotherapuetic drugs in vitro. Inorder to better understand observations of clonal heterogeneity a Growth Rate Diffusion model is outlined. In this model the growth rate of daughter cells take a random walk away from the growth rate of mother cells. Criteria are suggested for evaluating this model, and other models for describing

heterogeneity within proliferating populations. Several open questions are presented concerning our understanding of clonal heterogeneity of normal and tumor cells.

II. INTRODUCTION

Heterogeneity among cells in a population is the rule, rather than an exception. It might be expected that a population of cells each derived from a single cell, and growing within a common environment, would be homogeneous. However, such populations are frequently reported to be heterogeneous for cell growth rates, drug response, proliferative capacity, tumorigenicity, state of differentiation, and many other properties. Heterogeneity is commonly recognized as a property of mammalian tumor cell populations (1,2,3) but heterogeneity is also a property of non-tumor cells (4,5,6,7), yeasts (8,9) and bacteria (10).

This paper will be concerned with growth rate heterogeneity of mammalian tumor cells and of non-tumor cells. After a review of some relevant literature our experimental observations will be described for heterogeneity of cell life times, and for the heterogeneity of colony sizes. Results of Monte Carlo computer simulations of colony size distribution will be described, and a reference model (Growth Rate Diffusion Model) for clonal heterogeniety will be outlined. Finally, criteria will be suggested for the evaluation of models dealing with clonal heterogeneity.

Time lapse photography has been widely used to measure the life times of cells in pedigrees. The life times of individual cells can be determined, and the life times of related cells can be compared. The

distribution of cell life times (alpha curves) are skewed toward longer times, and resemble log-normal distributions (11) and rate- normal distributions (12). The heterogeneity of the distributions are indicated by coefficients of variation which have values of 10% to 30% (13). The distribution of differences of sister cell life times (beta curves) are exponentially distributed (14) and are parallel to the alpha curves at long times. Correlation coefficients of sister-sister cells is usually reported to be positive, and of mother-daughter cells to be negative, although positive and zero mother-daughter correlation coeffiecients have been reported (15).

These observations have been variously interpreted as being consistent with the idea that cell life time heterogeneity is controlled by one rate limiting step (11), two sequential steps (15,16,17,18,19), two or more oppposing steps (20,21), or by several steps, no one of which is critical (12). Some explanations of cell life time heterogeneity explicitly account for short term inheritance of cell life times by considering the possibility that one or more events in a mother cell affect subsequent events in daughter cells (17,22,23,24).

Many models can fit the cell life time data (25), so collecting more of the same kind of data can be useful but has limitations in choosing between different models to guide our understanding of the generation of heterogeneity (26). It has been emphasized that there are many possible ways in which distributions can arise which are exactly log-normal (27) and approximately log-normal (28).

The difficulty in using such observation and models to try to gain a better understanding of generation of heterogeneity of cell life times is illustrated by a comparison of the model of Kimmel et al. (29) with that of Losata and Mackey (30). Each of these are mathematically sophisticated cell cycle models which closely simulate life time distributions of unrelated cells (alpha curves), differences between sister cells (beta curves), and correlations between sister-sister and mother-daughter cells. However, the first model is based on the assumption of unequal division of daughter cells, and the second model is based on the opposite assumption of exactly equal division of sister cells. Clearly, we can not use these results to evalutate the contribution of unequal or equal partitioning of cell contents at division to the generation of cell life time heterogeneity. The difficulty lies not with the formulation of the models, but with the incompleteness of the kind of data which they describe. What is needed to further our understanding of the generation of heterogeneity is not more models attempting to fit the same kind of time lapse data, but different kinds of experimental observations.

In addition to collecting traditional time lapse data of cell life times, we have explored the possibility of using a different kind of experimental observation to study the generation of heterogeneity, viz, the distributions of sizes of microcolonies derived from single cells. We (31) and others (32,33,34) have detected a persistance of cell growth rates in pedigrees observed by time lapse photography. These results suggested to us that cells growing with different rates might give rise to colonies of different sizes, and that observations and analysis of colony size distributions might be informative about cell growth rate heterogeneity.

Heterogeneity of colony sizes has been observed previously. The heterogeneity of mammalian cell colony sizes has been reported for continuous cells lines which are immortal and do not differentiate (35,36), for immortal cell lines which can be induced to differentiate (37,38,39), and for non-immortalized cells which become senescent in culture (40). Primary cells from tumors form colonies with heterogeneous sizes, and this has been interpreted as reflecting the balance between stem cell terminal differentiation (death) and self-renewal (division) (41,42,43). Primary cells of non-tumorigenic hematopoietic origin form colonies which are heterogeneous in size, self-renewal capacity and expression of differentiated phenotypes (44). The heterogeneity of colony size and metastatic efficiency has been used as an indication of high frequency of diversity associated with this stage of maligancy (45,46). Colony size heterogeneity can be increased by X-irradiation, emphasizing the possibility of lethal and sublethal damage as a factor in colony size heterogeneity of some populations (47,48,49). An activated human ras oncogene has been shown to increase the heterogeneity btween secondary colonies (50). We are not aware of any treatment which can decrease colony size heterogeneity.

Since colony size heterogeneity is commonly observed in cells derived from tumors, taking it into account may improve the conclusions obtained from in vitro chemotherapuetic tests which depend on evaluating the ability of cells to clonally proliferate (clonogenic assays) (51,52,53). Taking into account colony size heterogeneity might also extend the efficacy of clonogenic assays used to study fundamental aspects of cell proliferation

(54).

The observed heterogeneity of colony sizes, and of other phenotypes, raises the question of the relative stability of these phenotypes. The experimental procedure most often used to investigate persistence and diversity of clonal phenotypes is subcloning. Typically, a single cell is allowed to divide to give a colony of 50 to 100 cells, and then the colony is dispersed into a suspension of non-aggregated single cells. Some or all of the single cells are then allowed to form secondary colonies. The size, or other properties, of the secondary colonies can then be compared to each other and to that of the primary colony.

Subcloning procedures have been informative in several situations. The self-renewal capacity of hematopoietic stem cells (55), T-lymphocytes (56), and mast cells (57) have been studied by observing colony sizes after replating cells in vitro. The self-renewal capacity of hematopoietic cell lines, which can be induced to differentiate in vitro, has also been studied this way (37,38). Hematopoietic cells injected into mice can form colonies in the spleen, and these colonies are hererogeneous in size (44,58). The self-renewal capacity of cells from several human solid tumors has also been determined by measuring colony sizes before and after replating, and then comparing the size of each primary colony with the sizes of the secondary colonies (59). In tumor biopsies, large primary colonies may be thought to be derived from cells with a larger probability of self renewal, however replating experiments show that both large and small primary colonies contain cells with high proliferative capacity (60). In other words, larger colonies are not necessarily made up cells with a

permenantly greater self-renewal capacity than smaller colonies. Similarly, populations of cultured cells with an apparently finite life time may not age uniformly, but may contain subpopulations of uncommited cells which can form large colonies (61,62). Clonal analysis of anchorage independence (36,63), metastasis (45), drug response (3), albumin content and cell surface antigen expression (64,65,66,67,68) has indicated that many populations rapidly generate new diversity upon subcloning and/or contain subpopulations that coexist in a dynamic state.

Modeling colony size heterogeneity can give some insight into the kinds of factors which could contribute to the diversity between subclones. Terminally differentiating populations have frequently been modeled as a "birth-and-death" process with differentiating cells undergoing "death" and loss of colony forming potential, and dividing cells giving rise to more cells by "birth". This kind of model has been used to fit data on spleen colony sizes (58,69) and has been modified to account for stem cell populations whose differentiation into two cells types can be observed in vitro (44). The success of these models in fitting data on colony size distributions suggested that self-renewal of pluripotential stem cells occurs in a stochastic manner. A similiar model has been used successfully to describe the proliferation of differentiated cells (57). Other models with stochastic elements assign variability to progressive loss of self-renewal capacity (43), to delay in onset (70,71), to clonal differences in growth rate (70), or to a combination of other factors (72,73).

A different tradition of modeling describes clonal heterogeneity in terms of known (or supposed) molecular mechanisms affecting cell

reproduction (7). Discrete and rapid clonal variation in albumin content of hepatoma cells has been modeled by envoking the possibility of discrete amplification and deamplification of relavant genes, and segregation by the rules of mitosis (64). The life times of T-lymphocytes is heterogeneous and log-normally distributed. It has been proposed that this life time distribution is a reflection of the log-normal density of cell surface receptors for the hormone interleukin-2. Seperated subpopulations behave as predicted by this model (74). Proliferation of other hematopoietic cells (75) and fibroblasts (76) are dependent on different hormone growth factors in serum. The existence of such extracellular factors has been incorporated into models which describe control of initiation of DNA synthesis by accumulation of a serum dependent protein with short half life (77,78). A decrease in proliferative capacity, cellular senescence, has been modeled by postulating molecules which are required for cell division, which can be partitioned unequally in daughter cells, and which are lost or inactivated during progressive division (79).

This short review indicates that clonal heterogeneity in populations has been studied by time lapse photography of individual cell life times, and independently by analysis of the distributions of phenotypes among colonies and subclones. Models have been proposed which account for clonal heterogeneity by assigning generation of variability to different factors. Additional experimental observations and modeling will be necessary before we have a firm understanding of the factors which generate clonal heterogeneity within populations of mammalian cells. New insights may be necessary before we can propose methods to ameliorate the consequences of increased clonal heterogeneity of tumor cell populations.

III EXPERIMENTAL OBSERVATIONS ON HETEROGENEOUS POPULATIONS

A. Heterogeneity of cell life times

We have observed heterogeneity of cell life times of two kinds of tumor cells, mouse erythroleukemia cells transformed with the Friend virus complex, and mouse fibroblast cells transformed with the human ras oncogene from a bladder carcinoma. For both cases, the cell life times were obtained by time lapse video photography. Time lapse photography has the advantages of providing the precise life time of each cell, and of providing the exact relationships between of cells in pedigrees. Time lapse photography has the disadvantages that it takes a long time to record many pedigrees, and it is tedious to observe the recordings and reduce the images to numbers.

Friend erythroleukemia cells are virus transformed mouse hemopoietic cells which proliferate continuously in culture (80). When exposed to dimethylsulfoxide they undergo terminal erythroid differentiation and cease division after about 5 days (81). We used these cells to ask how population heterogeneity is altered when a population of cells progresses from a high proliferative capacity to low proliferative capacity. Our experimental observations (82) indicate that median interdivisional times increased from 11.8 hours, before exposure to dimethylsulfoxide, to 24.0 hours at 72 hours after exposure. The fraction of dividing cells decreased from 1.0 to 0.807. The heterogeneity of interdivisional times, as indicated by the percent coefficient of variation, increased from 8.5 to

40.8%. The correlation coefficient of sister cell life times increased from 0.622 to 0.925. These changes in sister-sister correlation coefficients and the coefficients of variation of the entire population suggest that sister cells remained relatively similar to each other while unrelated cells became more diverse.

Inorder to better understand the control of leukemia cell proliferation, we sought a mathematical model that would fit the cell life time data, and whose features were suggestive of biological processes. We considered several mathematical models which have been used to described the distribution of cell life times. These included models which assume that cell cycle progression is influenced by a single rate-limiting step (11) or two or more rate controlling steps (12,17,19,20,83). After comparison of the data with several models we found that the data could be accounted for by a model which assumes two opposing steps (20) and which explicitly includes a fraction of non-proliferating cells (82). Our modification of the Murphy adaptation of the Erying-Stover formulation is

$$S = (F/1+\exp(-\text{alpha}(\text{tau}-t))) + (1-F)$$

where S is the probability that a cell has not divided (survives), alpha is a measure of population heterogeniety, tau is the median interdivisional time and F is the fraction of cells that will not divide at a time very long compared to the median interdivisional time.

A comparision of experimental data and mathematical models, cannot identify a "correct" model. But in this case, the comparison has been

instructive in four ways. One, it has emphasized to us that indicators of population heterogeneity can change as populations progress from being dominated by proliferating cells to being dominated by terminally differentiating non-proliferating cells. Secondly, it has reminded us that the fraction of cells which are observed not to divide can be large, and should not be ignored in analyzing real data or in formulating models. Thirdly, it has suggested to us that the cell cycle might be governed by several opposing steps rather than by a single rate limiting step. Fourthly, it has reminded us that there are many models which can generate exponential tails on distributions of cell life times.

The second kind of population of tumor cells that we observed by time lapse photography was mouse fibroblast cells transformed by the human $\underline{ras}^{EJ}$ oncogene. These transformed cells were compared to isogenic cells that were not transformed. We used this pair of cell lines to ask how a single gene alters population heterogeneity. The DNA base sequence of the ras proto-oncogene has been determined, its protein product is known, the molecular basis for its activation to an oncogene has been shown to be a single base change, and similar activated ras oncogenes have been found in many human tumors (84).

Our experimental observations indicate that the presence of an activated human $\underline{ras}^{EJ}$ oncogene decreases correlation coefficient of sister-sister cell life times from 0.558 to 0.288, and increases the mother-daughter correlation coefficient from -0.418 to -0.276. More specifically, it makes the life times of pairs of cells less related and more random.

The correlation coefficients were calculated after correcting for biases arising from pooling data from populations with clonal heterogeneity (31). Previous work with bacterial populations suggested that growth rates may run in families, and that combining data from different colonies may bias the estimates of correlation coefficients (31,85). The relationship between the uncorrected correlation coefficient (insert 1) and the corrected correlation coefficient (insert 2), which we used (31) is given by:

(insert 3)

where the observed variance (insert 4) is considered to be the sum of the variance components due to pooling experiments (insert 5) and pooling colonies (insert 6), as well as the intrinsic variance (insert 7), e.g.

(insert 8).

Analysis of variance, for both *ras* transformed and non-transformed cells, indicated that pooling data from different colonies, and from different experiments contributed to the estimates of total variance (31). This realization was important for two reasons. First, it allowed us to calculate corrected correlation coefficients estimated from pooled data. The corrected correlation coefficients differed from the uncorrected correlation coefficients by decrease in magnitude, and in some cases, by a change in sign! Secondly, it emphasized that there can be a tendency for cell life times to persist within families of eukaryotic cells as well as prokaryotic cells.

B. Heterogeneity of colony sizes

We have sought a more rapid method than time lapse photography for obtaining data on cell proliferation kinetics. The concept that cell life times perist in pedigrees, suggested to us that microcolonies derived from founder cells with different life times should give rise to microcolonies with different numbers of cells, when observed at the same time. Therefore, observations on the distribution of numbers of cells in colonies might be informative. Observing colony size distribution rather than cell life time distributions would have a practical advantage -- data on large numbers of colonies could be more easily obtained than data on large numbers of individual cells. We asked if there was a difference between the distributions of colony sizes of <u>ras</u>^{EJ} oncogene transformed and non-transformed cells, and if those differences persisted upon subcloning.

As expected, we observed a heterogeneous distribution of numbers of cells per colony, ranging from 2 to more than 50 (50). This range is greater than could be accounted for alone by cell cycle asynchrony of the initial cells, and was probably a reflection of the heterogeneity of cell life times previously observed by time lapse photography. The persistence of cell life times, suggested by the analysis of variance of time lapse data, was also observed when the sizes of secondary colonies were compared to primary colonies (50). Secondary colonies have an average number of cells per colony similar to that of the primary colony from which they were derived. The <u>ras</u>^{EJ} oncogene affects the persistence of cell life times, since it increases the variance of sizes between secondary colonies

compared to the size of their primary colony. Nevertheless, cells containing an activated oncogene give rise to secondary colonies whose average size continues to resemble the size of the primary colony from which they were derived.

These observations suggested to us a method of improving in vitro clonogenic assays for determining the drug response of tumor cells (51,53). We call this new method the Divided-Colony Assay (50). Previous test results had been confounded by the problem of sampling from a population which is heterogeneous for growth rate. Because of this heterogeneity, treated and untreated control subpopulations may not have equivalent mean growth rates. The recognition of the persistence of growth rates suggested a solution to this problem. We showed that primary colonies divided in half form two groups of secondary colonies, on two separate plates, with indistinguishable mean colony sizes. We exploited this observation by dividing primary colonies into two groups of secondary colonies, one group is left untreated as a control and the other group is treated with a chemotherapeutic drug. The size distribution of treated secondary colonies is then compared to the size distribution of untreated secondary colonies from the same primary colony.

This procedure has been shown to be able to detect response of heterogeneous $\underline{ras}^{EJ}$ transformed tumor cells to low concentrations of two different drugs, cycloheximide which affects protein synthesis,and cytosine arabinoside which affects DNA synthesis. The Divided-Colony Assay is proposed as a modification of the human tumor cloning system. It may be used to increase the sensitivity and reliability of clonogenic assays used

to determine the chemotherapeutic drug response of heterogeneous tumor cell populations.

IV MATHEMATICAL MODELING OF HETEROGENEOUS POPULATIONS

A. The Need for Models of Clonal Heterogeneity

Experiments with heterogeneous populations have lead to observations which appear, at first, to be contradictory. When cells from a proliferating population of tumor cells are dispersed and allowed to form colonies, the colonies are found to be different from each other in size. Yet when such primary colonies are dispersed, the secondary colonies resemble each other and the primary clone from which they were derived. The heterogeneity of the primary colonies indicates a tendency for growth rate to diversify, and the similarity of secondary colonies indicate a tendency for growth rate to persist. For clonal heterogeneity of growth rate to exist, there must be both a tendency for growth rate to diversify, as well as a tendency for growth rate to persist. Diversification and persistence of growth rate coexist.

Mathematical models can be useful for describing experimental results. Such models can serve several purposes. They can summarize the previously observed numerical data by explicit mathematical formulation, even without reference to biological mechanisms, or they can serve as a heuristic device to help suggest which biological mechanisms may govern. The models which are most useful to biologists summarize past data, provide a heuristic framework for biological mechanisms, and suggest new experiments. Even a non-rigorous outline of a mathematical model may be useful to a biologist if it is presented in an accessible way, and if its assumptions and

possible resulting behaviors are made explicit.

B. Computer Simulation of Colony Size Heterogeneity by a Cell Cycle Compartment Model

We have simulated the colony size distribution of non-transformed mouse NIH3T3 fibroblast cells using the CELLGROWII program written by E. Stubblefield and C.E. Donaghy (86). The purpose of these numerical simulations was to determine what factors had to be specified in order to generate results resembling experimental observations. The CELLGROWII program is a Monte Carlo simulation system in which means of cell cycle compartments (G_1, S, G_2, M, G_0) are specificied by the user. In our case the mean times were estimated from time lapse data and flow cytometry. Times spent in each cell cycle compartment are generated from built in distributions. S phase is Guassian distributed with a standard deviation of 0.2 times the mean, truncated at 1.7 times the mean, and the other states are exponentially distributed and truncated at 5 times the mean.

In the first round of simulation all colonies were initiated by cells in G_1, the proliferating fraction was equal to one, and cell life time variabilty was generated by the built-in distribution of time in states. Successive generations had no memory of parental times. This model resulted in a simulated colony size distribution, compared to the experimentally observed distribution, which had a larger median and a smaller variance. In six subsequent simulations, several factors were modified, seperately or together, inorder to reduce the median size and to increase the variance of the distribution. The final simulation, which

adequately simulated the observed colony size distribution, incorporated the following features: initial cells were in all stages of the cell cycle (asynchrony), the proliferating fraction was less than 1 and was proportional to the time spent in G_1, the G_1 time distribution was increased from the built-in distribution by using cell life time variances estimated from the life times observed by time lapse photography. When all of these factors were utilized the CELLGROWII cell cycle compartment model was able to satisfactorily simulate the heterogeneous distribution of colony sizes of NIH3T3 mouse fibroblast cells.

This series of simulations taught us several lessons about the heterogeneity of colony sizes. The first lesson is that the broad distribution of colony sizes may be partly the result of the broad distribution of individual cell life times observed in asynchronously proliferating cell populations. Secondly, cells which do not divide may constitute a considerable fraction within a proliferating population. Such cells may be ignored or not analyzed in time lapse records of dividing cells, but their existence increases the number of small colonies observed in the colony size distribution measurements. Thirdly, the classical cell compartment model ,as used in the CELLGROWII program, together with the features we have described, is an adequate description of proliferating heterogeneous populations, at least by the criteria of primary colony size distributions. Fourthly, the simple cell cycle compartment model generates diversity of cell life times, but it does not account for the persistence of life times. The life time of every cell is determined by the same probability distribution of time in states, regardless of the life time of its parent. The difference between cells results from a random number

generator which chooses from the probability distribution of time in states. No account is taken of the life time of the parent cell. However, experimental results demonstrate that secondary colonies resemble their parents and each other more than unrelated colonies. This observation requires a model that includes memory from one generation to another.

C. Growth Rate Diffusion Model for Clonal Heterogeneity

We have begun to develop a reference model to take into account the persistance, as well as the diversity, of cell life times detected by observing single cells in pedigrees and groups of cells in microcolonies. This model visualizes proliferating cells as taking a random walk in rate space i.e. cell growth rates diffuse from one generation to the next.

The Growth Rate Diffusion Model is subsumed under the the class of models refered to as multitype branching processes. This class of models has been frequently used to describe uniparental populations which are heterogeneous with respect to some characteristic(s). Specific multitype models have been developed to describe the persistence of cell growth rates (87,88), and the distribution of cell growth rates (12,89,90). Other random walk models have been described for related phenomena (24,29,65). Extensive techniques have been developed for the analysis of branching processes (91,92,93).

Our Growth Rate Diffusion Model has the following features:

a) Types. Each cell is born as one of i=1,2,...,n types.

b) Rate space. A cell born as type i has an associated rate λ = 1 / (expected lifelength), where $0 < \lambda_1 < \lambda_n$.

c) Random walk. A daughter of a cell of type i will be of type i + 1, i, or i - 1 with respective probabilities p, r and q, where p + q + r = 1.

d) Boundary condition. At states i = 1 or n, attempted steps to 0 or n + 1 are forbidden and a daughter cell will remain the same type as the mother. Steps from i = 1 to 2, or i = n to n - 1, are still possible.

The idea of random walk on a rate space provides a mechanism for short term persistence of rates, as well as for diversification. The short term persistence of rates occurs since daughter cells may take only one step from their mother cell.

It is useful to consider the behavior of the model for different values of the parameters. The parameters p and q are the probabilitities that daughter cells take a step up or down in rate respectively. The tendency for shiftup in rates can be described by setting p larger that q, shift down by q larger than p. The parameter r, the probability that a daughter cell grows at the same rate as the mother cell, captures the idea of rate of diversification. A large value of r, i.e. near 1, corresponds to a low rate of diversification since daughter cells are likely to be the same type as the mother cell, while low values of r, i.e. near 0, corresponds to rapid diversification since daughter cells are unlikely to be the same type as the mother cells. The model also captures the short

term inheritance of rates where progeny grow at rates more similar to mother rates than to rates of unrelated cells, while allowing for the eventual dispersion of rates of offspring as exemplified by the loss of synchrony of cells derived from on or many newborn cells. As long as there are a finite number of types the process will always converge to a unique steady state of distribution of types (i.e. growth rates), regardless of the initial type(s) of the ancestor(s).

The behavior of this model can be described in terms that can be directly compared with experimental observations on colony size distributions. According to this model, as well as some other models, the following would be expected:

a) Populations of cells of a wide variety of types will produce colonies growing at a variety of rates.

b) Two different cells, both of the same type i, will in a constant interval of time T (roughly a few generations) tend to produce colonies of comparable size.

c) The previous point remains true even if p, q, and r are different for each clonal line.

d) If differences in p, q and r in each clonal line exist, cells in each colony would drift away from each other in type by time T.

Behaviors similar to these have been observed experimentally (50).

First, a broad distribution of primary colony sizes was observed. Second, when cells from primary colonies are replated, secondary colonies are comparable in size to primary colonies. Third, drift in type to lower or higher rates in secondary colonies is detected when sizes of primary and secondary colonies are compared. Fourthly, when primary colonies are divided and replated onto separate plates, drifts in growth rates of both groups of secondary colonies are similar.

The difference in behavior of *ras*[EJ] transformed and non-transformed cells, greater intercolony variance of secondary colonies, may not be accounted for simply by different values of r. If this is so, then it would suggests that the two cell lines differ in the values of several parameters, or that the reference model outlined above needs to be refined. These possibilities will be evaluated by computer simulations based on this reference model and its refinements.

V. OPEN QUESTIONS

The Growth Rate Diffusion reference model outlined above provides a heuristic device for considering diversification and persistence of cell life times. While it is being developed formally, its major features are being incorporated into programs for computer simulation of cell life times and colony size distributions.

Our goal will be to determine if the model accounts for the experimental observations on cell life times and colony size distributions. If it is successful, we will consider its implications for understanding the molecular processes which govern cell proliferation. If not, it will be modified to improve the correspondence with experiments. It is useful to explicitly list the experimental observations against which the behavior of the model will be tested:

1. Cell life time heterogeneity,
    - a) sister-sister correlations are positive,
    - b) mother-daughter correlations are negative (although positive and zero correlations have been reported and this should be taken into account),
    - c) approximately log-normal or inverse normal distribution of cell life times, alpha curves,
    - d) approximately log distribution of differences between sister cell life times, beta curves,
    - e) beta curves approximately parallel to alpha curves,
    - f) altered slope of alpha and beta curves by modification of

medium components, inducers of terminal differentiation, gene mutation, or introduction of new genes such as activated oncogenes,

g) persistence of cell life times within pedigrees,

h) divergence of cell life times after a few generations,

i) difference in mean life times of different pedigrees within the same population,

j) not all cells give rise to two viable daughter cells.

2. Colony size heterogeneity,

a) colony size distributions are broader than would be expected by asynchrony of the initial cell only,

b) secondary colony sizes resemble the primary colony size from which they were derived,

c) subsets of secondary colonies resemble each other

d) the ras oncogene increases the variance between related secondary colonies.

Preliminary numerical experiments have indicated that there are several features of the Growth Rate Diffusion model which will have to be addressed carefully. These include the following:

1. Boundary conditions. Required to prevent explosion and to maintain stability. What is the fate of cells that reach the boundry? Do they die, do they pause and try again, or are they reflected back? Do cells behave the same at the upper and lower boundries? Do all cells behave the same at boundries?

2. Generation of diversity. For non-tumor cells and more so for tumor cells. What features, or numerical values, distinguish

non-tumor from tumor cells populations, and proliferating from differentiating cell populations? These may be size of step, probability of step or not step at each cell division, asymmetry of size or probabilty of step up or down, cell death linked or not to other features.

3. Incorporation of biochemical mechanisms which are known to affect cell proliferation, such as growth factors, growth factor receptors, signal transducing mechanisms, DNA replication and initiation signals, mutations in genes recognized as oncogenes, etc.

4. Suggest numbers or kinds of steps which could be experimentally and clinically manipulated to reduce the extra heterogeneity of tumor cells.

We are interested in developing this model to better understand the mechanisms governing cell proliferation of normal and tumor cells. It has already been useful in guiding us to carry out subcloning experiments, and to devise a practical improvement in a clinically relavent in vitro predictive test for tumor sensitivity to chemotherapeutic agents (50). Perhaps future work will indicate the molecular mechanisms which govern clonal heterogeneity, and allow us to suggest therapies which would reduce the heterogeneity of tumor cells.

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KEY WORDS FOR INDEXING

D.E. Axelrod and T. Kuczek, Clonal heterogeneity in populations of normal and tumor cells.

branching process	21
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cell cycle	2
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Divided-Colony Assay	2
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Growth Rate Diffusion Model	2
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tumor	2

INSERTS

now

① ρ

ρ^c

②

③ $\rho = [\rho^c(\sigma^2) + \sigma^2_{exp} + \sigma^2_{col(exp)}] / [\sigma^2 + \sigma^2_{exp} + \sigma^2_{col(exp)}]$

sigmas

④ σ_τ

⑤ σ^2_{exp}

⑥ $\sigma^2_{col(exp)}$

⑦ σ^2

⑧ $\sigma^2_\tau = \sigma^2_{exp} + \sigma^2_{col(exp)} + \sigma^2$