Mutation Research, 292 (1993) 17–24

MUTENV 08872

Use of mathematical models for understanding the dynamics of gene amplification

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(Received 12 January 1993)
(Accepted 3 February 1993)

Keywords: Gene amplification, dynamics; Gene amplification, mathematical models

Summary

Recently it has been suggested that high levels of cancer drug resistance and poor prognosis are strongly associated with gene or oncogene amplification (GA). It has been further suggested that the molecular mechanisms underlying GA may be different for different genes, and that different amplification mechanisms may function concurrently or sequentially in the same gene. The aim of this review is to demonstrate the use of mathematical models in studying these intricate dynamics. We have provided mathematical models for the generation of extrachromosomal elements, their autonomous replication and equal or unequal mitotic segregation, the integration of the extrachromosomal elements within the chromosomes, and chromosomal GA in one or many unlinked genes. Using this formal description one can examine the potential role of each GA mechanism in the generation of specific distributions of gene-copy number in a cell population, under various levels of selection stringency. Thus one can specify the conditions for the emergence of drug-resistant mutants prior to selection, as well as the relationships between the stringency of the selecting environment and the characteristics of the resultant cellular phenotype.

1. Introduction

DNA sequence amplification has important clinical consequences. Over the last 15 years, an intensive research has been carried out into its underlying molecular mechanisms, but as yet there is no consensus on how amplification occurs. It appears that the experimental techniques, which have been instrumental in documenting gene amplification (GA) at given moments, are not sufficient for yielding a full account of the complex dynamics that lead to an observed distribution of gene-copy number. For retrieving these dynamics it seems essential to translate to mathematical models the different assumptions about the underlying molecular events and to compare the computed distributions with experimental observations.

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Our study of GA involved the formulation in mathematical models of the different biological hypotheses about the underlying dynamics. By analyzing these models we hoped to be able to pinpoint the crucial parameters in the system and, ultimately, to provide a rigorous theoretical framework for testing the effect of GA on treatment protocols. The aim of this paper is to provide a concise description of GA models and their potential employment.

The structure of the paper will be as follows. Section 2.1 describes our mathematical description of the formation and amplification of extrachromosomal elements (episomes) as a sequence of events: formation of extrachromosomal elements, their replication and unequal mitotic segregation, and their eventual integration into chromosomes (Harnevo and Agur, 1991b). Section 2.2 reviews our work on chromosomal GA (Harnevo and Agur, 1991a). In section 2.3 it is shown how the model for extrachromosomal GA is combined with the chromosomal GA model in order to obtain a comprehensive description of the system as a whole.

2. Models for the dynamics of gene amplification

2.1. Extrachromosomal GA

Recently it has been suggested that GA in human cancers is often initiated by the production of acentric, circular, extrachromosomal DNA molecules (episomes), which replicate autonomously (Stark et al., 1989). Episomes are produced by deletion of sequences from the chromosome (Carroll et al., 1988), or by unscheduled DNA replication, followed by recombination (Schimke et al., 1986; Amler et al., 1992). It has been further suggested that the initially minuscule episome can gradually increase in size and in gene-copy number, to become a double minute chromosome (DM). DMs can integrate into the chromosome and further amplify (Carroll et al., 1988; Ruiz et al., 1989; Stark et al., 1989; Ruiz and Wahl, 1990).

Generation of extrachromosomal elements. Denote by r^j a cell with j extrachromosomal gene copies, so that r^0 is a wild-type cell. An extrachromosomal element is generated, with probability δ, so that the r^0 cell becomes an r^1 cell (top of Fig. 1). Underlying this formulation is the assumption that initiation occurs at random by deletion or by any other molecular mechanism. We have assumed that several independent initiations of extrachromosomal elements may occur in a single cell population.

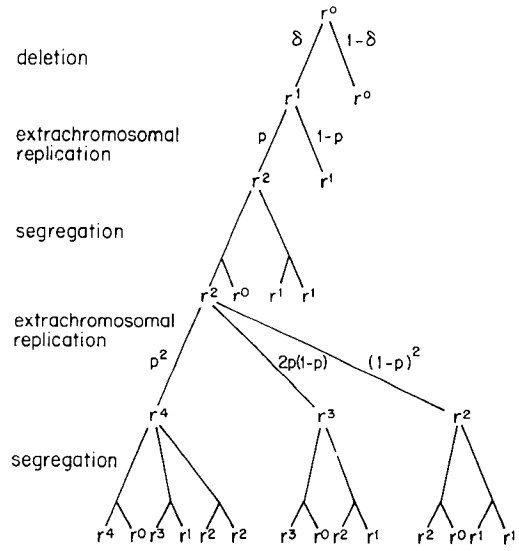

Fig. 1. A schematic description of the model for extrachromosomal GA. Extrachromosomal GA is initiated by the generation of an episome carrying a single extrachromosomal gene copy, with probability δ per cell division. The resulting r^1 cell undergoes episome amplification, with probability p, followed by mitosis. In mitosis each extrachromosomal copy segregates independently to generate two r^1 cells or one r^2 cell and one wild-type cell r^0. The r^2 cell undergoes a new process: (i) GA of both copies (with probability p^2); or (ii) GA of one copy (with probability $2p(1-p)$); or (iii) no GA (probability $(1-p)^2$); the segregation in mitosis of an r^2 cell may lead to cells with four extrachromosomal gene copies or fewer.

Autonomous replication and equal or unequal mitotic segregation of the extrachromosomal elements. Experimental observations suggest that extrachromosomal gene copies undergo fast independent replication (Wahl, 1989). Our model assumes that episome replication occurs once per cell cycle, with a probability p per copy, $p \leq 1$ (Ruiz et al., 1989). Thus, a cell having, initially, j extrachromosomal copies, can have after replication j, $j+1, \ldots, 2j$ copies, depending on the number of copies added by replication. Under

this assumption, the number of extrachromosomal copies added to the cell is a binomial random variable, $Bin(x, p)$, for which x is the maximal number of added gene copies. If we assume that the number of extrachromosomal gene copies has an upper bound, I, due to nucleotide or other energy constraints, then $x = I$, but if the number of gene copies is unbounded then $x = j$, j defined as above. The mean number of extrachromosomal gene copies and their variances were computed for both the bounded and unbounded cases (Harnevo, 1991; Harnevo and Agur, 1991b), and the corresponding distributions were generated. Results suggest that the assumption of an upper bound on the number of gene copies affects the dynamics only when the extrachromosomal GA process lasts for a relatively long period.

We also took account of the fraction of cycling cells, b, and the cells' natural mortality probability, μ. These two parameters influence the size of the cell population but not the amplification process itself.

Now we can formulate cell division, that is the random segregation of extrachromosomal gene copies into the newly formed daughter cells. Fig. 1 illustrates the model for cells with one extrachromosomal copy, r^1, and for cells with two extrachromosomal copies, r^2. The segregation process can also be described by the binomial distribution. Now the binomial random variable is $Bin(y, \frac{1}{2})$, where y is the number of extrachromosomal gene copies after replication, and the segregation probability for each copy is $\frac{1}{2}$.

The exact formulation and the analysis of the processes of extrachromosomal replication and unequal segregation are provided in Harnevo (1991) and Harnevo and Agur (1991b). Using these formulae we computed the distribution of extrachromosomal gene copies in a cell population for various assumptions about the parameter values. Our results suggest that, in general, the extrachromosomal GA process generates a relatively high proportion of cells with many gene copies (Fig. 2), (see section 2.2 for comparison with the chromosomal GA process).

However, this effect primarily depends on the probability of extrachromosomal DNA replication, p (Fig. 2). In addition, extrachromosomal gene-copy distribution may also be affected by

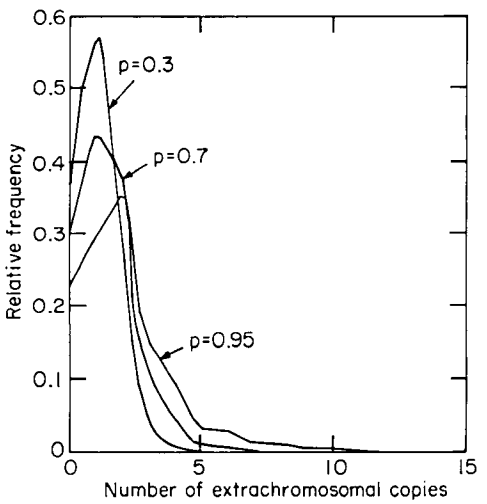

Fig. 2. The computed distribution of cells according to their extrachromosomal gene-copy number after 430 generations of extrachromosomal GA (approximately one year); upper bound on the copy number is $2I = 16$. Distributions are shown for extrachromosomal GA probabilities, $p = 0.3, 0.7, 0.95$, and for gene deletion probability, $\delta = 10^{-2}$, per cell division.

the probability of generating the extrachromosomal elements, δ (results not shown).

Integration of extrachromosomal elements into chromosomes. Observations suggest that the homogeneous staining region in the chromosome (HSR) may, in some cases, be formed by episomes and DMs that integrate into the chromosome and further amplify (Carroll et al., 1988; Ruiz et al., 1989; Stark et al., 1989; Ruiz and Wahl, 1990). Although it has been suggested that large sequences of extrachromosomal DNA are integrated at one time point into a specific locus on the chromosome (Stark et al., 1989), the possibility of progressive integration cannot be excluded. Thus, our model allows for two alternative possibilities: (i) in an individual cell, the integration of extrachromosomal elements of different sizes occurs at a single time point; (ii) integration of extrachromosomal elements may occur over an extended period of time.

The effect of these assumptions on the resulting extrachromosomal gene-copy number distributions is shown in Fig. 3. We note in this figure that progressive integration results in a narrower distribution of extrachromosomal gene-copy num-

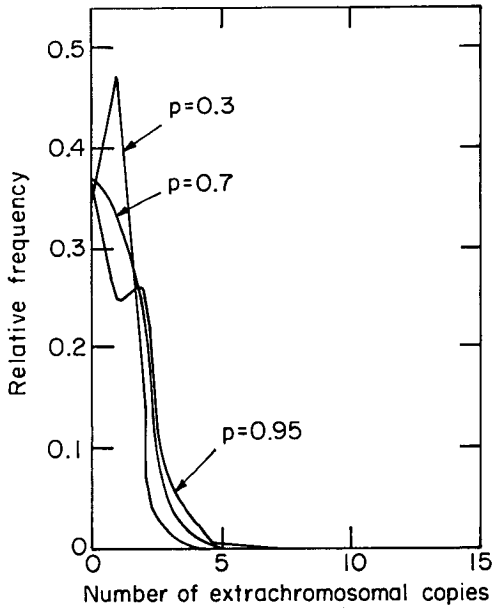

Fig. 3. As in Fig. 2 except that now we allow for progressive integration into the chromosome.

ber, and a smaller frequency of cells with more than five extrachromosomal gene copies. Our results indicate the need to obtain more information about the nature of the integration process before parameter estimation can be performed.

2.2. Chromosomal gene amplification

Studies in hamster and murine cells reveal that extrachromosomal GA is only one of several distinct GA mechanisms. Extrachromosomal GA is implicated in the case of *mdr1*, while the amplification of the *dhfr* or the *CAD* genes is suggested to involve either unequal sister-chromatid exchanges or a form of conservative transposition (Hamlin, 1992; Kopnin et al., 1992). To allow for the latter possibility, we describe chromosomal GA as an independent process.

Denote by α_i, $i = 1, 2, \ldots$ the *forward* chromosomal amplification probability, and by β_i the *backward* chromosomal amplification probability, where i is the number of added or subtracted gene copies per cell division. Note that here we allowed for one-copy or multi-copy increments, or decrements, per cell division.

Described in Fig. 4 is a model for one-copy increment, or decrement, chromosomal GA. For simplicity, we assumed that the process begins with a cell having no extra gene copies. Denote this cell by r, and by r_i a cell with i added chromosomal gene copies, $i = 1, 2, 3, \ldots$ An r cell may divide into one r_1 cell plus one r cell, with probability α_1, or into one r_2 cell plus one r cell, with probability α_2, or into one r_3 cell plus one r cell, with probability α_3, and so on; an r cell will divide into two r cells with probability $1 - \sum_{j=1}^{k} \alpha_j$. Each of the r cells undergoes the same process again. An r_1 cell will divide into one r_2 cell plus one r_1 cell, with probability α_1, or into one r_3 cell plus one r_1 cell, with probability α_2, or into one r_4 cell plus one r_1 cell, with probability α_3, and so on. An r_1 cell may also lose its extra copy by backward amplification and divide into one r cell and one r_1 cell, with probability β_1. Alternatively, it may divide into two r_1 cells with probability $1 - [\sum_{j=1}^{k} \alpha_j + \beta_1]$. In the same way, we take account of all r_k cells, $k = 1, 2, \ldots$, as described in Fig. 4.

Note that in the above description we assumed that GA is asymmetric, i.e., that cell division

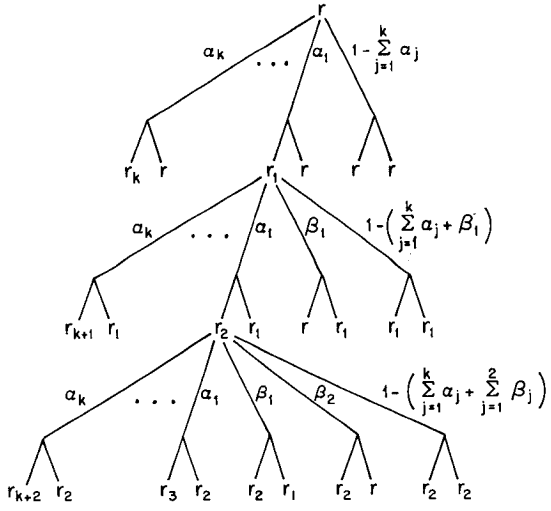

Fig. 4. A schematic description of the model for chromosomal GA, in a wild-type cell, r. This cell may divide into one cell identical to the mother cell, and another cell bearing i additional gene copies, $i = 1, 2, \ldots$; an r_1 cell may divide into one cell identical to the mother cell, and another cell bearing i additional gene copies, $i = 2, 3, 4 \ldots$; an r_1 cell may lose its extra copy during division to become an r cell; alternatively an r_1 cell may divide with no change in gene-copy number. Similar processes for all r_j, $j = 2, 3, \ldots$ may be described.

results in one daughter cell identical to the mother cell, and one cell with a different number of gene copies. In Harnevo and Agur (1991a) we also take account of symmetric GA. Natural loss of cells, constant or dependent on the number of chromosomal gene copies, and spatial heterogeneity in amplification probability (e.g., due to hypoxia), are additional elements in our models (Harnevo and Agur, (1991a).

Using the branching process approach, we calculated changes over time in the distribution of cells (means and variances) according to their gene-copy number. The relative frequency of cells bearing no extra copies was calculated as well, and was found to be decreasing to zero at a rate that depended on the amplification probability. This was so when the natural cell loss was taken to be constant. In contrast, when cell loss was copy-number-dependent, the number of cells with no extra gene copies had a lower bound, which can be evaluated numerically.

The calculated distribution of cells according to their gene-copy number after 430 generations (equivalent to one calendar year if cell generation

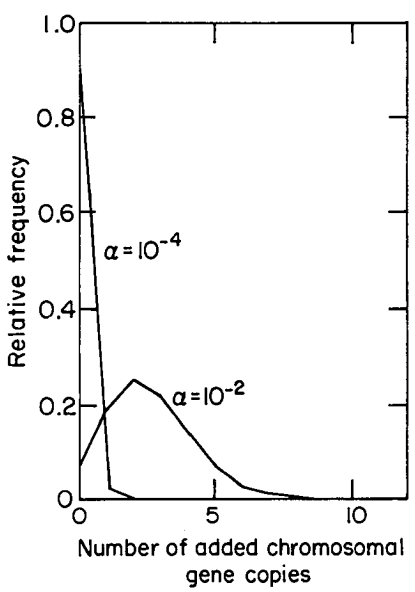

Fig. 5. The computed distribution of cells according to their chromosomal gene-copy number after 430 generations of chromosomal GA (approximately one year); upper bound on the copy number per cell is 20. Distributions are shown for chromosomal GA probability, $\alpha = 10^{-2}, 10^{-4}$.

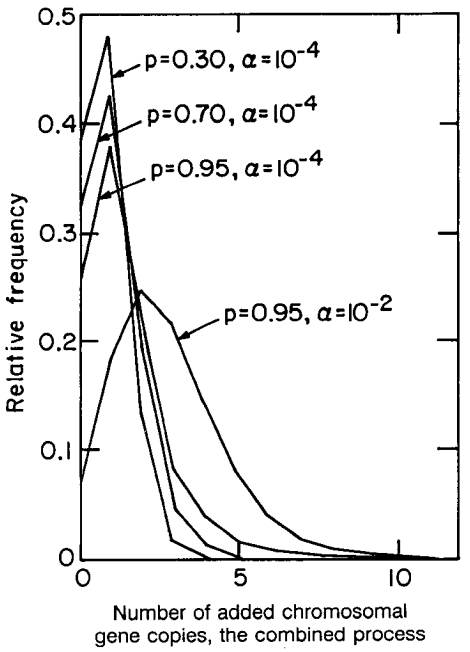

Fig. 6. Numeric computations of the combined extrachromosomal (200 generations) and chromosomal GA (230 generations). The distributions of cells are shown according to their gene-copy number (upper bound on the extrachromosomal and chromosomal copy number is 16 and 20, respectively). Extrachromosomal GA probabilities are, $p = 0.3, 0.7, 0.95$. Gene deletion probability is $\delta = 10^{-2}$ per cell division, and chromosomal GA probabilities are $\alpha = 10^{-2}, 10^{-4}$.

time is 20 h) in a single-copy increment chromosomal GA process is described in Fig. 5. This distribution appears to be strongly dependent on the amplification probability. For example, for chromosomal GA probability, $\alpha = 10^{-4}$ (see Schimke (1984) and Stark and Wahl (1984) for justification), the proportion of cells bearing additional chromosomal gene copies is smaller than 5%; note that all these cells carry only one additional copy of the amplified gene. In contrast, if GA probability is $\alpha = 10^{-2}$, most of the cells carry amplified genes, the average number of added copies being three. Comparing these results with the extrachromosomal process (Fig. 2), we note that, for realistic parameters, the chromosomal process alone yields a relatively narrow distribution of cellular gene copies. This is so because we assumed that chromosomal GA has a smaller probability than extrachromosomal replication.

2.3. The combined process: extrachromosomal and chromosomal GA

Integration may initiate a wave of chromosome instabilities, which may lead to further chromosomal amplifications (Ruiz and Wahl, 1990). To account for these dynamics we took the final distribution of extrachromosomal gene copies as the initial condition for the integration and the chromosomal GA process. Here the extrachromosomal amplification episode is taken as a little shorter than the chromosomal amplification (Windle et al., 1991) (we assumed 200 generations of extrachromosomal GA followed by 230 generations of chromosomal GA).

Results, presented in Fig. 6, suggest that the frequency of cells with a large copy number is higher in 430 generations of the combined process than it is in 430 generations of the chromosomal process alone. Our model suggests that under non-selective conditions, an observable proportion of cells with three or more chromosomal gene copies can most probably be obtained by the combined extrachromosomal-chromosomal amplification process; the chromosomal process alone will generate a comparable distribution only if the probability of amplification is larger than or equal to 10^{-2}, that is, much larger than estimated in laboratory experiments (Schimke, 1984; Stark and Wahl, 1984).

3. Discussion

Gene amplification is a pervasive phenomenon in nature, with especially adverse effects on the prognosis of cancer and parasitic diseases. We have provided an exact formal description of extrachromosomal and chromosomal GA process.

Our extrachromosomal GA model allows for the stochastic generation of an extrachromosomal element, its replication and its unequal mitotic segregation. Using this model one can formally describe the temporal changes in the mean and variance of extrachromosomal gene-copy distribution of a cell lineage. This can be done for various assumptions about the system's parameters. On this model we can superimpose the integration of extrachromosomal elements into the chromosomes, so as to evaluate the contribution of extrachromosomal amplification to the overall distribution of gene-copy number in cancer cells. Our results suggest that when GA is initiated by the generation of episomes, their amplification and the subsequent integration of DMs into chromosomes, the cell population will consist of a relatively large proportion of cells with more than one copy of the gene. If the amplified gene is related to cancer drug resistance, this effect may result in a continuously growing resistant part in the tumor (Harnevo and Agur, 1992), and if it is related to oncogene amplification, it may result in poor prognosis (Brodeur et al., 1984; Films and Buick, 1985).

Our model for chromosomal GA allows to compute (i) the full dynamic distributions of cells according to their gene-copy number; (ii) the variance in these distributions over time; and (iii) the probability of eliminating all cells bearing no additional gene copies. The relative frequency of cells with no additional copies, computed for constant loss of cells or for copy-number-dependent cell loss, illuminates the significance of this factor with respect to chemotherapeutic treatments. If GA is associated with drug resistance, then under constant cell loss the wild-type cells will eventually disappear, while a copy-number-dependent cell loss will yield a bounded compartment of these cells and a complementary bound on the number of cells bearing additional copies.

Our models were employed for studying a well-defined drug-resistance mechanism, i.e., drug resistance due to GA, resistance threshold depending on the cellular gene-copy number (Agur and Harnevo, 1992; Harnevo and Agur, 1992). Previous drug-resistance models ignored the possibility that drug dose may determine not only the fraction of susceptible cells that are killed by the drug, but also drug susceptibility itself, since the number of copies of the gene (or gene products) that render a cell resistant may be dose-dependent. We have incorporated drug resistance into the models, as a dynamic process (Harnevo and Agur, 1992), and the optimal control problem of chemotherapy was attacked (Agur and Harnevo, 1992).

Recently, another mathematical model for extrachromosomal GA has been suggested by Kimmel et al. (1992) following Windle et al. (1991). This model differs from our model for extrachro-

mosomal GA (section 2.1) in a number of assumptions.

(i) Kimmel et al. assume that a cell with i acentric elements, $i = 1, 2, \ldots$, has $2i$ elements, after replication, so that all extrachromosomal elements are assumed to replicate with probability, $p = 1$. In contrast, our model assumes that the autonomous replication of extrachromosomal elements is not necessarily successful, so that $p \leq 1$.

(ii) Implicit in Kimmel et al.'s model is the assumption that the generation probability of an acentric element is so small that this event cannot occur more than once in a cell population; we allow for the possibility that more than one cell-line in the population may undergo extrachromosomal GA.

(iii) Kimmel et al.'s model counts the number of acentric elements, and is not concerned with the number of copies each element bears, whereas we take account of the number of gene copies per element. If, and only if, all elements consist of one copy each, Kimmel et al.'s model is identical to the model by Harnevo (1991).

(iv) Kimmel et al.'s model does not allow for loss of DNA during integration whereas our model assumes that the extrachromosomal elements have some probability of getting lost in the process of integration. In addition, we showed that different results are obtained if integration is a brief or a prolonged process.

Above we have shown that the theoretical distributions of cellular gene-copy number are sensitive to the assumptions about the molecular events underlying this process. Since to date there is no consensus about these events we feel that time is not ripe for parameter estimation. Rather, at the present stage, mathematical modeling should be employed for elucidating the molecular mechanisms of GA, and for evaluating the prospects of obtaining different distributions of gene copies under different levels of selection stringency.

Acknowledgements

This work was supported by the Rashi Foundation and by the Sherman Foundation.

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