Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3711–3715, June 1983
Cell Biology

Rapid spontaneous dihydrofolate reductase gene amplification shown by fluorescence-activated cell sorting

(methotrexate/rapid mutation rate)

RANDAL N. JOHNSTON, STEPHEN M. BEVERLEY, AND ROBERT T. SCHIMKE

Department of Biological Sciences, Stanford University, Stanford, California 94305

Contributed by Robert T. Schimke, March 4, 1983

ABSTRACT **We have determined whether the gene encoding dihydrofolate reductase (5,6,7,8-tetrahydrofolate:NADP$^+$ oxidoreductase, EC 1.5.1.3) varies spontaneously in gene copy number in cells *in vitro*. Cells were stained under nonselective conditions with fluoresceinated methotrexate, which binds quantitatively to dihydrofolate reductase. Cells with the highest fluorescence were collected by a fluorescence-activated cell sorter and subsequently grown in the absence of methotrexate. At no time during the experiment were the cells placed under metabolic stress. After 10 successive rounds of growth and sorting, the derived population showed a 50-fold increase in fluorescence intensity, was highly resistant to methotrexate, and was amplified 40-fold in content of dihydrofolate reductase gene. We also found that cells already having amplified genes can undergo increases or decreases in their fluorescence and in gene copy number even more rapidly (at rates as high as 3×10^{-2} amplification events per cell division) than do parental cells (*ca.* 10^{-3} events per division). We therefore conclude that gene amplification can occur spontaneously in cells and that the rate of its occurrence varies with gene copy number.**

The phenomena of drug resistance as conferred by gene amplification (for a review, see ref. 1) suggest that changes in gene copy number may be a common response of cells to cytotoxic selection with drugs. A major question to be considered is whether gene amplification is itself a consequence of the drug treatment. It is possible, for example, that the damage and fragmentation of chromosomes that is caused (2, 3) by methotrexate [MTX, an inhibitor of dihydrofolate reductase (DHFR)] may actually facilitate the amplification of the DHFR gene (*dhfr*) (4, 5). Alternatively, the observed gene amplification events may reflect processes occurring spontaneously, even in untreated cells, that would confer resistance detectable during later selection with cytotoxic drugs such as MTX.

One way to examine the hypothesis of spontaneous variations in gene copy number is to perform a classical fluctuation analysis of cells (6). For example, Kempe *et al.* (7) determined the frequencies of formation of colonies resistant to *N*-phosphonacetyl-L-aspartate (an inhibitor of aspartate transcarbamoylase) in different subpopulations of a Syrian hamster cell line, and they concluded that resistance to 0.1 mM *N*-phosphonacetyl-L-aspartate can indeed arise spontaneously, though at an apparently low frequency ($2–5 \times 10^{-5}$). Similarly, Terzi and Hawkins (8) conducted a fluctuation analysis for cells exposed to MTX and found that resistance to 220 nM MTX arose at a frequency of 4×10^{-5}.

The fluctuation analysis, however, is insensitive to some features of gene amplification in that it is most suitable for detecting rare, random, stable mutations that confer complete resistance to a particular selective agent. Gene amplification, on the other hand, is frequently unstable in character and confers graded resistance to selective agents (9). We were therefore led to employ an alternative method for the detection of spontaneous changes (both increases and decreases) in gene copy number. This method depends on the use of the fluorescence-activated cell sorter (FACS) and may be applicable to a wide variety of systems. We find that gene amplification and loss occur much more frequently than previously suspected and that the frequency of occurrence of these events varies as a function of gene copy number.

MATERIALS AND METHODS

Chinese hamster ovary (CHO K_1) cells were obtained directly from the American Type Culture Collection and were not previously exposed to selection with MTX. Two other cell lines, CHO K_1 B_{11} [0.1] and CHO K_1 B_{11} [0.5], were derived from the CHO K_1 line by stepwise selection with MTX (9). The B_{11} lines are resistant to 0.1 and 0.5 μM MTX, respectively, and *dhfr* in them is amplified approximately 20- and 50-fold (Table 2). We routinely maintain the CHO lines in F12 medium (GIBCO) prepared without glycine, hypoxanthine, or thymidine, to which 10% dialyzed fetal bovine serum (GIBCO) and 0, 0.1, or 0.5 μM MTX are added. During experiments the cells were grown in a supplemented medium that includes 30 μM each of glycine, hypoxanthine, and thymidine (Sigma) but lacks MTX, except as noted. The supplements were included to reverse MTX cytotoxicity (ref. 3; also see Table 1).

Fluorescence analysis and sorting of cells were conducted on a Becton Dickinson FACS-II instrument. [Fluoresceinated MTX (F-MTX) was prepared as described (10) and was further purified by elution from a preparative Rainin RP_{18} Microsorb HPLC column with a 0–100% gradient of methanol, retaining the material eluting at 40–60% methanol.] Cells growing in supplemented medium were exposed for 24 hr to 30 μM F-MTX, then treated with trypsin and suspended in medium lacking F-MTX. In a typical experiment, the distribution of fluorescence for a cell population was determined from 10,000 cells. Cells displaying extremes of fluorescence, representing the upper or lower 2–5% of the population, were sorted and then returned to the supplemented medium. The new cell population arising after 7–12 days of proliferation was sorted again, recollecting for further growth the cells of extreme fluorescence. As multiple successive populations were derived in this way, cells were frozen at each stage and stored at −80°C in supplemented medium with 10% (vol/vol) dimethyl sulfoxide. After the final cell population in an experimental series had been obtained, the intermediate populations were thawed and restored to growth,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DHFR, dihydrofolate reductase (5,6,7,8-tetrahydrofolate:NADP$^+$ oxidoreductase, EC 1.5.1.3); *dhfr*, DHFR gene; MTX, methotrexate; F-MTX, fluoresceinated methotrexate; FACS, fluorescence-activated cell sorter; CHO cells, Chinese hamster ovary cells.

permitting concurrent analysis of the fluorescence characteristics of all the populations.

To determine sensitivities to MTX, cells from each population were plated at low densities in nonsupplemented medium, between 10 and 1,000 per cm^2, and after 24 hr they were exposed to MTX (0–20 μM) for 2–4 weeks. Surviving colonies were counted, and the degree of resistance was expressed as the concentration of MTX required to reduce the frequency of surviving colonies to 25% of controls (ED_{25}). For measurements of *dhfr* gene copy number, equivalent amounts of DNA from each cell population were digested with *Eco*RI, then electrophoresed in 0.7% agarose, transferred to nitrocellulose, and hybridized to ^{32}P-labeled *dhfr* cDNA cloned in pBR322 (11). The degree of hybridization was estimated by scanning exposed x-ray film with a Joyce–Loebl microdensitometer.

RESULTS

The distribution of fluorescence for CHO K_1 cells when stained with F-MTX is shown in Fig. 1. These cells contain little DHFR and are therefore only weakly fluorescent. A few cells (0.6% of the total), however, have 3-fold or greater fluorescence intensity than the mean of the population. Much of the variation in cellular fluorescence arises from the distribution of cells in the cell cycle (12), with premitotic cells staining more brightly than recently divided cells. We speculated, however, that some of the variation might result from genetic heterogeneity within the population, such that some cells would synthesize additional *DHFR* and therefore stain with greater intensity.

In order to evaluate this hypothesis, CHO K_1 cells were grown and stained with F-MTX in medium supplemented with glycine, hypoxanthine, and thymidine. These supplements were included to circumvent the inhibition of cellular growth that otherwise occurs during MTX inhibition of DHFR activity (3). Cells in the supplemented medium, even when treated for up to 2 months with 1–30 μM MTX or F-MTX, show neither a decrease in rate of proliferation or in plating efficiency nor an increase in the frequency of colonies resistant to MTX when the supplements were removed (Table 1). We therefore conclude that cells grown in this manner do not experience selection pressure favoring the overproduction of DHFR. The F-MTX-stained cells were then sorted, retaining for growth as a new population the cells with highest fluorescence (indicated by a bracket in Fig. 1*a*). The 7- to 12-day cycle of growth, staining, sorting, and regrowth of the cells highest in fluorescence was repeated 10 times. We reasoned that, if position in the cell cycle completely accounted for the variable fluorescence, then each new population of cells should display the same pattern of fluorescence as the parental cells. Alternatively, if the highly fluorescent cells were genetically distinct from the rest of the population, then the populations derived from these cells should display a net increase in mean fluorescence intensity.

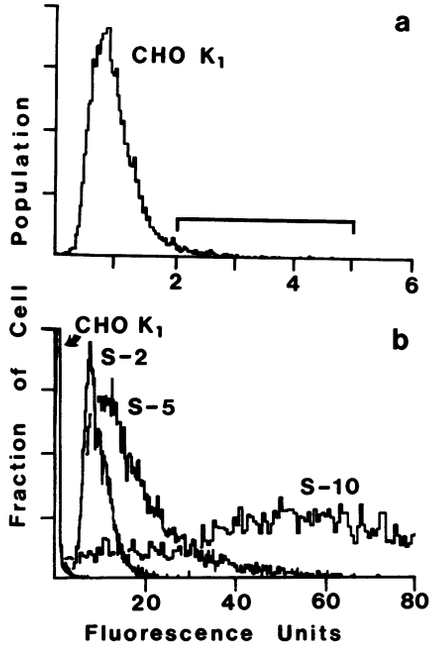

FIG. 1. Rapid increase in cellular fluorescence. The fluorescence profile of untreated CHO K_1 cells is shown in *a*. We define a fluorescence unit as the mean fluorescence of this population; the fluorescence intensities of other cell populations are expressed in multiples of this unit. The cells of fluorescence within the range indicated by the bracket (3.0% of the total population) were sorted by FACS and subsequently grown in nonselective medium. This sorted population was grown and resorted 10 times in succession, yielding the new populations (S-2, S-5, and S-10) shown on a compressed scale in *b*. In each case, the area under the curve is adjusted to represent 100% of the population. The mean fluorescence for each population is shown in Table 2.

After only two rounds of sorting on the FACS, the mean fluorescence of the newly derived cells was already increased 5-fold relative to the starting population (Fig. 1*b*, curve S-2). Therefore, substantial heterogeneity, of a heritable nature, either existed among the parental cells or was generated during the experiment. To further demonstrate this point, we analyzed the fluorescence distributions of five randomly cloned cell lines, which were isolated directly from the untreated CHO K_1 population. The mean fluorescence of these subpopulations varied between 0.6 unit and 1.5 units (parental cells = 1.0); the sensitivities to MTX varied accordingly, between ED_{25} = 0.05 μM and 0.1 μM MTX (parental cell ED_{25} = 0.08 μM). These observations are consistent with the existence of clonal heterogeneity within the parental cell population.

As further rounds of highly fluorescent cells were sorted, we found that both the spread in fluorescence staining intensity and the mean fluorescence continued to increase. By the tenth sort (Fig. 1*b*, curve S-10), the derived cells were on average 50-fold more fluorescent than the parental CHO K_1 cells. We then determined both the ED_{25} and the *dhfr* gene copy number for each of the intermediate and final populations (Table 2, Fig. 2*a*). We find that the mean fluorescence intensity, the degree of resistance to MTX, and the *dhfr* copy number correspond well, and they are comparable to values obtained for the stably resistant cell lines CHO K_1 B_{11} [0.1] and B_{11} [0.5]. Because at no time during their derivation were the highly fluorescent cells exposed to cytotoxic selection for DHFR overproduction, we interpret these results as demonstrating spontaneous increases in DHFR, in MTX resistance, and in *dhfr* copy number.

To examine how the rate of change in *dhfr* copy number might

Table 1. Growth characteristics of CHO K_1 cells

Growth medium	Population doubling time, hr	% resistant to 0.2 μM MTX*	Plating efficiency, %
Supplemented medium	20 ± 1	0.33 ± 0.09	58 ± 12
Supplemented medium + MTX	16 ± 2 (n.s.)	0.46 ± 0.26 (n.s.)	51 ± 7 (n.s.)

Data are presented as mean ± SD. n.s., Not different from control by one-tailed *t* test at $P < 0.05$.

*Cells treated in supplemented medium ± MTX for 40 days, then selected in nonsupplemented medium with 0.2 μM MTX as described in *Materials and Methods*.

Table 2. DHFR-related characteristics of CHO K_1 cells and derivatives

Cell population	Relative mean fluorescence	ED_{25}, μM	Relative *dhfr* copy no.
CHO K_1 (uncloned)	1.0	0.08	1.0
Sort-2	9.6	0.6	3.0
Sort-5	12	1.2	6.7
Sort-10	48	10	39
CHO K_1 B_{11} [0.1]	8.4	1.0	17
CHO K_1 B_{11} [0.5]	56	10	53

vary among different populations, we used freshly isolated subcloned populations prepared from the CHO K_1 parental line and from the CHO K_1 B_{11} [0.1] and B_{11} [0.5] lines. From the B_{11} [0.1] subclone we repeatedly sorted two populations—cells of maximal and of minimal fluorescence. From the CHO K_1 subclone, we sorted (as before) cells of maximal fluorescence, and from the B_{11} [0.5] subclone we sorted only cells of minimal fluorescence. We were unable to collect cells of increasing fluorescence from the B_{11} [0.5] subclone because cells beyond this level of amplification do not saturate well with stain.

Fig. 3 shows the shifts in mean fluorescence during successive sorts for each of these subclones. We found that the changes in fluorescence—either increasing or decreasing—were more rapid for cells already amplified than for the nonamplified parental cells. Thus, whereas the CHO K_1 subclone increased its mean fluorescence by 8 units in 12 rounds of sorting, the B_{11} [0.1] subclone increased its fluorescence by 6 units in only 5 rounds; the decrease in fluorescence for the B_{11} [0.1] subclone was also 6 units in 5 rounds of sorting. The B_{11} [0.5] subclone shifted most rapidly of all, and showed a decrease in mean fluorescence of 35 units in 5 rounds. These differences among the subclones can also be measured by examining the rate of appearance of variant cells during each round of sorting (see *Appendix*). By this analysis, variant cells arise in the subcloned

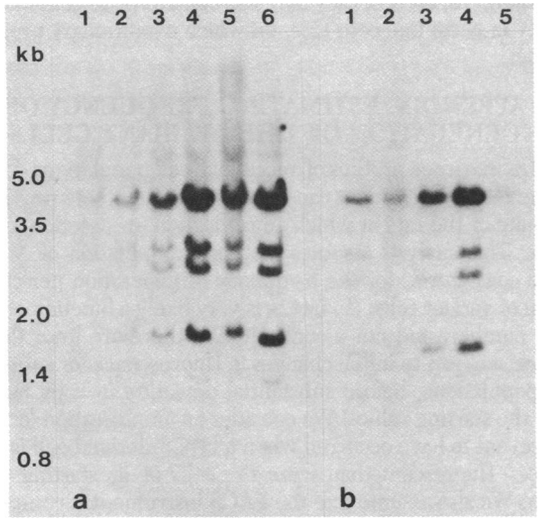

FIG. 2. Variation in *dhfr* copy number. Hybridization to *dhfr* cDNA was measured (Table 2) for each cell population from Fig. 1 (*a*) and from Fig. 3 (*b*). In each case, DNA was digested with *Eco*RI and loaded at 10 (*a*) or 5 (*b*) μg per lane. (*a*) Lane 1, CHO K_1; lane 2, sort-2; lane 3, sort-5; lane 4, sort-10; lane 5, CHO K_1 B_{11} [0.1]; and lane 6, CHO K_1 B_{11} [0.5]. (*b*) Lane 1, CHO K_1 B_{11} [0.1] subclone; lane 2, B_{11} [0.1] low-fluorescence sort-5; lane 3, B_{11} [0.1] high-fluorescence sort-5; lane 4, CHO K_1 B_{11} [0.5] subclone; lane 5, B_{11} [0.5] low-fluorescence sort-5.

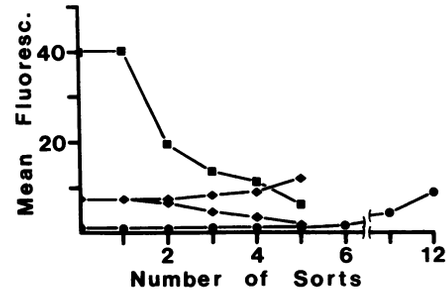

FIG. 3. Shift in mean population fluorescence. Successive populations of increasing or decreasing fluorescence were derived by FACS from subclones of CHO K_1 (●), CHO K_1 B_{11} [0.1] (◆), and CHO K_1 B_{11} [0.5] (■). The mean fluorescence of each derived population is displayed as a function of the number of rounds of cell sorting in its derivation. The mean fluorescence shifts more rapidly during selection by FACS as gene copy number increases.

CHO K_1 population at a rate (averaged over the first five rounds of sorting) of 8×10^{-4} per cell division, in the B_{11} [0.1] subclone at a rate of 2×10^{-2}, and in the B_{11} [0.5] subclone at a rate of 3×10^{-2}. Analysis of DNA from these cell populations confirmed that in each case the *dhfr* copy number varied in accordance with the changes in cellular fluorescence (Fig. 2*b*). Thus, we conclude that the rates of increase or loss in *dhfr* genes vary as a function of *dhfr* gene copy number.

DISCUSSION

Previously, cells with amplified *dhfr* genes have been derived only as a consequence of cytotoxic selection of cells with drugs. In recognition of this, we and others have suggested that the metabolic stress endured by such cells may actually contribute to errors in DNA replication leading ultimately to gene amplification (4, 13). However, we wished also to examine the extent to which changes in *dhfr* copy number might occur spontaneously, without perturbations of cells by drugs. We therefore conducted the experiments described here under conditions that were specifically chosen to avoid cytotoxicity or metabolic stress for the cell populations (see Table 1). We nevertheless observe the appearance of an amplified-*dhfr* genotype even in these nonperturbing conditions. In particular, we readily obtain cells of increasing or decreasing *dhfr* copy number even from recently subcloned populations of cells (Fig. 3). We therefore conclude that the detected amplification events arise during the normal growth of cells *in vitro* and are to this extent spontaneous in occurrence.

If so, then one would predict that a cell line not recently subcloned should be heterogeneous, containing within it cells with *dhfr* copy number differing from the mean value. Indeed, we find that cells sorted from an uncloned CHO K_1 population show rapid initial increases in mean fluorescence (Fig. 1*b*) when compared with populations sorted from recently cloned CHO K_1 cells (Fig. 3). It is likely, therefore, that the initially sorted populations of Fig. 1 were derived from a few variant cells already present within the uncloned CHO K_1 line. Consistent with this interpretation, we find that even randomly selected subclones of CHO K_1 cells, not previously exposed to selection with MTX, show substantial variation in resistance to MTX and in *DHFR* protein content (also see refs. 1 and 8). Thus, although exposure of cells to MTX or to other drugs may indeed stimulate events of gene amplification (13), such events must be considered as an increase with respect to a background of spontaneous, noninduced variations in gene copy number.

The rapid variation in gene copy number we observe in the amplified-*dhfr* CHO cells at first appears inconsistent with our

description of them as stably amplified, retaining many *dhfr* copies even after prolonged growth in the absence of MTX selection (14). However, when cells with chromosomally amplified *dhfr* are grown for 6–12 months in the absence of MTX, there is in fact a gradual decline in mean gene copy number of the population (B. Mariani, personal communication). Similarly, Biedler *et al.* have reported that chromosomal homogeneously staining regions of MTX-resistant Chinese hamster cells diminish in length when the cells are grown in MTX-free medium for long periods of time (15). Thus, "stable" and "unstable" are only relative terms and are not absolute characteristics of cells. Our present results suggest that heterogeneity arises rapidly in even "stably" amplified cell populations and that this heterogeneity is readily detectable by FACS.

Spontaneous variations in gene copy number may be of widespread occurrence in animal cells. Both Law (16) and Terzi and Hawkins (8) have concluded from fluctuation analyses that MTX resistance (though not necessarily by virtue of *dhfr* amplification) can arise spontaneously. In other systems, the gene for aspartate transcarbamoylase amplifies spontaneously in Syrian hamster cells (17), the cellular oncogene *c-myc* is present in increased copy number in human HL-60 cells (18, 19), an *Alu*-associated sequence (20) varies in copy number among different human somatic tissues, and genes for rRNA undergo compensatory increases in copy number in bobbed mutants of *Drosophila melanogaster* (21). Also, Miller and Whitlock (22) have previously described the derivation by FACS of cells with greatly enhanced ability to metabolize benzo[*a*]pyrene that exhibit corresponding increases in aryl hydrocarbon hydroxylase activity. (It is not known, however, whether the enhanced hydroxylase activity is a consequence of gene amplification.) More recently, P. Kavathas and L. A. Herzenberg (personal communication) have used the FACS to derive successive populations of mouse L cells, after transfection with human DNA, that display increases in staining intensity with antibody directed against human Leu-2 antigen. Karyotypes of the intensely staining cells show many double-minute chromosomes; these cells are therefore strong candidates for amplification of the gene for Leu-2.

The fluorescence distributions obtained for each population can be analyzed to yield estimates for the frequency of appearance of variant cells (see *Appendix*). We find that the calculated frequencies vary considerably among different populations (Fig. 3), from 8×10^{-4} events per cell division for *dhfr*-nonamplified CHO K_1 cells to as high as 3×10^{-2} per division for the 50-fold *dhfr*-amplified CHO K_1 B_{11} [0.5] cells. (Note that in the latter case we are measuring the loss of gene copies; we do not know whether the events associated with increases and decreases in gene copy number are actually related at the molecular level.) The rate of change in mean fluorescence varies accordingly, from <1 fluorescence unit in five rounds of cell sorting for the CHO K_1 cells, to 35 units in five rounds for the B_{11} [0.5] cells. Thus, it appears that amplification (or deletion) events occur surprisingly often, with a frequency that increases as gene copy number increases.

These estimates for the frequency of generation of variant cells are much higher (*ca.* 100-fold) than are previous estimates from other systems (e.g., see refs. 7 and 23). It is possible that the *dhfr* gene may be unusually susceptible to amplification processes and that other genes may amplify less frequently. However, we suspect that the FACS instrument may markedly facilitate the detection of amplified cells that might otherwise be missed during classical cytotoxic selection experiments. This is because the FACS permits collection of variant cells, which may be less robust than normal cells, without overt metabolic stress. In addition, the FACS can easily identify and isolate cells in which the genes are as little as 2-fold amplified or that may exhibit only transient or unstable amplification. Cytotoxic selection experiments, on the other hand, would more easily detect relatively stable amplification events of greater than 2-fold magnitude. In this view, these latter events may be only a subset of the total amplification events detectable with the FACS.

The availability of F-MTX as a specific stain for DHFR has rendered the FACS instrument particularly useful in the study of *dhfr* gene amplification. However, the FACS may also be useful in studying amplification of other genes. Given a chromophore that assays for a specific molecule or metabolic process, it should generally be possible to derive with the FACS successive cell populations that exhibit increasingly enhanced or diminished staining with the chromophore, with appropriate genetic changes. Therefore, one may even generate cells that have amplified or deleted genes for which there is no available cytotoxic selective agent. In addition, the FACS provides a specificity of approach in the study of gene amplification that is not necessarily available with a cytotoxic agent. Thus, we have derived with the FACS only cell populations that are resistant to MTX by virtue of their overproduction of native *DHFR*; MTX resistance by alterations in *DHFR* enzyme kinetics (24) or in transport of MTX (25) is not obtained by this approach. In principle, the protocol described here should also give rise to cells that do not have specific genes amplified but instead exhibit aberrant regulation (positive or negative) of the genes. We have not yet observed this behavior in the *dhfr* system. In each case, heritable changes in levels of DHFR protein have always been accompanied by corresponding changes in *dhfr* copy number.

If spontaneous amplification and deletion events occur throughout the cellular genome at rates comparable to that for *dhfr* (*ca.* 10^{-3} events per locus per cell division), then the genome may be much more variable than previously thought. Such variability could account for much of the clonal heterogeneity routinely observed in the morphologies, behaviors, and metabolic activities of cells in culture. More importantly, the processes we describe here may contribute to the variations in gene copy number occurring in somatic cells during development and aging (18, 26, 27); they may also contribute to genomic variability in germ line cells (21), on which evolutionary pressures can act.

APPENDIX: ESTIMATING FREQUENCY OF GENERATION OF DHFR VARIANT CELLS

The fluorescence profiles of successive cell populations derived by the FACS instrument can be analyzed directly to provide an estimate of the rate at which amplification (or deletion) events occur. This analysis assumes a starting population of N cells, and a constant, k, for the frequency of generation per cell division of variant cells. (In fact, k is very likely a function of gene copy number, and not a constant. We therefore limit the numerical analysis to initial changes in fluorescence for subcloned cell populations, before substantial deviation in copy number from the starting value.) We consider an amplification (or deletion) event to have occurred when a FACS-derived cell is more (or less) fluorescent than were the cells of its starting population. We also assume that the FACS instrument is completely efficient at detecting and isolating variant cells against a background of many normal cells, that variant cells do not suffer reduced rates of growth, and that no loss in gene copies occurs during the 7- to 12-day interval between sorting and analysis. These latter assumptions, and particularly the last, may well be invalid. If so, it may be shown that the true value for the rate of generation of variant cells with amplified *dhfr* genes will be higher than the calculated rate. Thus, we consider our estimates of k for each cell population to be minimal ones.

Given these assumptions, after one generation we have $2N - kN$ normal cells. After x generations we have $(2 - k)^x N$ normal cells and $2^x N - (2 - k)^x N$ variant cells. The fraction F of the total cell population that is variant after x generations is therefore $1 - (1 - k/2)^x$ and is independent of N. We can obtain numerical estimates of F directly from the FACS, by determining the fraction of cells at the end of each period of growth that display more extreme fluorescence than the cells from the beginning of the period. In addition, the number of generations experienced by the population can be estimated from the growth interval and the population doubling time of *ca.* 20 hr. Thus, k can be calculated as $2[1 - (1 - F)^{1/x}]$. As an example, we find that among the cells of the fifth population sorted for increasing fluorescence from the B_{11} [0.1] subclone (Fig. 3), 17% (= F) were higher in fluorescence than were the starting cells prepared from the fourth sorted population. From the above equation, we find that $k = 2.5 \times 10^{-2}$ when $x = 15$. By averaging the values for k calculated for each of the populations sorted from the B_{11} subclone, we obtain a mean value for k of 2×10^{-2} amplification events per cell division.

We thank Gene Filson and Len Herzenberg for assistance with the Shared Cell Sorter Facility at Stanford, Dan Santi for assistance with the HPLC, and E. Mosse for technical assistance. R.N.J. was supported in part by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada. This work was also supported by grants to R.T.S. from the National Cancer Institute (CA 16318) and from the National Institute of General Medical Sciences (GM 14931).

1. Brown, P. C., Johnston, R. N. & Schimke, R. T. (1983) *Symp. Soc. Dev. Biol.* **41**, in press.
2. Murcia, C. R. & Nombela, J. J. A. (1972) *Mutat. Res.* **14**, 405–412.
3. Hittelman, W. N. (1973) *Mutat. Res.* **18**, 93–102.
4. Biedler, J. L., Albrecht, A. M. & Hutchinson, D. J. (1965) *Cancer Res.* **25**, 246–257.
5. Schimke, R. T. (1982) *Harvey Lect.* **76**, 1–25.
6. Luria, S. E. & Delbruck, M. (1943) *Genetics* **28**, 491–511.
7. Kempe, T. D., Swyryd, E. A., Bruist, M. & Stark, G. R. (1976) *Cell* **9**, 541–550.
8. Terzi, M. & Hawkins, T. S. C. (1974) *Biochem. Exp. Biol.* **11**, 245–254.
9. Kaufman, R. J. & Schimke, R. T. (1981) *Mol. Cell. Biol.* **1**, 1069–1076.
10. Kaufman, R. J., Bertino, J. R. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 5852–5860.
11. Chang, A. C. Y., Nunberg, J. H., Kaufman, R. J., Erlich, H. A., Schimke, R. T. & Cohen, S. N. (1978) *Nature (London)* **275**, 617–624.
12. Mariani, B. D., Slate, D. L. & Schimke, R. T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4985–4989.
13. Tlsty, T., Brown, P. C., Johnston, R. & Schimke, R. T. (1981) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 231–238.
14. Schimke, R. T., Brown, P. C., Kaufman, R. J., McGrogan, M. & Slate, D. L. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 785–797.
15. Biedler, J. L., Melera, P. W. & Spengler, B. A. (1980) *Cancer Genet. Cytogenet.* **2**, 47–60.
16. Law, L. W. (1952) *Nature (London)* **169**, 628–629.
17. Wahl, G. M., Padgett, R. A. & Stark, G. R. (1979) *J. Biol. Chem.* **254**, 8679–8689.
18. Collins, S. & Groudine, M. (1982) *Nature (London)* **298**, 679–681.
19. Dalla Favera, R., Wong-Staal, F. & Gallo, R. C. (1982) *Nature (London)* **299**, 61–63.
20. Calabretta, B., Robberson, D. L., Barrera-Saldana, H. A., Lambrou, T. P. & Saunders, G. F. (1982) *Nature (London)* **296**, 219–225.
21. Tartof, K. D. (1975) *Annu. Rev. Genet.* **9**, 355–385.
22. Miller, A. G. & Whitlock, J. P. (1982) *Mol. Cell. Biol.* **2**, 625–632.
23. Roberts, J. M. & Axel, R. (1982) *Cell* **29**, 109–119.
24. Haber, D. A. & Schimke, R. T. (1981) *Cell* **26**, 355–362.
25. Sirotnak, F. M., Moccio, D. M., Kelleher, L. E. & Goutas, L. J. (1981) *Cancer Res.* **41**, 4447–4452.
26. Spradling, A. C. & Mahowald, A. P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1096–1100.
27. Shmookler Reis, R. J., Lumpkin, C. K., Jr., McGill, J. R., Riabowol, K. T. & Goldstein, S. (1983) *Nature (London)* **301**, 394–398.