

REVIEW ARTICLE

MITOTIC PERTURBATION AND G₂ CELL CHROMATID ABERRATIONS*

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ABSTRACT

There is no cell system in current usage that has a uniform radiosensitivity for the production of chromatid-type aberrations. Consequently, aberration frequencies fluctuate with sample-time after exposure; there is no unique yield to set against absorbed dose.

Given this situation, the *observed* yields, and their time-profiles, are subject to modification by cell kinetic factors, in particular, the mitotic delay which inevitably accompanies all clastogenic treatments.

This paper discusses some cell-cycle modelling methods which attempt to explore the effects of these perturbations on aberration frequencies, and which, in time, may allow us to correct observed yields to obtain reliable, and useable, scores of chromatid structural changes.

Within most asynchronous, steady-state cell populations, the time taken for a cell to transit the post-S, G₂P compartment varies between cells by a factor of at least five.

As each cell transits this G₂P compartment, the sensitivity with which it *produces* chromatid-type aberrations fluctuates. This, in part, probably reflects the spatial changes which accompany the contraction/compaction of chromatin in preparation for mitosis, and also the effects of lesion repair (Savage, 1989).

Thus, as we sample sequentially at intervals, following an acute ionizing radiation exposure, a wider and wider spread of G₂P positions and an ever-changing-aberration/cell estimates. This is illustrated in Figure 1 by means of a "range diagram" (Savage and Papworth, 1973).

It follows, therefore, that the *observed* fluctuations in aberration frequency with time after exposure (the

profile of the "yield-time" curve) are not simply related to intrinsic changes in sensitivity for aberration production in G₂P. *Observed* frequencies are confounded with the cell-kinetic characteristics of the cell population.

The application of any clastogen perturbs the orderly progression of cells towards mitosis ("*mitotic delay*") - in the case of radiation, producing a differential lengthening of G₂P compartmental transit times such that some cells are delayed (in certain cases stopped altogether) whilst others overtake them (Lea, 1946; Elkind and Whitmore, 1967; Dewey and Highfield, 1976). In general, delay is greater the closer the cell is to mitosis. This exacerbates the kinetic confounding, altering resultant aberration frequencies even more.

Since the degree of perturbation is dose dependent (and is also influenced by any additional treatments), there can be no guarantee that the same mixture of cells can be obtained in two experimental situations from which to determine aberration frequencies, and thus render comparisons legitimate.

In sum, any simple relationship between the intrinsic transit sensitivity pattern and the observed yield-time profile is severed by these kinetic complexities (Savage and Papworth, 1991).

These are obvious facts, but we have very little first-hand knowledge of the kind, or magnitude, of the effects involved.

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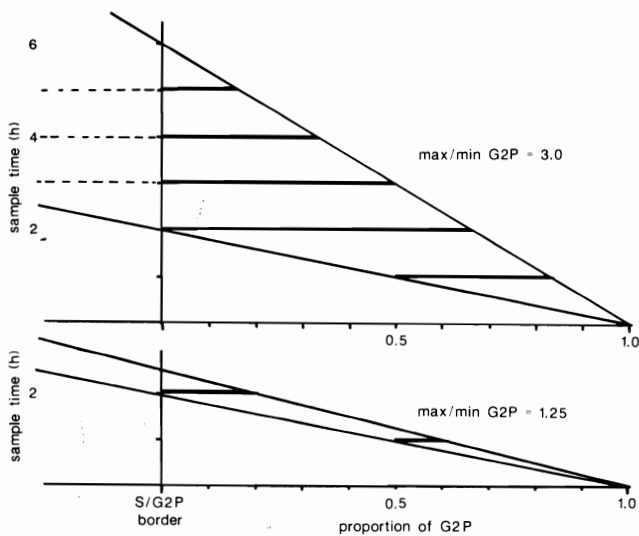


Figure 1 - Range Diagrams. The thick horizontal bars indicate the range of G₂P cell positions which will be contemporary in metaphase at hourly times after treatment. Mitotic Delay is absent and there is no colcemid accumulation period. Two populations are shown: Both have a minimum G₂P of 2 h but differ in the maximum G₂P (spread of transit times). Note that the spread of cell positions changes with sample time and with transit time range and that the same position can be present at several times. Only when the transit time spread is very narrow can different positions in G₂P be examined with precision.

Normally, we are confined to experimental observations - we see the fluctuating yield-time curve, we see that different aberration categories can behave differently, we know that there must be some relationship between observed frequency and the underlying G₂P sensitivity pattern - but we have no means of de-convoluting to find out how close that relationship is.

We have, therefore, to go to work from the opposite direction, we must set up a model cell population, with known plausible (but adjustable) kinetic parameters and produce from it a yield-time aberration profile for the cells that were irradiated in G₂P. Having established what that profile is like in the absence of any mitotic perturbation, we may then stress it with various models of mitotic delay and make some assessment of the form and magnitude of the distortion that might result.

Cell population modelling has been a popular pastime for many years, and a large number of very sophisticated mathematical approaches are in regular use for such things as determining cycle and phase times from FLM or flow-cytometric data, and for the study of tumour kinetics (White, 1981; Easton and Schneiderman, 1987). The advent of powerful computers enables the use of very complex numeration and, when many interacting parameters have to be considered, the Monte-Carlo methods are applied.

We have used both an analytical and a Monte-Carlo approach, the latter to act as a test of the former. Our model is adapted for obtaining aberration yield/G₂P metaphase cell at any time after radiation exposure. All usual population parameters (like cycle times, transit-time distributions etc.) can be altered at will. The G₂P compartment is divided into 10 equal length segments which allow the setting of any desired intrinsic sensitivity pattern. The assigned sensitivity in each is construed as the mean of a Poisson distribution and the aberration burden for each cell in the segment is allocated on this basis.

In addition, various models of mitotic delay can be superimposed in G₂P to distort the observed yield-time curve. Currently, three broad groups of delay model are being investigated:

Model I: *Generalised Delay*. A cell is delayed according to its position in G₂P at the time of irradiation. The amount of delay is considered to be maximal close to the G₂/mitosis border and minimal at the S/G₂P border. The grade between maximum and minimum is either linear or exponential.

Model II: *Barrier Delay*. time-related. A specific "Transition Point" (TP) is present in G₂P at which cells are temporarily stopped, the duration of stoppage being related to the distance the cell is from the TP at the time of radiation. Cells that are past this position are undelayed.

In this model, the barrier is placed at a specific time before metaphase, i.e. it will be at different developmental positions in different cells.

Model III: *Barrier Delay*. Position-related. As Model II, but the TP is placed at a precise developmental position in G₂P; i.e. it will occur at different times before metaphase.

We illustrate the effects of these factors by a set of examples, using a population model based upon typical cultured fibroblasts. The relevant population parameters are as follows:

- Minimum G₂P = 1.5 h.
- Maximum G₂P = 9.0 h.
- G₂P transit-time distribution; moderately positive skewed beta distribution ($p = 3, q = 6$); modal G₂P = 3.64 h.

The intrinsic (segmental) pattern of sensitivity through G₂P for the production of aberrations is the same for all the examples shown.

For Delay model I, the maximum delay at the end of G₂ is 2 h in both examples. The minimum delay at the S/G₂P border is either 0.5 h or zero. The amount of delay changes linearly between the extremes.

For Delay model II, TP is placed 1.0 h back from metaphase (i.e. from the end of G₂P). The maximum delay experienced by cells at this position is 2.0 h. A rapid (2.0

h/h) and a slow (0.5 h/h) time-decay at the barrier are considered.

For Delay model III, TP is placed at 0.75 of the transit of G_2P . Again, the maximum delay experienced is 2.0 h, and the same two time-decay parameters are investigated.

Figure 2 illustrates the actual profiles of the yield-time curves, first when there is no delay, then two examples from each of the three Delay models with parameters as defined above.

The curves shown are built on the basis of 0.1 h sample-time increments. This is, of course, much finer than we ordinarily use for experiments, so they need to be integrated and averaged into sample-time blocks that correspond to the colcemid accumulations routinely

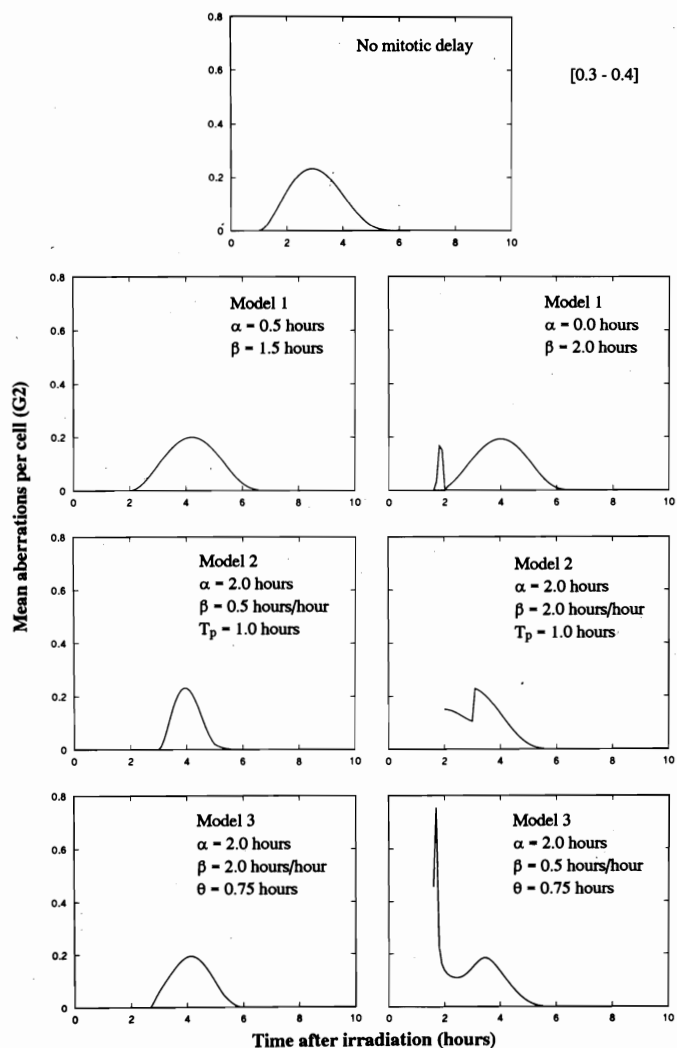


Figure 2 - Yield-time profiles of chromatid aberrations produced exclusively in G_2P cells. The intrinsic sensitivity pattern is the same for all populations. Three models of mitotic delay have been tested, the details of which are discussed in the text. In model II, T_p , the Transition point, has been placed 1.0 h back from metaphase. In model III, it is set 0.75 of the way through G_2P .

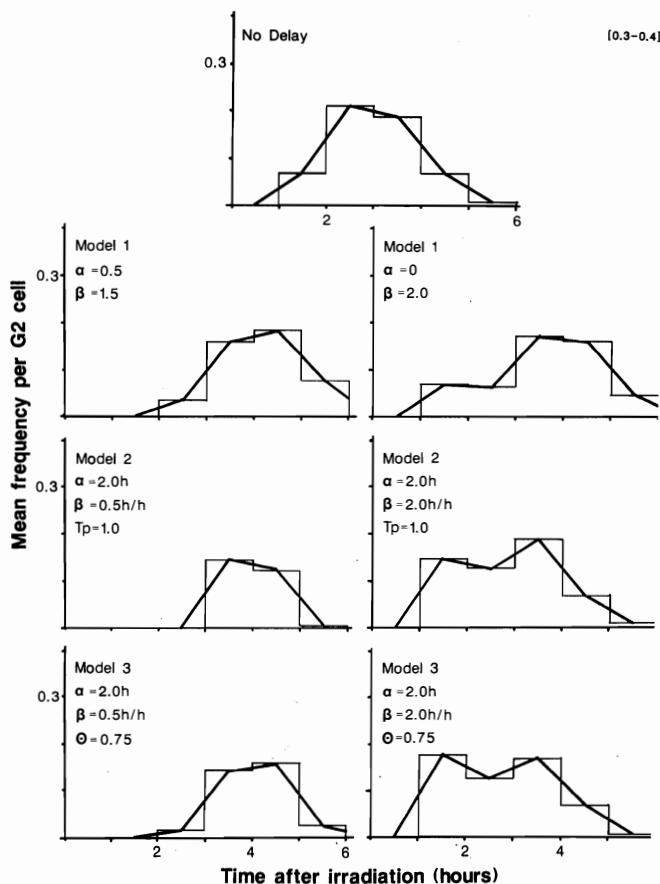


Figure 3 - The data of Figure 2 has been integrated into hourly time blocks to simulate colcemid accumulations. The means of the time blocks are plotted as bar charts, and polygons have been drawn to the mid-intervals. This represents the more usual form in which experimental data is seen for interpretation. Differences between the models are still apparent, but some of the more dramatic fluctuations have been dampened out.

employed. This has been done in Figure 3 for hourly collection periods, starting immediately after irradiation.

It is obvious that quite a variety of yield-time curves result, with differences in fine-structure profile, frequency maxima, integrated area and sample-time positioning in G_2P .

Yet these all arise from one and the same sensitivity pattern in G_2P ! This happens to be a very simple all-or-nothing pattern; aberrations can *only* be produced in cells lying between 0.3-0.4 of their G_2P transit, and *not otherwise*. Figure 4 shows such a pattern and also the dramatic effect that changing the position of this "all-or-nothing" segment can have upon the yield-time aberration profile. The assigned sensitivity in this segment (Poisson mean) is 1.0. Consequently, if the total G_2P cells were to be collected, the grand aberrations mean (from a sensitivity segment in this position) would be 0.1009.

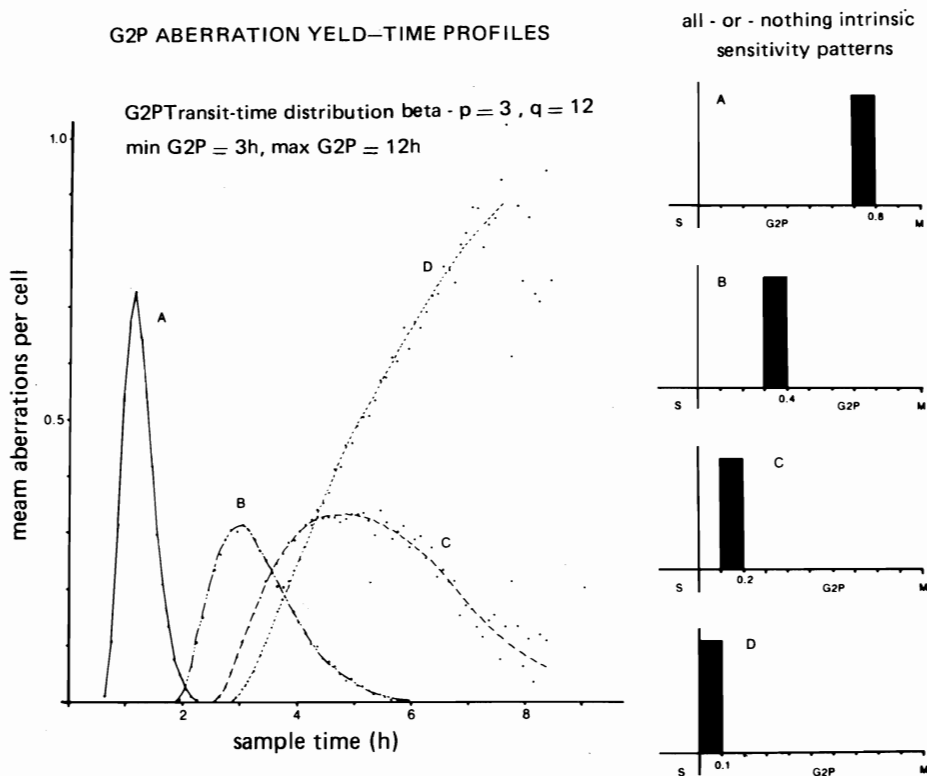


Figure 4 - The aberration yield-time profile in an "all-or-nothing" sensitivity situation is markedly dependent upon where the sensitive segment is placed in G₂P. For *all the curves of Figures 2 and 3*, an "all-or-nothing" segment with mean sensitivity of 1.0 per cell was present 0.3-0.4 of the way through the compartment (compare B above). Note that the population parameters here are different from those used for the delay studies so that the undelayed profile is somewhat sharper.

In conclusion, one or two comments need to be made.

1) We have analysed for "chromatid aberrations" as a single class. In reality, there are many different categories, some of which clearly have different sensitivity patterns in G₂P.

The actual profile of a yield-time curve, (and hence the family of distorted curves derivable from it by mitotic perturbations) is very dependent upon the position of the sensitive segment, as is easily demonstrable from simple all-or-nothing situations. Thus categories differing in sensitivity pattern (e.g. gaps, intrachanges, interchanges) will show rather different profiles for the *same* delay sequence. This has implications for both combining and comparing the observed frequencies of different categories.

2) Only G₂P cells have been considered in the analyses. In a real situation covering this sampling period, S-phase (and ultimately pre-S) cells are also present, and unless steps are taken to distinguish them, their scores will be included in constructing the yield-time curve.

In some of the delay models used here, sample-time regions occur where there are no G₂P cells

present to score (i.e. the mitotic index for G₂P cells is zero). S-cells, however, will be present, having overtaken the G₂P cells, because we have assumed in most cases that delay does not extend beyond the S/G₂P border.

In reality, of course, it usually does at higher doses, but its magnitude is normally much less. In general, S-cells are much less sensitive for the production of chromatid-type aberrations, so their inclusion is likely to have a dramatic effect upon observed frequencies and the profile of the yield-time curve. Eventually, we plan to extend our investigations into this area.

3) The results presented are purely illustrative of the effects that mitotic perturbation can have upon aberration frequencies. We believe that the models we have used are plausible, but there is still much confusion in the literature about the exact nature and form of the phenomenon.

One area we have left untouched is the consequences or relationship of delay to the aberration frequency itself: Does a delayed cell have opportunity to repair lesions which otherwise would form aberrations? Alternatively, Is the amount of delay *related* to the (potential) aberration burden of a cell? In either case, our

simplistic yield-time curves become invalid and, frequencies become even further removed from reality. If these are important factors, then it should be noted that fluctuations in yield-time curves could arise from this source alone, without the need to invoke intrinsic transit-time-related changes in G₂P sensitivity.

4) These studies, thoughts and observations raise a very important question: What is a meaningful measure of chromatid-type damage? It is obvious that there is no unique yield to set against a given dose, and that the frequencies we observe have been modified by the changing kinetic status of our sampled population.

If we isolate only the cohort of sensitive cells (and strictly, these are the only cells at-risk for aberration production) we have a mean frequency of 1.0 per cell, aberrations being distributed as a Poisson.

If we collect all and only the G₂P cells, the overall mean would be 0.1009 per cell. The value would differ very slightly depending upon the position of the segment.

However, these possibilities can only be realised in model populations. In the real world, we are confined to yield-time curves of the type shown in Figure 3. Depending on the delay conditions, peak frequencies range from 0.15 to 0.21. The integrated areas likewise vary from 0.06 to 0.12, but lie around the overall mean.

What therefore should one use for comparative or experimental purposes? This is a question of considerable importance if chromatid-type aberrations are to be used for serious work and, as yet, there is no simple or satisfactory answer to it.

RESUMO

Não há sistema celular que tenha radiosensitividade uniforme para a produção de aberrações tipo cromátide. Conseqüentemente, a frequência de aberração flutua com o tempo após exposição; não há um resultado uniforme para correlacionar com a dose absorvida.

Devido a esta situação, os resultados *observados* e seus perfis de tempo estão sujeitos a modificação por fatores cinéticos celulares, em particular, o atraso mitótico que inevitavelmente acompanha todo o trabalho clastogênico.

Este trabalho discute métodos de construir modelos do ciclo celular o qual explora os efeitos destas perturbações na frequência de aberrações, e o qual, em tempo, pode permitir-nos corrigir efeitos observados para obter dados de mudanças estruturais da cromátide, confiáveis e usáveis.

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