Unequal Cell Division as a Driving Force During Differentiation

Millet Treinin* Dror G. Feitelson†

*Department of Genetics and †Department of Computer Science

The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

Abstract

Unequal cell division is a common event during development. We have formulated a model that predicts significant differences in the expression of an activator gene that are strictly dependent on cell size. The model is based on well known aspects of the regulation of gene expression, specifically the existence of multiple regulatory sites, positive autoregulation, and the short half-life of regulatory proteins.

1 Introduction

Differentiation is a basic process during the development of multicellular organisms, leading to the creation of diverse cell types. This requires that the two products of a cell division assume different identities. There is evidence for two mechanisms which may be responsible for the determination of cell identity: one is unequal distribution of genetic information, proteins, and RNA in the mother cell, and the other is cell-cell interactions that provide different environments for the two cells. We wish to show how unequal cell division may serve as a mechanism that drives cell fate determination, through its effect on gene expression.

In many cases it seems that cell differentiation is a hierarchical process, governed by master regulatory genes. These genes regulate the expression of specialized gene sets whose products determine the identity of the differentiated cell (Herskowitz, 1989). Thus it is possible to view the expression of a master regulatory gene (henceforth called the "activator") as the determinative event in the differentiation process. We will therefore focus on the

expression of the activator, and show how very different levels of expression may arise in small and large cells.

We assume that the two products of cell division express basal levels of the activator gene. This low-level expression is not sufficient for the activation of the genes that require the activator's product for their expression, and the product does not accumulate due to degradation (it is often the case that regulatory proteins have short life times). The activator is also assumed to have positive autoregulation. We assume that the rate of transcription and the rate of degradation do not depend on cell size. However, the concentration of the gene's product will be larger in a smaller volume. Thus unequal cell division will cause a certain difference in the concentration of the activator gene's product. If the concentration of the product in the smaller cell passes a certain threshold, then autoregulation will take effect. Autoregulation can then amplify this small difference, by a positive feedback effect, leading to a high level of expression. This will switch on all the genes that depend on the activator. The nonlinear dependence of the autoregulation on the concentration is modeled by assuming strongly cooperative binding in multiple binding sites. This threshold mechanism is essentially the same as that proposed by Lewis *et. al.* (1977).

Sporulation in the usually unicellular prokaryote *B. subtilis* is one example of a system that matches many aspects of our model. The process starts with unequal cell division (Kunkel, 1991). The smaller cell differentiates into the spore cell, and the larger cell develops into the mother cell. The *spoIIIG* gene is the activator of the spore specific genes. Two modes of expression where shown for this gene: basal expression which is not specific to the spore cell, and a higher spore-specific expression which is dependent on the product of the *spoIIIG* gene itself (Karmazyn-Campelli *et. al.*, 1989). It is possible that the difference in size between the mother cell and the spore cell may trigger the change in the mode of expression of the *spoIIIG* gene. The analogy between this system and the model breaks down when the nonlinear autoregulation is considered, because *spoIIIG* has a single binding site. This issue is elaborated in the discussion, where other possibilities are presented.

The patterns of cell division during the development of multicellular organisms are highly regulated. Unequal cell division is a well documented phenomenon during the early development of many organisms. For example, it is seen in *C. elegans* (Kimble, 1981b; Sulston and Horvitz, 1977) and sea urchin (Hörstadius, 1973). There are a number of examples where cell size correlates with cell fate determination. In *C. elegans* unequal cell divisions

create cells that differ in the cell lineages that they produce. Laser ablation of neighboring cells can sometimes change the polarity of these unequal cell divisions, and this correlates with changes in the polarity of the ensuing cell lineages (Kimble, 1981a). A possible interpretation for this is that cell-cell interactions regulate the polarity of the division, and that the unequal cell division directly determines the developmental fate, as our model suggests. Another example is provided by the *lin-17* mutation. This mutation may cause cell divisions that are normally unequal to produce daughter cells with equal sizes (Sternberg and Horvitz, 1988). The products of such equal divisions have the same developmental fates, whereas the unequal cells produced normally have different fates. Again, a possible interpretation is that the *lin-17* mutation affects the equality of the division, which determines the daughter cells' fates.

In sea urchin, four cells undergo unequal cell division in the forth cleavage, and their smaller daughter cells undergo unequal division again in the fifth cleavage. The larger products of this second unequal division are the founder cells of the primary skeletogenic mesenchyme, while their smaller sisters are the founders of the small micromers which produce the coelomic pouches (Cameron and Davidson, 1991). Again the correlation between cell size and cell fate seems to agree with our model; however, the close proximity of these cells may also be the cause for their undertaking the same fates.

The basic ideas underlying our model (that cell size may influence gene expression and that an amplification mechanism is required) were suggested by Sternberg in the context of mother-daughter asymmetric *HO* expression in the yeast *S. cerevisiae* (Sternberg, 1987). However, it seems that in this specific case a mechanism other than cell size is at work (Nasmyth *et. al.*, 1987).

2 Model and Results

Let us now formalize the proposed model. Positive autoregulation exists when the promoter for a gene's transcription includes a binding site for its own product. If there are multiple binding sites, the gene is transcribed only when all the sites are occupied. Assuming strong cooperativity of binding (a Hill coefficient of n), the probability that n binding sites are occupied

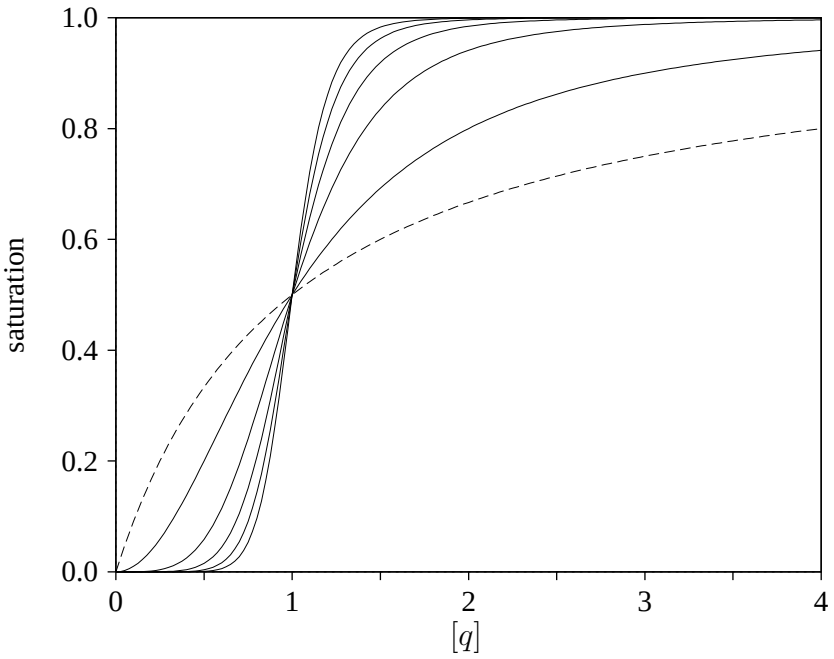

Figure 1: A plot of the saturation as a function of product concentration, given by equation (1). The dashed line is for $n = 1$, and the others are for $n = 2$, 4, 6, 8, and 10. The dissociation constant is $k = 1$; it dictates the point at which all the curves intersect. Note that for $n > 1$ these curves are sigmoids, indicating that in practice the n binding sites will be occupied simultaneously only if the concentration exceeds a certain threshold.

simultaneously is given by

$$Y = \frac{[q]^n}{k + [q]^n}, \tag{1}$$

where $[q]$ denotes the concentration of the product, k is the dissociation constant, and Y is the saturation. This is plotted in figure 1 for different values of n, showing that for $n > 1$ autoregulation can only take effect if the concentration passes a certain threshold. This equation, which is well known in enzymology (Creighton, 1984), assumes that all the binding sites are equivalent and that binding is an "all or nothing" event.

Apart from the induced transcription due to autoregulation, there is also some basal transcription that does not depend on the gene's product. The total rate at which the product is generated is therefore

$$\left.\frac{\partial q}{\partial t}\right|_{\text{gen}} = \beta + \alpha \left(\frac{(q/V)^n}{k + (q/V)^n} \right), \tag{2}$$

where α is the rate of induced transcription (when all the binding sites are occupied), β is the rate of basal transcription, and we use $[q] = q/V$ to introduce the dependency on the cell volume.

Countering this generative process is a process by which the product degrades. We make the simplest possible assumption about this process, namely that the rate of degradation is proportional to the current quantity of the product:

$$\left.\frac{\partial q}{\partial t}\right|_{\text{deg}} = -\frac{q}{\tau}. \tag{3}$$

This means that with no generation the product quantity would decrease exponentially with a half-life time of τ.

The Two Stable States

A stable state is achieved when the transcription rate is equal to the degradation rate, i.e. when equation (2) equals the absolute value of equation (3). The two functions are plotted together in figure 2. It is seen that there may be either one or three intersections, depending on the values of various parameters (the critical cases of two intersections are of no practical interest, except to delimit the range in which three intersections occur). Stable states are represented by intersections such that at slightly higher quantities of the product the degradation is larger than the production, while at lower quantities the production is higher than the degradation.

Keeping all the parameters fixed, we find that there are two critical values that divide the volumes into three ranges (figure 3 (a)). For small volumes there is only one stable state, in which the quantity of the product is high due to autoregulation: it is approximately $q = (\alpha + \beta)\tau$. For large volumes there is also only one stable state, but in this case the quantity of the product is low, corresponding to the basal transcription rate: $q = \beta\tau$. For intermediate volumes both stable states are possible (the middle intersection is not stable). Assuming that the quantity of the product is low to begin with, the cell will enter the low stable state and stay there. However, if the cell starts out small, so that the product reaches the high level, it will stay in the high level even if the cell subsequently grows into the middle range (dashed line in figure). This property is a result of the positive feedback, and is important for the maintenance of the cell's new identity (Lewis *et. al.*, 1977). In particular, it means that the state that is observed depends on the *history* of the cell's

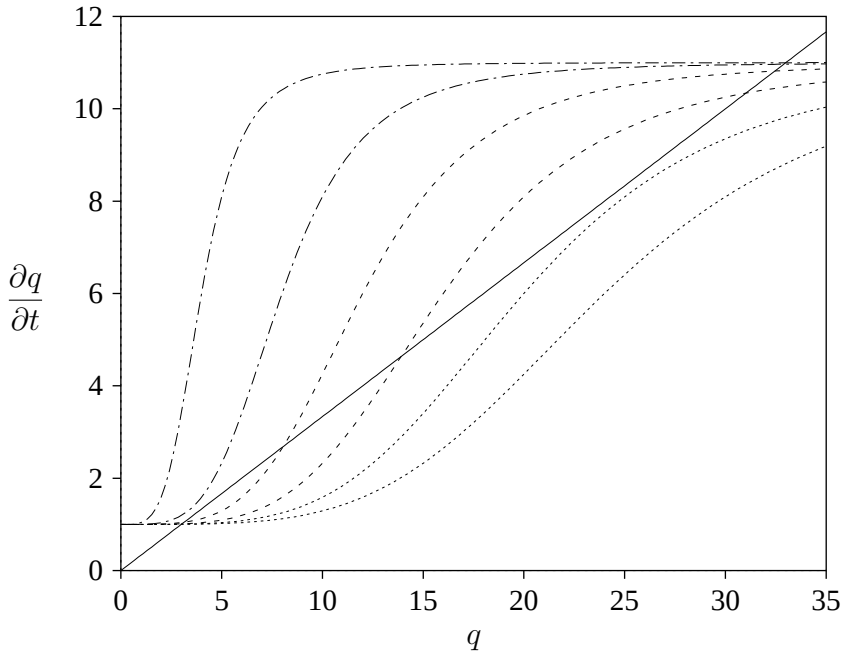

Figure 2: Plotting the rate at which the product is produced against the rate in which it degrades identifies intersections that denote steady states. The six curves show the rate that the product is generated at volumes of $V = 4$, 8, 12, 16, 20, and 24, from left to right (equation (2), with $\alpha = 10$, $\beta = 1$, $k = 1$, and $n = 4$). The diagonal line represents the linear degradation (absolute value of equation (3), with $\tau = 3$). For low volumes (—·—· lines) there is only one stable state, where the quantity of the product is high due to autoregulation. For higher volumes (– – – lines) there are three intersections, two of which represent stable states. Assuming that we start with a small amount of the product, the lower one will be observed. In this state the quantity of the product corresponds to the basal transcription. For very large volumes (------ lines) there is only one stable state, with the same low quantity.

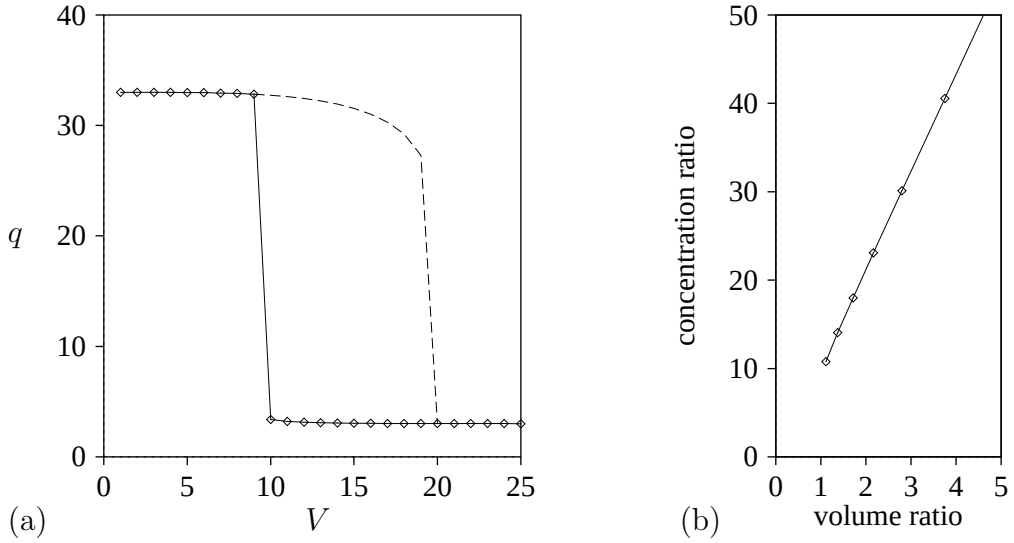

Figure 3: The steady state of the product was found by starting with $q = 0$, and using equations (2) and (3) iteratively until a steady state was reached. (a) shows the results as a function of V, for $\alpha = 10$, $\beta = 1$, $k = 1$, $\tau = 3$, and $n = 4$. The three ranges of volumes are $V \leq 9$, $10 \leq V \leq 19$, and $V \geq 20$. In the first range the high steady state is observed, while in the other two cells settle into the low steady state. However, if a small cell subsequently grows into the middle range, it will remain in the high steady state (dashed line). The concentration of the product in small cells is larger than in big cells both because the absolute quantity is larger, and because the volume is smaller. This effect is plotted in (b). Pairs of data points symmetrical on both sides of $V = 9$ were used.

size.

Figure 3 (a) gives the *quantity* of the product that is found in cells of various sizes, and figure 4 shows how it depends on the ratio of α to β. But the activity of the product depends on the *concentration*, not on the absolute quantity. It is therefore interesting to see how the concentration changes. Near the critical volume, very small changes in cell size can cause very large changes in the concentration. Figure 3 (b) plots the ratio of product concentration in the small and large cells as a function of the ratio of volumes. It should also be remembered that the volume is cubic in the diameter. Thus an increase of only 26% in the cell diameter leads to a doubling of the volume ($1.26^3 = 2.000376$), and a 21-fold decrease in the concentration (for the

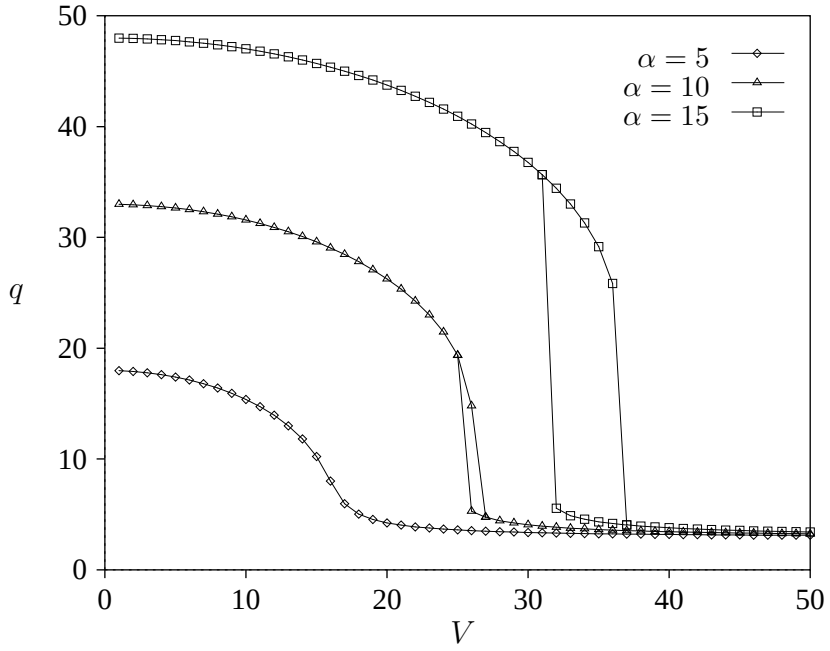

Figure 4: The calculation used in fig. 3 (a) is repeated here for different ratios of α/β. The parameters are $n = 2$, $\tau = 3$, $k = 0.5$, $\beta = 1$, and $\alpha = 5, 10$, and 15. For $\alpha = 5$ there is no sharp threshold, as expected from the data in table 1. When α is big enough, a bistable region is formed, and the larger the ratio of α/β, the wider this region is.

parameters in our example). In general, the concentration ratio is

$$\frac{[q]_{\text{small}}}{[q]_{\text{large}}} = \frac{(\alpha+\beta)V_{\text{large}}}{\beta V_{\text{small}}}. \tag{4}$$

If the smaller cell subsequently grows, the concentration ratio will be reduced accordingly.

Finding the Threshold Volumes

The dynamics of the system depend of course on the model's parameters. It is especially interesting to find the threshold volumes as a function of these parameters. To do so, we note that the threshold volumes are characterized by the fact that the graph of equation (2) is *tangential* to the graph of equation (3) (see figure 2). This leads to the following two conditions. First, the

n	2	3	4	5	6
α/β	8	3	1.78	1.25	0.96

Table 1: Minimal ratios of α to β needed for bistability.

generation rate must equal the degradation rate:

$$\beta + \alpha \left(\frac{(q/V)^n}{k + (q/V)^n} \right) = \frac{q}{\tau}, \tag{5}$$

and second, the derivatives of these two functions must be equal:

$$\frac{\frac{\alpha n}{V}(q/V)^{n-1}}{k + (q/V)^n} \left(1 - \frac{(q/V)^n}{k + (q/V)^n} \right) = \frac{1}{\tau}. \tag{6}$$

By substituting the left-hand side of equation (6) for $\frac{1}{\tau}$ in the right-hand side of equation (5), we get the following quadratic equation in the saturation:

$$\alpha n \left(\frac{(q/V)^n}{k + (q/V)^n} \right)^2 - \alpha(n-1) \left(\frac{(q/V)^n}{k + (q/V)^n} \right) + \beta = 0. \tag{7}$$

Using the formula for the solution of a quadratic equation, we can express the two solutions as

$$Y_{1,2} = \frac{\alpha(n-1) \pm \sqrt{\alpha^2(n-1)^2 - 4\alpha n\beta}}{2\alpha n}. \tag{8}$$

Obviously, if the discriminant is negative, there is no solution. When this happens there is no range of volumes where the cell can have two stable states. In particular, there is no threshold volume such that very different levels of expression will be observed on its two sides. This immediately leads to a necessary condition for bistability and the resulting threshold effect, namely that

$$\frac{\alpha}{\beta} > \frac{4n}{(n-1)^2} \tag{9}$$

The minimal allowed ratios of α to β for different values of n are shown in table 1. It is seen that in most cases this is not a severe restriction, as the transcription rate with autoregulation may be expected to be substantially larger than the basal rate. If the ratio is too small, we still get a higher level

of expression in smaller cells, but the transition from "small" to "large" is gradual rather than abrupt (figure 4).

returning to equation (8), we complete the discriminant to a square by adding a factor of $\left(\frac{2n\beta}{n-1}\right)^2$. Assuming as we do that α is much larger than β, this introduces a negligible error. The solutions are then found to be

$$Y_1 = \frac{\beta}{\alpha(n-1)} \qquad \text{and} \qquad Y_2 = \frac{n-1}{n} - \frac{\beta}{\alpha(n-1)}. \tag{10}$$

These values may be plugged into equation (5) to yield solutions in terms of q rather than Y. Finally, based on the knowledge of q and Y, we can use the original expression for Y (equation (1)) to express the result in terms of the volume V. The two thresholds are found to occur at

$$\begin{aligned} V_1 &= \tau\ \beta\ \tfrac{n}{n-1}\ \sqrt[n]{\tfrac{\alpha(n-1)-\beta}{k\,\beta}} \\ V_2 &= \tau\left(\tfrac{n-1}{n}\ \alpha + \tfrac{n-2}{n-1}\ \beta\right)\ \sqrt[n]{\tfrac{\alpha(n-1)+\beta n}{\alpha k(n-1)^2-\beta k n}} \end{aligned} \tag{11}$$

The second expression especially is ungainly. We can arrive at a simpler one by using $\frac{n-1}{n}$ as an approximation for Y_2, rather than the original expression in equation (10). This is again reasonable because α is much larger than β. The result using this approximation is

$$V_2 = \tau\left(\tfrac{n-1}{n}\ \alpha + \beta\right)\ \frac{1}{\sqrt[n]{k(n-1)}}. \tag{12}$$

We have conducted extensive tests for a large number of combinations of parameters to validate these approximations and results. In each case, the real threshold values were found by using equations (2) and (3) directly, as described in the caption of figure 3. These were compared with the the values calculated from equations (11) and (12) above. The agreement in all cases was excellent. For example, the numbers for the parameters used in figure 3 are $V_1 = 9.28$ and $V_2 = 19.38$.

An important point is that the threshold volume that distinguishes between small and large cells, V_1 in equation (11), may be quite large for certain sets of parameters. This means that the smaller daughter cell does not have to be extremely small for the model to work. The ratio of α to β dictates the ratio of product levels in the two steady states. The absolute levels are given by these parameters multiplied by τ. Thus a large τ raises the product

quantities at steady state. As a result, it also raises the threshold volumes, and allows larger cells to enter the high steady state. A small k also raises the threshold volumes, because it indicates that binding occurs at lower levels of the product. A large n, on the other hand, reduces the threshold volume, because when more binding sites need to be occupied higher concentrations are required.

3 Discussion

An important aspect of the model is that the activator gene may have two very different stable states of expression over a relatively wide range of volumes. This bistable behavior implies that the observed state depends on the cell size history, and not only on the current size. It can also explain how a transitive difference in cell size can lead to an epigenetic change which is stably inherited.

Stable inheritance can work in two ways: either the same mechanism may continue to work whenever a cell divides, or the initial differentiation may trigger some other change that is then permanently maintained (Heemskerk *et. al.*, 1991). In cases of epigenetic inheritance where there are switches back and forth, it seems plausible that the same mechanism is at work. This requires that the top threshold be at least twice as large as the bottom one, i.e. that $V_2 \geq 2V_1$. To see this, consider what happens during normal cell division. To guarantee stable inheritance, a growing cell should not pass the V_2 threshold. In addition, when a cell divides, its daughter cells should not be smaller than the V_1 threshold. This implies that the sizes of both the mature cell which is dividing and its newly formed daughter cells must be within the bistable region. As the mother cell is at least twice the size of the smaller daughter cell, the condition follows.

Occasional switching may occur due to fluctuations in cell size under the following conditions. The mature cell size should be just a little bit smaller than the V_2 threshold, which is just a little bit larger than twice the V_1 threshold. Whenever a mature cell happens to grow beyond V_2, the level of expression in it will drop to the basal level, and this will be inherited by its descendants. On the other hand, whenever a cell divides and one of the daughter cells happens to be smaller than V_1, the level of expression in it will go up due to autoregulation. this too will be inherited by its descendants.

We can find the ratio of V_2 to V_1 using equations (11) and (12). The

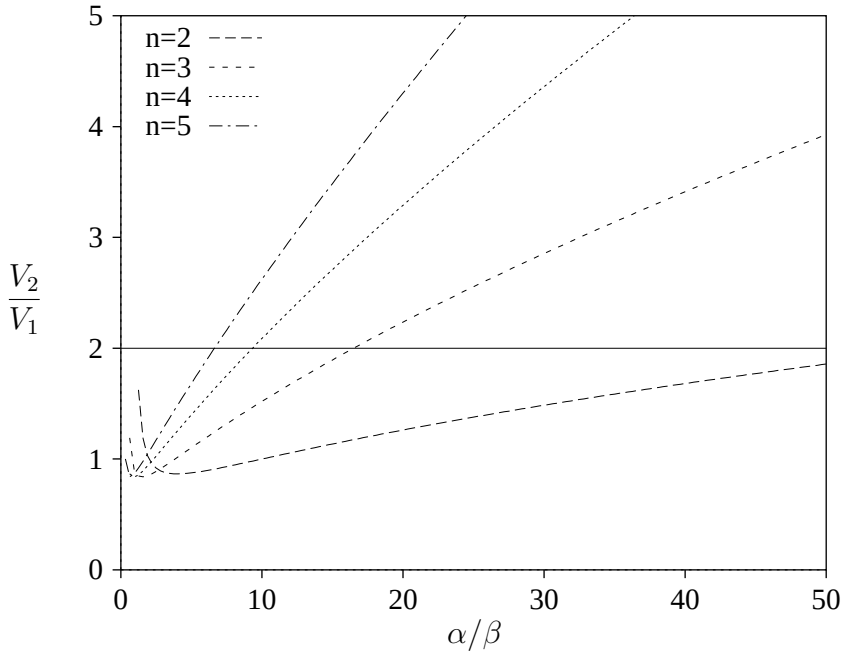

Figure 5: Stable inheritance can occur if $V_2/V_1 \geq 2$. This ratio is expressed by equation (13). Note that it depends only on n and on the ratio α/β. It is plotted here for several values of n.

result is

$$\frac{V_2}{V_1} = \left[\frac{n-1}{n} + \left(\frac{n-1}{n}\right)^2 \frac{\alpha}{\beta}\right] \frac{1}{\sqrt[n]{(n-1)^2\frac{\alpha}{\beta} - (n-1)}}. \tag{13}$$

Figure 5 shows how this ratio depends on the ratio α/β for different values of n. For small n, rather large ratios of α to β are needed to make this model applicable, but for larger n the necessary ratios are perfectly reasonable.

The weakest point in the model is the assumption that the autoregulation depends on multiple binding sites with highly cooperative binding. Multiple binding sites with strong cooperativity are known to exist in the regulatory regions of various eukaryotic genes (Struhl *et. al.*, 1989; Fromental *et. al.*, 1988). For example, it has been suggested that cooperative binding and the resulting threshold property provide a mechanism for the differential regulation of downstream genes by the *bicoid* morphogen gradient in early *Drosophila* development (Struhl *et. al.*, 1989). Likewise, cooperative binding of the λ repressor plays a role in the switch between lysogeny and lytic growth in phage λ (Johnson *et. al.*, 1981).

However, in real biological systems one rarely finds such simple and clear-cut situations as the one used in our model. Rather, the different binding sites may have different affinities, and binding at one site may affect the affinity of another. In addition, various complexes may be required for the binding to succeed. Obviously, a detailed model of such a system would be very hard to analyze, and would also require a large number of parameters. The highly cooperative binding model that we used has only one parameter, k, and leads to tractable formulas. In addition, it is reasonable to believe that all other binding patterns that have a similar (i.e. sigmoid) nature would give similar results. For example, it has been suggested that multi-component complexes might also require that the concentration of their components pass a certain threshold for their formation (Frankel and Kim, 1991). The mechanism in *B. subtilis* may be of this type, since *spoIIIG* has only one binding site in its 5' upstream region.

Our model demonstrates that unequal cell division may cause changes in a gene's expression in the smaller cell. Changing the rate of transcription of a transcriptional activator is the first step in the process that changes the whole pattern of gene expression in the smaller cell. Differentiation of the larger cell can be a result of a default mechanism, or alternatively cell-cell interactions with the sister cell may determine the larger cell's identity. The *lin-17* mutations in *C. elegans* sometimes result in both (equal) daughter cells producing abnormal cell lineages (Sternberg and Horvitz, 1988), indicating that interactions with the differentiated smaller cell may be important in this case.

It should be noted that the question of what causes unequal cell divisions is left open. However, this phenomenon is known to exist. The main contribution of our model is to show how easily this phenomenon can affect gene expression, to identify the necessary components (especially nonlinear autoregulation), and to quantify the effect. Assuming that size differences indeed influence differentiation as our model suggests they might, this means that additional regulatory mechanisms are not needed. We therefore believe that this possibility deserves more attention than it has received so far.

Morphogenesis is part of the developmental process which is usually considered to be a product of the differentiation process. We have now shown that morphogenesis can also influence differentiation. The interplay between morphogenesis and differentiation might be an important factor during development.

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