Stochastic model of transcription factor-regulated gene expression

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Abstract

We consider a stochastic model of transcription factor (TF)-regulated gene expression. The model describes two genes: Gene A and Gene B which synthesize the TFs and the target gene proteins respectively. We show through analytic calculations that the TF fluctuations have a significant effect on the distribution of the target gene protein levels when the mean TF level falls in the highest sensitive region of the dose-response curve. We further study the effect of reducing the copy number of Gene A from two to one. The enhanced TF fluctuations yield results different from those in the deterministic case. The probability that the target gene protein level exceeds a threshold value is calculated with a knowledge of the probability density functions associated with the TF and target gene protein levels. Numerical simulation results for a more detailed stochastic model are shown to be in agreement with those obtained through analytic calculations. The relevance of these results in the context of the genetic disorder haploinsufficiency is pointed out. Some experimental observations on the haploinsufficiency of the tumour suppressor gene, $Nkx\,3.1$, are explained with the help of the stochastic model of TF-regulated gene expression.

1 Introduction

Transcription factors (TFs) are proteins which are involved in regulating gene expression in eukaryotes. The genetic code provides the blueprint for gene expression, i.e., protein synthesis. Proteins and their complexes perform several essential functions in the living organism. The TFs bind at the appropriate regions of the target gene and regulate its expression by activating/inhibiting the first step in gene expression, namely, transcription [1]. Gene expression involves a series of biochemical reactions/events which are inherently probabilistic in nature. Several recent studies, both theoretical and experimental, highlight the significant influence of stochasticity on gene expression and its regulation [2]. Stochasticity gives rise to fluctuations around the mean protein level. This is also true for the

TFs which are synthesized by specific genes. In this context, an issue of particular interest is how the TF fluctuations affect the expression of the regulated (target) gene. The TFs constitute the "dose" or "input signal" which induces a nonlinear response measured in terms of the amount of proteins synthesized by the target gene. The dose-response curve, depicting steady state average values, is in general a sigmoid with maximum steepness at intermediate levels (region of highest signal sensitivity) of the input signal. Experiments on synthetic transcription cascades in *S. cerevisiae* and *E. coli* show that the effect of TF fluctuations on the target gene protein levels is the most prominent when the mean TF level falls in the region of highest signal sensitivity [3, 4].

One aspect of the TF-regulated gene expression which is not well explored relates to the consequences of reducing the copy number of the regulating, i.e., the TF-synthesizing gene. Eukaryotes, which include higher organisms, are characterized as diploids in which the set of genes in a cell has two copies. Haploids, in contrast, have single gene copies. If one of the copies of a specific gene in an eukaryotic cell is mutated, i.e., the gene copy number is reduced, the amount of proteins synthesized is diminished. The functional activity of proteins is often linked to the requirement that the protein amount cross a threshold level. If the diminished protein level falls below the threshold, the protein function is hampered. The loss of a vital protein function may in certain instances give rise to haploinsufficiency which includes several genetic diseases [5, 6, 7]. In TF haploinsufficiency, one copy of the gene synthesizing the TFs is mutated and the lower amount of TFs is not sufficient for the functional activity of the proteins synthesized by the target gene. The p53 tumour suppressor gene is a case in point. In normal cells, the level of p53 proteins is low. On DNA damage or under genotoxic stress, the p53 proteins are activated. These proteins function as TFs and initiate the expression of several target genes resulting in the activation of a number of pathways. There are two possible outcomes: either the DNA damage is repaired (the cell cycle progression is halted temporarily for this purpose) or if that is not possible, apoptosis, i.e., programmed cell death occurs. In the absence of any of these outcomes, there is a proliferation of cells containing damaged DNA through repeated rounds of the cell division cycle. This triggers the formation and growth of tumours which may ultimately lead to cancer. Recent experiments [8] show that the mutation of only one of the copies of the p53 tumour suppressor gene is in many cases sufficient to give rise to cancer. The cancer is thus a result of TF haploinsufficiency. Ghosh and Bose [9] have shown through model calculations that in response to DNA damage the transition from the G2 to the mitotic phase of the cell cycle is delayed, i.e., the cell cycle is arrested temporarily. This happens when the copy number of the $p53$ tumour suppressor gene is two. The cell cycle is, however, not arrested when the copy number is reduced to one. In this case, the DNA damage is not repaired and a proliferation of cells containing damaged DNA occurs. In the last few years, several examples of TF haploinsufficiency, involving a number of tumour suppressor genes, have appeared in the literature [9, 10, 11, 12, 13]. The proteins synthesized by the tumour suppressor genes function as TFs and regulate the expression of the target genes which further activate the relevant signalling pathways. As in the case of the p53 gene, a reduced gene dosage leads in certain instances to a loss in the desired outcome, i.e., the arrest of cell proliferation or apoptosis, resulting in the formation and growth of tumours. In the case of

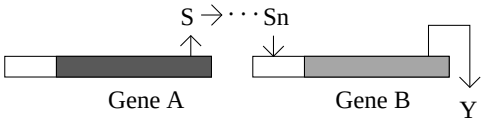


Figure 1. TF-regulated gene expression. Gene A synthesizes the TFs, S proteins, which regulate the synthesis of Y proteins from Gene B. S_n is the bound complex of n TFs.

prostate cancer, tumour initiation is often brought about by the mutation of one copy of the tumour suppressor gene $Nkx\,3.1$. There is now considerable experimental evidence to support the view that both reduced gene copy number (dosage) and stochasticity in gene expression are essential factors for the inactivation of the $Nkx\,3.1$- regulated pathways in a fraction of affected cells [11].

Cook et al. [5] have considered a minimal model of gene expression and shown that stochasticity may be an important contributing factor in the occurrence of haploinsufficiency. They have obtained a number of interesting results on the stochastic origins of haploinsufficiency via numerical simulation. When the gene copy number is reduced, the fluctuations around the mean protein level are enhanced. This sometimes results in transient excursions of the protein level below the threshold for the onset of protein activity. In the model of TF-regulated gene expression, the only stochasticity that is taken into account is that associated with the expression of the regulated gene. The TFs are assumed to be present in constant amounts. In this paper, we propose a stochastic model of TF-regulated gene expression in which Gene A synthesizes TFs (S proteins) which in turn activate the expression of Gene B leading to the synthesis of Y proteins (figure 1). The concentrations of the respective proteins are also denoted by S and Y. In our model, the expressions of both Gene A and Gene B are considered to be stochastic in nature. Using an analytical formalism, we explore how the fluctuations in the amount of TFs affect the distribution of the Y protein levels. We further study the effect of a reduction in the copy number of Gene A from two to one (the case of TF haploinsufficiency) on the expression of the target gene (Gene B). For simplicity, the copy number of Gene B is assumed to be one. The results from the analytical calculation are shown to be consistent with those obtained through stochastic simulations based on the Gillespie algorithm [14]. Some experimental observations on the haploinsufficiency of the tumour suppressor gene, $Nkx\,3.1$, are explained using the stochastic model of TF-regulated gene expression.

2 Stochastic Model

In the minimal model of stochastic gene expression [5], a gene can be in two possible states: inactive (G) and active (G^*). Random transitions occur between the states G and

G^* according to the Scheme (Scheme 1),

$$G \underset{k_d}{\overset{k_a}{\rightleftharpoons}} G^* \overset{j_p}{\longrightarrow} p \overset{k_p}{\longrightarrow} \Phi \tag{1}$$

where k_a and k_d are the stochastic activation and deactivation rate constants. In the active state G^*, the gene synthesizes a protein (p) with the rate constant j_p. The protein product degrades with a rate constant k_p, the degradation product being represented by Φ. The model (equation (1)) describes constitutive gene expression. In the case of eukaryotes, activation from the state G to the state G^* is brought about by the binding of a complex S_n of n individual TFs to the gene. Gene expression now takes place according to the Scheme (Scheme 2)

$$G + S_n \underset{k_2}{\overset{k_1}{\rightleftharpoons}} GS_n \underset{k_d^{'}}{\overset{k_a^{'}}{\rightleftharpoons}} G^* \overset{j_p^{'}}{\longrightarrow} p \overset{k_p^{'}}{\longrightarrow} \Phi \tag{2}$$

where GS_n represents the bound complex of G and S_n. Though the Schemes 1 and 2 do not fully capture the complexity of gene expression, they include the essential features. The simple schemes provide the theoretical framework for gaining important insight on stochastic gene expression and also for interpreting experimental results [2]. As has been shown earlier [5, 15, 16], the Schemes 1 and 2 are equivalent with effective rate constants $k_a^{''}$ and $k_d^{''}$ given by

$$k_a^{''} = k_a^{'} \frac{(S/K)^n}{1 + (S/K)^n} \ , \qquad k_d^{''} = k_d^{'} \tag{3}$$

where $K^n = \frac{k_2}{k_1} K_n$, K_n being the equilibrium dissociation constant for the reaction $n\, S \rightleftharpoons S_n$. The effective activation rate constant $k_a^{''}$ has the form of a Hill function.

We now consider the stochastic model corresponding to the Scheme 1. In the model, the only stochasticity arises from the random transitions of a gene between the active and inactive states as in the minimal model of Cook et al. [5]. Protein synthesis and degradation occur in a deterministic manner. In each state of the gene, the concentration X of proteins evolves according to the equation

$$\frac{dX}{dt} = j_p z - k_p X \tag{4}$$

where $z = 1(0)$ when the gene is in the active (inactive) state.
Let $p(X)$ be the probability density function (PDF) of the protein levels in the steady state. This is given by [15]

$$p(X) = C\, (k_p X)^{r_1 - 1} (j_p - k_p X)^{r_2 - 1} \tag{5}$$

where $r_1 = k_a/k_p$, $r_2 = k_d/k_p$ and C is the normalization constant.

In the model of TF-regulated gene expression, the synthesis of the TFs (S proteins) and that of the Y proteins occur according to the Schemes 1 and 2 respectively. The different

rate constants in the two cases are as specified in equations (1)-(3). In the deterministic formalism, the steady state concentrations of the TFs and the Y proteins are given by

$$S_{mean} = \frac{n_G\, j_p}{k_p}\, \frac{k_a}{k_a + k_d}\ , \quad Y_{mean} = \frac{j_p^{'}}{k_p^{'}}\, \frac{k_a^{''}}{k_a^{''} + k_d^{''}} \tag{6}$$

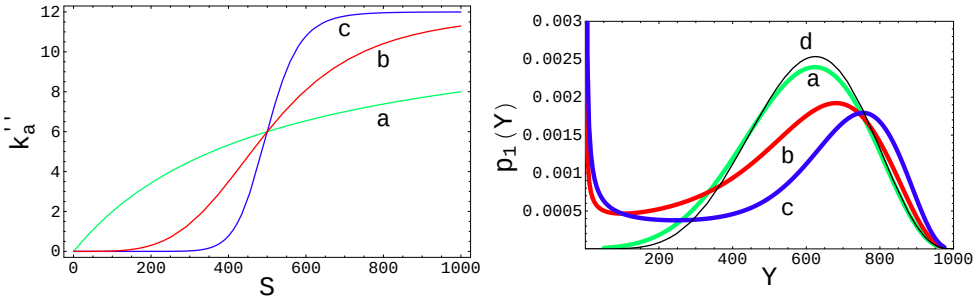
where n_G is the copy number of Gene A. Let Y_{thr} be the threshold level for the onset of activity of the Y proteins. If Y_{mean} is $< Y_{thr}$, a loss in the protein function occurs. If Y_{mean} is $> Y_{thr}$, the normal protein activity is not hampered. With the stochastic expression of both the Genes A and B taken into account, the steady state distributions of the TF and the Y protein levels are spread around the mean values S_{mean} and Y_{mean}. Let $q(S)$ and $Q(S)$ be the PDFs of the TF levels when the copy number of Gene A is 1 and 2 respectively. The corresponding PDFs of the Y proteins are $p_1(Y)$ and $p_2(Y)$. If the distribution of the Y protein levels overlaps with Y_{thr}, the steady state probability, $p(Y < Y_{thr})$ ($p(Y > Y_{thr})$), that the protein level Y is $< Y_{thr}$ ($> Y_{thr}$), is non-zero even if Y_{mean} is $> Y_{thr}$ ($<Y_{thr}$).

We now study the effect of TF fluctuations on the distribution of the Y protein levels. We consider one copy each of the Genes A and B and assume the parameter values to be $k_a = k_d = 4$, $j_p = 1000$, $k_p = 1$ (Gene A) and $k_a^{'} = 12$, $k_d^{'} = 4$, $j_p^{'} = 1000$, $k_p^{'} = 1$ (Gene B) in appropriate units. The parameter K is chosen to be $K = 500$ so that $S_{mean} = K$ (S_{mean} is given by equation (6) with $n_G = 1$). Let $p_1(Y, S)$ be the steady state PDF of the Y protein levels for a fixed amount S of TFs. The PDF has the same form as in equation (5) but with k_a, k_d, j_p and k_p replaced by $k_a^{''}$, $k_d^{''}$, $j_p^{'}$ and $k_p^{'}$. Similarly, the PDF $q(S)$ has the form as in equation (5). The steady state PDF is given by

$$p_1(Y) = \int_{all\ S} p_1(Y, S)\, q(S)\, dS \tag{7}$$

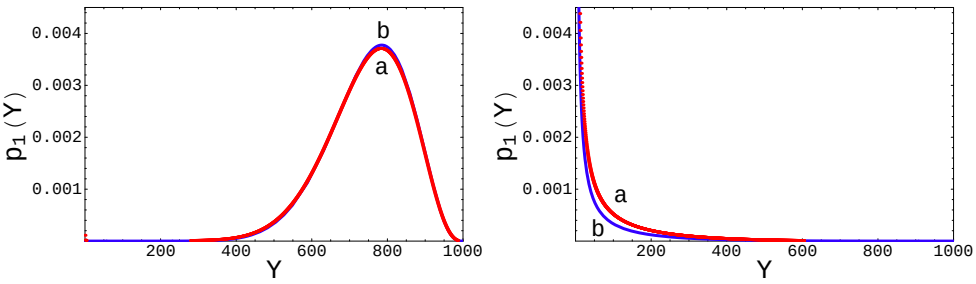
Since $q(S)$ and $p_1(Y, S)$ are known analytically, $p_1(Y)$ can be calculated using Mathematica. Figure 2(a) shows the variation of $k_a^{''}$ (equation (3)) with S for different values of the Hill coefficient, $n = 1$ (curve "a"), $n = 4$ (curve "b") and $n = 12$ (curve "c"). Figure 2(b) shows the steady state distribution $p_1(Y)$ versus Y for $n = 1$ (curve "a"), $n = 4$ (curve "b") and $n = 12$ (curve "c"). The curve "d" describes the distribution of $p_1(Y, S_{mean})$ with S fixed at S_{mean}, the mean amount of the TFs in the steady state i.e., the TF fluctuations are ignored. From figure 2(b), one finds that as the value of the Hill coefficient n increases, the effect of the TF fluctuations on the distribution of $p_1(Y)$ becomes more and more prominent. The reason for this is not difficult to find. For $n > 1$, the sharpest change in the $k_a^{''}$ versus S curve (equation (3)) occurs around $S \sim K$. Figure 2 has been obtained for $K = S_{mean} = 500$ so that for large n even small fluctuations around S_{mean} give rise to considerable changes in the value of the effective rate constant $k_a^{''}$. The region close to this point defines the highest signal sensitive region of the dose-response curve.

For $S_{mean}/K << 1$ or $>> 1$, the TF fluctuations have a less marked effect as the change in the value of $k_a^{''}$ is not as much as in the case when $S_{mean}/K \sim 1$. This is shown in figures 3(a) and (b). The parameter values are the same as in the case of figure 2 with the Hill coefficient $n = 4$ except that $S_{mean}/K = 4$ (figure 3(a)) and $S_{mean}/K = 0.25$



(a) (b)

Figure 2. (a) Variation of the effective rate constant k_a''as a function of S (equation (3)) for different Hill coefficients, $n = 1$ (curve "a"), $n = 4$ (curve "b"), $n = 12$ (curve "c"), (b) The distribution $p_1(Y)$ versus Y of protein levels for different Hill coefficients, $n = 1$ (curve "a"), $n = 4$ (curve "b") and $n = 12$ (curve "c"). Curve "d" curve describes the distribution for a fixed amount, S_{mean}, of TFs.

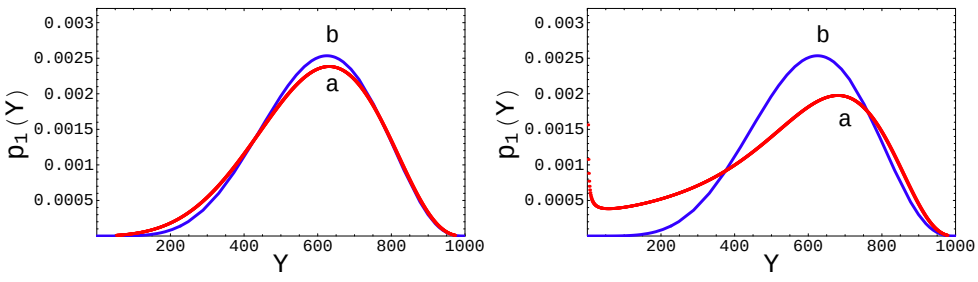


(a) (b)

Figure 3. Steady state distribution of the Y protein level for (a) $S_{mean}/K = 4$ and (b) $S_{mean}/K = 0.25$. Curve "a" ("b") is obtained with the TF fluctuations taken (not taken) into account.

(figure 3(b)). Curve "a" ("b") describes the steady state distribution $p_1(Y)$ versus Y with the TF fluctuations taken (not taken) into account. The findings from figures 2 and 3 are consistent with the earlier observation [2, 3] that the effect of TF fluctuations on the target gene protein levels is most clearly seen in the region of highest signal sensitivity ($S_{mean}/K \sim 1$). Figure 2(b) ($n = 4$ and $n = 12$) shows that the TF fluctuations give rise to a bimodal distribution in the Y protein levels. This is in agreement with the experimental observations of Blake et al. [3]. We also find that the higher the value of n, the greater is the range of values of S_{mean}/K for which the TF fluctuations can be ignored. Furthermore, with lesser fluctuations in the TF levels (narrower distribution), the effect of the TF fluctuations on the Y protein levels becomes significant for higher values of n. This is demonstrated in figure 4. A narrower distribution in the TF protein levels, than in the cases of figures 2 and 3, is obtained with the choice of the parameter values $k_a = k_d = 40$, $j_p = 1000$, $k_p = 1$. The target gene has the parameter values $k_a^{'} = 12$, $k_d^{'} = 4$, $j_p^{'} = 1000$, $k_p^{'} = 1$ and $S_{mean} = K = 500$. Figure 4(a) shows that for the Hill coefficient $n = 4$, the $p_1(Y)$ versus Y curve is little affected by the TF fluctuations even when $S_{mean}/K \sim 1$. The effect becomes prominent for higher values of n (figure 4(b), $n = 12$).

The major result obtained in this section is to demonstrate through analytical calculations that the TF fluctuations may considerably affect the distribution of the target gene protein levels. This is particularly so when the mean TF level falls in the highest sensitive region of the dose-response ($k_a^{''}$ vs S) curve describing the activation of the target gene. The result, though based on a specific set of parameter values, illustrates a general feature of TF-regulated gene expression. In many such cases, the TFs bind the target gene at multiple sites. This, combined with cooperative interactions between the TFs, imparts an ultrasensitive character to the dose-response curve. The same is true if a bound complex of TF molecules binds the operator region. If the mean TF level falls in the regions around the steepest part of the curve, the fluctuations around the mean level give rise to fluctuations in the effective activation rate constant, $k_a^{''}$, of the target gene. For sufficiently strong TF fluctuations, the distribution of the target gene protein levels is significantly altered from the case when the TF fluctuations are ignored. There have been earlier studies [5, 17, 18], based on simple models of TF-regulated gene expression, which did not take the TF fluctuations explicitly into account. Simpson et al. [19] have carried out a comprehensive analysis of stochasticity in gene transcriptional regulation based on the frequency domain Langevin approach. The transcriptional regulation occurs via protein (inducer)-DNA interactions at an operator site of the target gene. A significant achievement of the study is to obtain the frequency distribution of the target gene expression noise and identify the impact of noise originating from operator fluctuations. The operator fluctuations constitute an important component of the stochasticity associated with TF-regulated gene expression. The noise associated with the expression of a specific gene has two components: intrinsic and extrinsic of which the latter has the more dominated contribution to the total noise [2]. Recent reports suggest that this is particularly so for highly expressed genes [20, 21]. Both numerical computations and experiments [2, 3] show that a major component of the extrinsic noise stems from the fluctuations in the TF



(a) (b)

Figure 4. Steady state distribution of the Y protein levels for Hill coefficient $n = 4$ (a) and $n = 12$ (b) respectively. Curve "a" ("b") is obtained with the TF fluctuations taken (not taken) into account. The distribution of the TF levels is less noisy than that in the case of figure 2.

activity. Our simple stochastic model of TF-regulated gene expression yields analytical expressions for the PDFs describing the distributions of the TF and target gene protein levels. This makes it particularly convenient to study the effect of the TF fluctuations on the expression of the target gene.

The analytical tractability of the model arises from two assumptions. Firstly, the two major steps of gene expression, namely, transcription (synthesis of mRNAs) and translation (synthesis of proteins) have been combined into a single step leading to protein production. Secondly, the only source of stochasticity in the model lies in the random activation and deactivation of the target gene expression. The first assumption provides the basis for several studies of stochastic gene expression [5, 17, 18, 22]. The second assumption is strictly valid when the dominant source of noise is associated with the random activation and deactivation of gene expression. This holds true in the case of large steady state gene product level studied by Kepler and Elston [17]. In this limit, slow promoter kinetics (rates of gene activation and deactivation lower than the synthesis rate of the gene product) constitute a major source of noise. Fast transitions between the promoter states, on the other hand, generate low amounts of noise. The effect of the TF fluctuations on the target gene expression is prominent in the case of slow promoter kinetics. As discussed in detail in Ref. [2], slow promoter kinetics give rise to increased heterogeneity within a cell population including bimodal population distributions. Slow transitions between the promoter states are particularly relevant in the case of eukaryotic gene expression resulting in transcriptional bursts of mRNA synthesis (the production of mRNA occurs in pulses) [2, 23].

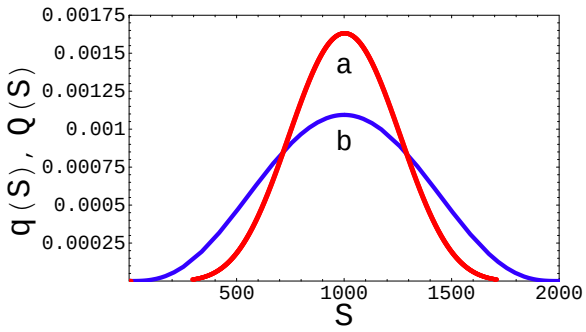


Figure 5. Steady state distribution of protein Y levels when the copy number of Gene A is two (curve "a") and one (curve "b")

3 Reduced Gene Copy Number

We next consider the effect of reducing the copy number of Gene A from two to one on the distribution of target gene protein levels. Figure 5 shows that a reduction in the copy number n_G of Gene A from two (red curve) to one (blue curve) gives rise to a wider distribution in the TF levels, i.e., the fluctuations around the mean protein level are larger. The steady state PDF $Q(S)$ ($n_G = 2$) is given by

$$Q(S) = \int_{all\ s_1} q(s_1)\, q(S - s_1)\, ds_1 \tag{8}$$

where S denotes the total TF concentration with $S = s_1 + s_2$, the sum of the concentrations of the TFs synthesized by the individual copies of the Gene A. The PDF $q(s_i)$ ($i = 1,\ 2$) has the form given in equation (5). The parameter values for both the one-gene and the two-gene cases are the same as in the case of figure 2 except that $j_p = 2000$ in the one-gene case. This has been done to keep S_{mean} fixed and facilitate the comparison of the two distributions. A measure of noise is given by $\chi = \textit{standard deviation/mean}$. If $j_p = 1000$ in both the one- and two-gene cases, χ has the values $\chi = 0.236$ (two gene copies) and $\chi = 0.333$ (one gene copy). Figure 6 shows the steady state distributions $p_1(Y)$ (curve "b") and $p_2(Y)$ (curve "a") when the copy number of Gene A is 1 and 2 respectively. The parameter values are the same as in the case of figure 2 with the Hill coefficient $n = 4$. One finds that the distribution of the Y protein levels is considerably changed when the copy number of the regulating gene is reduced. In the one-gene case, the value of S_{mean} falls to 500 i.e., $S_{mean}/K = 1$. In the parameter region close to this point, the effect of fluctuations is the most prominent. Again, one finds that the TF fluctuations give rise to a bimodal distribution in the Y protein levels.

Figure 7 shows the steady state probability $p(Y > Y_{thr})$ that the protein level Y exceeds a threshold value, Y_{thr}, versus the activation rate constant k_a of Gene A. The dotted (dot-dashed) curve corresponds to the case when the copy number of Gene A is 2 (1). In the

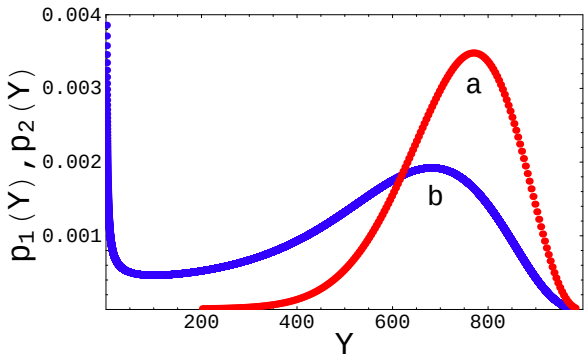


Figure 6. Steady state distribution of protein Y levels when the copy number of Gene A is two (curve "a") and one (curve "b")

one-gene case,

$$p(Y > Y_{thr}) = 1 - \int_0^{Y_{thr}} \int_{all\, S} p_1(Y, S)\, q(S)\, dS\, dY \tag{9}$$

The expression for the two-gene case is obtained by substituting $p_1(Y, S)$ and $q(S)$ by $p_2(Y, S)$ and $Q(S)$ respectively. The threshold value Y_{thr} is set at 25 percent of the maximum amount Y_{max} of the Y protein when the copy number of Gene A is two ($Y_{max} = j_p^{'}/k_p^{'} = 1000$ so that $Y_{thr} = 250$). From figure 7 one finds that the reduction of the copy number of Gene A diminishes the probability of maintaining the output protein level above a threshold value. Variation of the activation rate constant k_a changes S_{mean}, the mean amount of TFs (Eq. (6)) regulating the expression of Gene B. Figure 7 also includes the curves for $p(Y > Y_{thr})$ when the fluctuations in the TF amounts are ignored, i.e., the TF amount is kept fixed at S_{mean}. The dashed (solid) curve describes the two-gene (one-gene) case. For the same gene copy number, the curves, with and without the TF fluctuations, intersect at a value of $k_a = k_{ac}$ for which $Y_{mean} = Y_{thr}$. When k_a is $< k_{ac}$, Y_{mean} is $< Y_{thr}$. The distribution of the Y protein levels around Y_{mean} becomes broader when the TF fluctuations are taken into account, i.e., the TF levels are assumed to have a distribution around S_{mean}. Thus, $p(Y > Y_{thr})$ in this case is larger than when the TF concentration is kept fixed at S_{mean}. When k_a is $> k_{ac}$, Y_{mean} is $> Y_{thr}$. In this case, $p(Y > Y_{thr})$, with the TF fluctuations taken into account, is smaller than $p(Y > Y_{thr})$, with the TF concentration kept fixed. One thus has the interesting result that the TF fluctuations can both augment and diminish the probability $p(Y > Y_{thr})$. The $p(Y > Y_{thr})$ versus k_a curve is also found to be less steep in the presence of the TF fluctuations. One can further show that the curve is steeper for higher values of the Hill coefficient n.

In section 2, we have outlined some arguments for the validity of our stochastic model. We now show that similar results regarding the effect of TF fluctuations are obtained when a more detailed stochastic model is considered. In the model, the expression of both the Genes A and B take place through two steps: transcription and translation, i.e., the syntheses of mRNAs and proteins are treated separately. The production and the degradation of the mRNAs and the proteins are considered to be stochastic processes. The

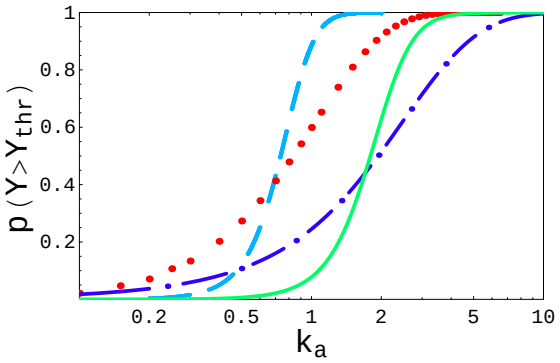
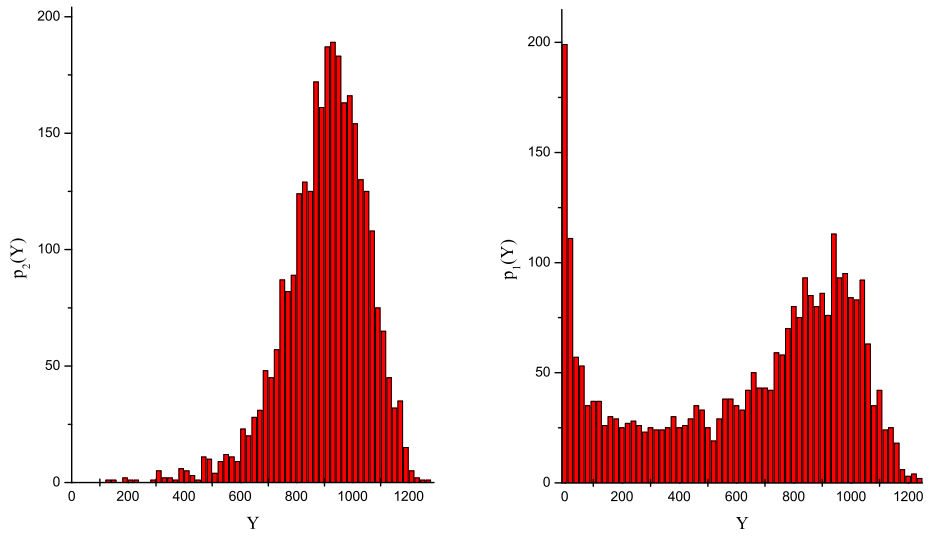


Figure 7. The probability $p(Y > Y_{thr})$ versus the activation rate constant, k_a, of Gene A when the copy number of Gene A is two (dotted and dashed curves) and one (dot-dashed and solid curves). The dashed and solid curves represent the cases in which the TF fluctuations are ignored.

formation of a complex of n TF molecules which regulates the expression of the target gene is also taken to be a stochastic event. With full stochasticity taken into account, the model is no longer analytically tractable. We undertake numerical simulation based on the Gillespie algorithm [14] to obtain the distribution of protein Y levels. The results are displayed in figure 8. The target gene protein levels have a unimodal distribution when the copy number of the regulating gene (Gene A) is two (figure 8(a)), a bimodal distribution (figure 8(b)) is obtained when the copy number of Gene A is 1 (increased TF fluctuations). The results have been obtained for slow promoter kinetics leading to the production of mRNAs in bursts. The amount of proteins synthesized is also not small. A heterogeneous distribution of the levels of expression, including bimodality, is obtained if the mRNA and protein half lives are shorter than the average time between the successive bursts of transcription [2, 15, 18, 23]. The simulation and analytical model results are in good agreement for low transcriptional burst rates. Higher transcriptional burst rates demand faster transitions between the inactive and active states of the gene. In this case, the noise contributed by the promoter activation-inactivation kinetics is lower. Depending on the gene product level, the noise associated with transcription and translation may become more dominant so that the analytical model ceases to be valid.

We now discuss the occurrence of haploinsufficiency, due to the loss of one copy of the tumour suppressor gene *Nkx* 3.1. In this case, haploinsufficiency is manifest in tumour initiation in the prostate leading to cancer [11, 13]. The tumour suppressor genes are generally activated under DNA damage or genotoxic stress. The function of the proteins synthesized by these genes is to limit cell growth or survival. Mice lacking one copy of such a gene (examples include the *p*27, *p*53, *Dmp*1 and *Nkx* 3.1 genes) are known to develop cancerous or pre-cancerous lesions despite protein synthesis from the remaining copy of the gene. This indicates a failure in checking cell proliferation or bringing about cell death. The *Nkx* 3.1 gene has several positively and negatively regulated target genes which exhibit a variety of responses to the loss of one copy of the *Nkx* 3.1 gene. We

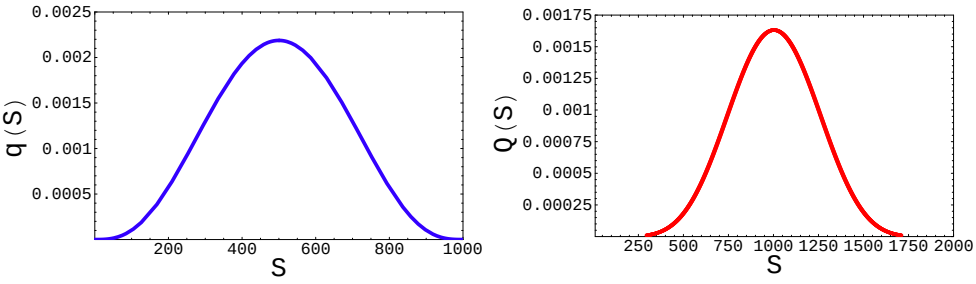


(a) (b)

Figure 8. Steady state distribution of protein Y levels, obtained through numerical simulation based on the Gillespie algorithm, when the copy number of Gene A is two (a) and one (b) respectively. The corresponding distributions of protein Y levels are $p_1(Y)$ and $p_2(Y)$.

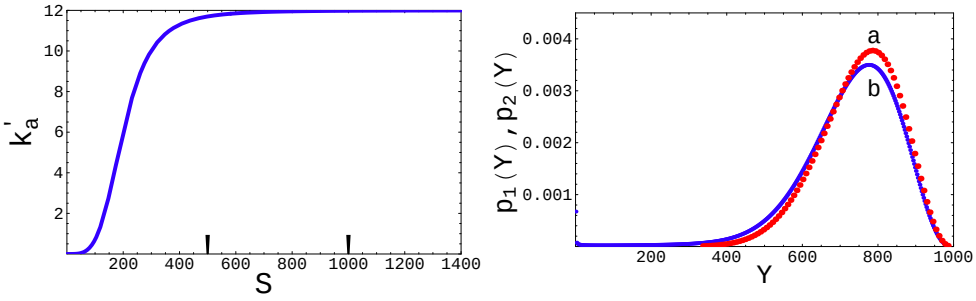
consider the examples of two of the positively regulated genes, probasin and intelectin. Probasin is relatively insensitive to the loss of one $Nkx\,3.1$ copy, i.e., both the wild-type and $Nkx\,3.1^{+/-}$prostates witness high levels of probasin expression. The expression is retained even in $Nkx\,3.1^{-/-}$ (loss of both gene copies) indicating a basal level of probasin expression. Intelectin is, however, highly dosage sensitive and is not expressed in either the $Nkx\,3.1^{+/-}$ or $Nkx\,3.1^{-/-}$ prostate. In situ hybridization experiments reveal heterogeneous expression patterns for both probasin and intelectin in a population of cells [11]. In wild-type and $Nkx\,3.1^{+/-}$ prostate, probasin is expressed uniformly whereas in the case of the $Nkx\,3.1^{-/-}$ prostate, a heterogeneous population of probasin-expressing and nonexpressing cells is obtained. In the case of intelectin expression, a considerable heterogeneity is observed even in the wild-type prostate. This contrasts with the relatively uniform expression of the $Nkx\,3.1$ gene in both the wild-type and $Nkx\,3.1^{+/-}$ prostates.

We now provide an explanation for the dosage response of the probasin and intelectin genes. The TF ($Nkx\,3.1$ protein)-regulated promoter activity can be either graded or binary. In the first case, the activity increases in a graded fashion in response to increasing levels of TFs. Reductions in the target gene protein levels due to the loss of one copy of the regulating gene are expected to be uniform in all cells. In the binary mode, a gene can be in a transcriptionally active or inactive state and the TFs regulate the probability of the gene being in either state. Reduced $Nkx\,3.1$ gene dosage leads to a decrease in the



(a) (b)

Figure 9. Steady state distribution of TF levels in the one-gene (a) and two-gene cases (b).

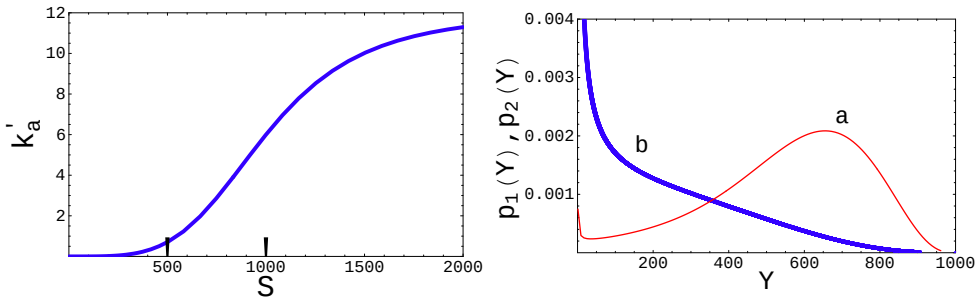


(a) (b)

Figure 10. A possible scenario for probasin gene expression. (a) The dose-response curve, (b) the distributions $p_2(Y)$ ("a") and $p_1(Y)$("b") of target gene protein levels when the copy number of Gene A is two and one respectively.

fraction of transcriptionally active cells. At the population level, a bimodal distribution of the target gene levels is obtained. In a fraction of cells, the target gene activity is null, i.e., the signalling pathways leading to the arrest of cell proliferation or apoptosis remain inactivated. Gene copy reduction combined with stochastic effects are responsible for the heterogeneous gene activity in an ensemble of cells.

We now illustrate the experimentally observed responses of the probasin and intelectin genes to the reduced copy number of the *Nkx* 3.1 gene in the analytical framework of our stochastic model. Gene A in the model now represents the *Nkx* 3.1 gene and Gene B the probasin or intelectin gene. The particular values of the different rate constants and the parameters are not mentioned as the aim is to simply demonstrate how the differences between the responses of the probasin and intelectin genes arise. Figure 9 shows the distributions $q(S)$ and $Q(S)$ of the TF protein levels when the copy number of Gene A is one and two respectively. Figure 10(a) shows the dose-response curve k_a'' versus S of the target gene. One finds that the effective activation rate constant k_a'' (Eq. (3)) does not change appreciably when the copy number of the regulating gene is reduced from two to



(a) (b)

Figure 11. A possible scenario for intelectin gene expression. (a) The dose-response curve, (b) the distributions $p_2(Y)$ ("a") and $p_1(Y)$("b") of target gene protein levels when the copy number of Gene A is two and one respectively.

one ($S = S_{mean}$ is reduced from 1000 to 500, these two points are marked by vertical lines on the $x - axis$ of figures 10(a) and 11(a)). This is reflected in the distributions $p_1(Y)$ (curve "b") and $p_2(Y)$ (curve "a") of the target gene protein levels (Figure 10(b)). Figure 10 represents the case of probasin for which experimental observations show that the probasin protein level is relatively insensitive to the loss of one copy of the $Nkx\,3.1$ gene and the probasin gene is expressed uniformly (unimodal distribution of the protein levels) in both the wild-type and $Nkx\,3.1^{+/-}$ prostate. Figure 11 depicts the possible scenario for the intelectin gene. In this case, the magnitude of k_a'' is significantly reduced (figure 11(a)) when the copy number of Gene A is reduced from two to one. In the two-gene case, the effective rate constant k_a'' falls in the most sensitive region of the dose-response curve. The effect of the TF fluctuations is the most prominent in this case giving rise to increased heterogeneity in the target gene expression (the bimodal distribution $p_2(Y)$, curve "a", in figure 11(b)). A bimodal distribution of intelectin-expressing and nonexpressing cells has been experimentally observed [11] in the wild-type prostate (gene copy number of the $Nkx\,3.1$ gene is two). In the one gene case, the intelectin expression is found to be essentially lost. The distribution $p_1(Y)$ of the intelectin protein levels in figure 11(b) (curve "b") shows a prominent peak at zero protein level, in agreement with experimental findings.

4 Summary and Discussion

In this paper, we have considered a stochastic model of TF-regulated gene expression and studied the effect of the TF fluctuations on the distribution of the target gene protein levels. We have shown that the TF fluctuations associated with the highest signal sensitive region of the dose-response curve have the strongest influence on the distribution of the target gene protein levels, consistent with experimental observations [2, 3, 4]. In fact, the TF fluctuations can give rise to a bimodal distribution in the output protein levels. This is an experimentally observed effect [2, 3]. The effect of the TF fluctuations is more prominent

for higher values of the Hill coefficient n. The TF fluctuations may be ignored away from the region of highest signal sensitivity. We have reported the results for one set of parameter values but the results are of general validity. The analytical results obtained in the paper are valid when the random transitions between the active and inactive gene expression states constitute the dominant source of noise which is often true for eukaryotic systems. It will be of interest to extend the results of the model to more general situations. In our analytical formalism, the PDF associated with the distribution of the TF levels in the steady state is used to determine the steady state distribution of the target gene protein levels (see equation (7)). This is similar in spirit to the Static Noise Approximation studied recently by Scott et al. [24]. The method does not take into account the dynamical aspect of the TF noise like the frequency of fluctuations in the TF amounts. The numerical simulation based on the Gillespie algorithm (figure 8) is more exact in nature and incorporates the dynamical effects.

We have further studied the consequences of reducing the copy number of Gene A, synthesizing TFs, from two to one. The TF fluctuations are greater in magnitude when the copy number of gene A is one. As before, the fluctuations have the strongest effect when the mean TF protein level, S_{mean} ($n_G = 1$), is close to K, the threshold parameter for activation of the target gene expression. This is the region where the dose-response or equivalently the k_a'' versus S curve has the highest slope, i.e., maximal sensitivity. As illustrated in figure 6, the TF fluctuations can give rise to a bimodal distribution in the output protein levels. The appearance of a bimodal distribution, when the copy number of the gene synthesizing the TFs is reduced from two to one, is a prediction of our model and should be verified experimentally. The result, obtained through analytical calculations, is supported by the results of numerical simulation based on the Gillespie algorithm. The variance in protein levels may be underestimated by a significant factor if transcription and translation are combined into a single step [20, 25]. In the light of this possibility, transcription and translation are treated as separate stochastic processes in the numerical simulation. The use of Hill functions introduces errors in a stochastic analysis [19]. In the simulation, the formation of a TF complex occurs through stochastic processes. The agreement between the analytical and numerical results confers validity on the reported result.

The issue of whether a protein level exceeds a threshold value is of crucial importance as the functional activity of proteins, in general, depends on this. Our study shows that results, significantly altered from those in the deterministic case, may be obtained when stochasticity is taken into account. As seen in figure 7, the TF fluctuations can increase as well as reduce the steady state probability $p(Y > Y_{thr})$, that the Y protein level exceeds a threshold value Y_{thr}, from the values obtained when the TF fluctuations are ignored. Our stochastic model enables us to obtain analytical expressions for the probability distributions. With the knowledge of the distributions, the calculation of the probability that the target gene protein level exceeds a threshold value is straightforward. Such calculations provide the basis for the study of problems related to TF haploinsufficiency. There are already suggestions in the biomedical literature [5, 10, 11, 12, 13, 16, 26] that stochasticity is a key contributing factor in the occurrence of haploinsufficiency. Our study lends

support to this notion and shows that the gene copy number is an important criterion in determining the relationship between stochasticity and desired protein activity. We have specifically considered the case of $Nkx\,3.1$-regulated gene expression in the prostate. The loss of one copy of the $Nkx\,3.1$ gene can trigger the initiation and growth of tumour leading to cancer. Our stochastic model of TF-regulated gene expression provides an explanation, at a qualitative level, for the experimental observations on the target gene responses to gene (TF) dosage reduction. A recent experiment [27] shows that the loss of one allele of the $p53$ tumour suppressor gene results in a four-fold reduction of $p53$ mRNA and protein, thus providing the basis for haploinsufficiency. As in the case of the $Nkx\,3.1$-regulated probasin and intelectin genes, the $p53$-dependent transcriptional response after genetoxic stress can be either homogeneous or heterogeneous [28]. A heterogeneous response (only some cells of a population respond, the fraction of such cells increases as the TF amount increases) can be explained only if stochastic gene expression is taken into account. A reduction in the gene copy number accenuates the stochastic effects. Investigation of the stochastic origins of haploinsufficiency is expected to yield useful information and insight on a host of human diseases.

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