Biological implications of $\mathfrak{so}(2,1)$ symmetry in exact solutions for a self-repressing gene

Alexandre F. Ramos

Escola de Artes, Ciências e Humanidades,
Núcleo de Estudos Interdisciplinares em Sistemas Complexos,
Departamento de Radiologia – Faculdade de Medicina,
Universidade de São Paulo – Instituto do Câncer
do Estado de São Paulo – Av. Arlindo Bétio,
1000 CEP 03828-000, São Paulo, SP, Brazil

John Reinitz

Department of Statistics, Department of Ecology and Evolution,
Department of Molecular Genetics and Cell Biology,
University of Chicago 5747 South Ellis Av, Jones, Chicago, IL 60637, USA

Abstract

We chemically characterize the symmetries underlying the exact solutions of a stochastic negatively self-regulating gene. The breaking of symmetry at low molecular number causes three effects. Average protein number differs from the deterministically expected value. Bimodal probability distributions appear as the protein number becomes a readout of the ON/OFF state of the gene. Two branches of the solution exist, having high and low switching rates, such that the low switching rate branch approaches deterministic behavior and the high switching rate branch exhibits sub-Fano behavior.

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Dedicated to the memory of José Eduardo Martinho Hornos, 1953–2013.

Following the development of Gillespie’s exact simulation method for the chemical master equation (CME) \[1\] and its application to the lysis/lysogeny decision of phage λ \[2\], there has been extensive interest in the application of stochastic techniques to gene regulation and other problems in biology. Study of such systems by the CME is hampered by the necessity of employing computational techniques, and for that reason exact solutions of the CME, when they can be obtained, have been sources of insight into the behavior of stochastic genetic systems \[3\]. It was previously shown that the exact solutions of the CME for a self-repressing gene possess a symmetry described by the \(\mathfrak{so}(2,1)\) Lie algebra. This is evident from the form of the equations for the generating functions when written as an eigenvalue problem \[4\]. The biological and chemical implications of these symmetries remained unclear, however, and differed from the usual interpretation of Lie symmetries in quantum mechanics. The essential stochastic characteristics of the self-repressing gene system arise from the existence of two coupled random variables in the CME, one describing the ON/OFF state of the gene and one describing the state of its protein product. In this Letter, we will show that these symmetries are obeyed at the deterministic limit but are broken in three ways in the regime of small molecular numbers.

We conceive a deterministic model for a negative self-regulating gene as an ensemble of genes (operators, in the case of a prokaryote) at concentration \([O_T]\). The operators may be in the ON or OFF state if they are, respectively, unbound or bound to the regulatory protein. The concentration of ON (OFF) operators is indicated by \([O]\) \(([OP])\), with \([O_T] = [O]+[OP]\), and the protein concentration is given by \([P]\). The macroscopic reaction scheme is given by

\[
\begin{align*}
\text{Protein synthesis:} & \quad O \xrightleftharpoons{k} P + O \\
\text{Protein decay:} & \quad P \xrightleftharpoons{\hat{\rho}} \emptyset \\
\text{Switching OFF:} & \quad P + O \xrightleftharpoons{\hat{h}} OP \\
\text{Switching ON:} & \quad OP \xrightleftharpoons{\hat{f}} P + O.
\end{align*}
\]

(1)

All macroscopic rate constants are written with hats and have units of moles liter\(^{-1}\). \(\boxed{1}\)
implies that
\[
\frac{d[P]}{dt} = \hat{k}[O] + \hat{f}[OP] - (\hat{\rho} + \hat{h}[O])[P],
\]
(2)
\[
\frac{d[O]}{dt} = \hat{f}[OP] - \hat{h}[O][P],
\]
(3)
\[
\frac{d[OP]}{dt} = -\hat{f}[OP] + \hat{h}[O][P].
\]
(4)

Let us indicate the steady state concentrations by \([P] = X, [O] = A,\) and \([OP] = B,\) with \(T = A + B,\) so that
\[
\frac{A}{T} = \frac{\hat{f}}{\hat{f} + \hat{h}X}, \quad \frac{B}{T} = 1 - \frac{A}{T},
\]
(5)
\[
X = NA = \frac{\sqrt{1 + 4KNT} - 1}{2K},
\]
(6)

where \(K,\) the equilibrium affinity, is given by \(K = \frac{\hat{h}}{\hat{f}}\) and \(N = \frac{\hat{k}}{\hat{\rho}}.\) Eq. (5) indicates that the flow of operators going from the OFF to ON and ON to OFF states is, respectively, given by \(\hat{f}\) and \(\hat{h}X.\) Thus, the total flow of operator states in both directions is \(\hat{f} + \hat{h}X.\) Note that the expected number of proteins is given by the product between the ratio between the protein synthesis and degradation rates and the concentration of ON operators. Hence, at the limit of small affinity of the repressor for the operator \((K \to 0),\) \(X/T = N\) as expected. Furthermore, the relationships in Eq. (5) enable us to write an expression for \(X\) in Eq. (6) that does not involve the concentration of DNA, a standard feature of near equilibrium models of transcription [5, 6].

A stochastic model for the negative self-regulating gene has been proposed in terms of two random variables, the protein number, denoted by \(n,\) and the operator state, which can be ON or OFF [3, 7]. The probability of finding \(n\) proteins when the operator is ON or OFF is denoted by \(\alpha_n\) or \(\beta_n,\) respectively. In the stochastic model, we replace the reaction rate constants of Eq. (1) by propensities represented by unhatted symbols. Each propensity is related to its corresponding macroscopic rate constant by multiplying by the volume of the system, so \(k = \hat{k}V\) etc. Note that now we may consider a single gene instead of an ensemble and the proportion of ON operators of the deterministic model becomes the marginal probability of finding the operator ON, \(P_\alpha = \sum_{n=0}^{\infty} \alpha_n.\) In the steady state regime, the marginal probability of finding \(n\) proteins in the cytoplasm independently of the operator state being ON or OFF is given by \(\phi_n = \alpha_n + \beta_n\) and is computed in terms of the
KummerM functions, so that
\[ \phi_n = \frac{(Nz_0)^n (a)_n}{n! (b)_n} c M(a + n, b + n, -Nz_0^2), \quad (7) \]
where
\[ c^{-1} = M(a, b, Nz_0(1 - z_0)), \quad z_0 = \frac{\rho}{\rho + h}, \quad (8) \]
\[ N = \frac{k}{\rho}, \quad a = \frac{f}{\rho}, \quad b = \frac{f}{\rho + h} + \frac{hk}{(\rho + h)^2}. \quad (9) \]

$N$ is the average number of proteins at the steady state regime if the operator is fully ON. $z_0$ gives the proportion of protein removal from cytoplasm by first order decay. $a$ is the ratio of the OFF to ON transition rate to the protein degradation rate. $a \gg 1$ indicates a regime where, on average, the OFF operator switches back to the ON state faster than the time required for protein degradation. $a \ll 1$ describes the opposite situation. $b$ gives the ratio of the operator switching to the protein removal rates. The operator switching rate is the sum of the average OFF to ON switching rate $f$ and the ON to OFF rate. The latter is defined in analogy with the deterministic case to be $hk/(\rho + h)$. For $b \gg 1$ the operator switches multiple times between the ON and OFF states during the average time for protein removal. In that case, the probability distributions for protein number are unimodal. On average, for $b \approx 1$ or smaller, the operator takes longer to switch from ON to OFF to ON (or vice-versa) than the average protein removal time. In that case, and for $a \approx k/(\rho + h)$, the probability distributions for protein number are bimodal because most of the proteins synthesized when the operator is ON decay before the operator switches OFF.

This stochastic model is a combination of two stochastic processes, and hence approaches equilibrium at the two rates $\rho$ and $b(\rho + h)$, the former related to the protein degradation and the latter to operator switching. The smallest of those two rates determine when the system reaches equilibrium. The time dependent solutions have the form
\[ \phi(z, t) \propto e^{-j\rho t} \mathcal{H}_1(z) + e^{-(\rho + h)(b + j)t} \mathcal{H}_2(z), \quad (10) \]
where $j$ is a non-negative integer and $\mathcal{H}_1$ and $\mathcal{H}_2$ are confluent Heun functions.

The exact solutions of the stochastic model indicated the existence of $so(2, 1)$ symmetries. The probability distributions were obtained by means of generating functions,
\[ \phi(z) = c M(a, b, Nz_0(z - z_0)) = \phi_{b,a}, \quad (11) \]
FIG. 1. (A) and (B) show $z_0$ and $\langle n \rangle$, respectively, as functions of $a$ for fixed values of $N$ as indicated by the keys in A and B. Dashed-dot (dashed) lines correspond to $z_0^+$ ($z_0^-$). The vertical black line at $a = 25$ separates the sub-Fano and super-Fano noise regimes of the steady state probability distribution. In graph A, the golden non-dashed line indicates $z_0^+ = z_0^-$, and $P_1$ and $P_2$ show the $(a, z_0)$ values for two distributions shown in (C). (B) shows the dependence of $\langle n \rangle = NP_\alpha$ on $a$, where $P_\alpha = \frac{az_0}{b}M(a + 1, b + 1, Nz_0(1 - z_0))$ is the steady state probability for the operator to be ON. (C) shows probability distributions with $(N, b) = (150, 25)$. $(a, z_0)$ for each distribution are $P_1 = (8.3, 0.86)$, $P_2 = (8.3, 0.19)$, $P_3 = (2.2832, 0.14)$, $P_4 = (50, 0.81)$ with $z_0$ calculated from Eq. (14), where $P_1$, $P_3$ (or $P_2$, $P_4$) were calculated with $z_0^+$ (or $z_0^-$). The Fano factor of each probability distribution is indicated by $F$.

that span irreducible representations of $\mathfrak{so}(2,1)$, which in the Cartan basis has its operators denoted by $H$, and $E_\pm$. The Casimir operator is defined as $C = -H^2 + H + E_+ E_-$ and the commutation relations are

$$[H, E_\pm] = \pm E_\pm, \quad [E_+, E_-] = -H, \quad [C, H] = [C, E_\pm] = 0.$$  

The action of the algebraic operators on the generating functions $\phi_{b,a}$ is:

$$C\phi_{b,a} = \frac{1 - b^2}{4}\phi_{b,a}, \quad H\phi_{b,a} = \frac{2a + 1 - b}{2}\phi_{b,a}, \quad (12)$$

$$E_+\phi_{b,a} = a\phi_{b,a+1}, \quad E_-\phi_{b,a+1} = (b - a)\phi_{b,a}. \quad (13)$$

The invariant of the algebra is determined by the eigenvalue of the Casimir operator and Eq. (12) implies that $b$ is constant. The Cartan operator’s eigenvalue determines the OFF to ON switching rate in relation to the protein degradation rate while the ladder operators change the value of $a$ by one.
We start building the biological interpretation of the symmetries of the model by writing its invariant as \( b = az_0 + Nz_0(1 - z_0) \). A fixed \( b \) leads to a 3D locus embedded in a 4D space. For fixed values of \( N \) we obtain two possible values for \( z_0 \), given by

\[
    z_0^\pm = \frac{(1 + a/N)}{2} \left( 1 \pm \sqrt{1 - 4bN(N + a)^{-2}} \right).
\] (14)

FIG [1A] shows the possible values of \( z_0^\pm \) as functions of \( a \). \( a \geq b \) implies \( z_0^+ > 1 \) which is biologically meaningless and only \( z_0^- \) has acceptable values (Eq. [8]). For a given \( a \leq b \) the dynamical regime of the system is degenerate and two values of \( z_0 \) distinguish those regimes in terms of the ON to OFF operator state transition. The first regime, \((z_0^-)\), has strong self-repression (high value of \( h \)) and low steady state protein number. The second regime, \((z_0^+)\), is characterized by a high steady state protein number and weak self-repression (low value of \( h \)).

FIG [1B] shows a further consequence of this degeneracy on the average protein number. For sufficiently low \( \langle n \rangle \), one has two possible values of \( a \) and \( z_0^- \), both characterized by the same value of \( b \). Those values indicate two regimes of operator switching, with lower (or higher) values for the switching rates \( f \) and \( h \), that is, slow or fast switching. For the specific condition when one regime has \( a > b \) and the other has \( a < b \) the noise on the protein numbers is characterized, respectively, as sub-Fano and super-Fano.

The stochastic model exhibits splitting between the deterministic and stochastic solutions to the dynamics of the negative self-regulating gene when the average protein numbers are low. FIG [2A] shows a comparison between the steady state concentration of proteins predicted by the deterministic model in Eq. [6] and the average protein number as given by the stochastic model. For high values of \( X \) there is a good agreement for the steady state number of proteins predicted by both the stochastic and deterministic approaches. As the steady state number of proteins decreases, discrepancies between the two approaches start to appear as \( f \to 0 \). For \( h \) sufficiently high, the probability for the gene being OFF increases and when \( f \) becomes very small the stochastic and deterministic solutions diverge. The correspondence principle breaks down when the molecular number is extremely small.

FIG [2A] shows that for \( K = 10^4 \), large values of \( f \) cause the protein number to approach 1. This is a consequence of the fact that a protein bound to the operator does not decay in the reaction scheme given in Eq (1), and we have shown elsewhere that this case can give rise to Fano factors arbitrarily close to zero [9].
FIG. 2. (A) shows a comparison of the expectation of the steady state protein number of the stochastic model and the protein number from the deterministic model as a function of the parameter \( f \), assuming volume \( V = 10^{-15} \) liters. Synthesis and degradation rates are \( k = 500, \rho = 1 \). (B) shows the distributions when \( \langle n \rangle \) and \( X \) are comparable. The relative error \( E \) is given by

\[
E = \frac{\| \langle n \rangle - X \|}{\max(\langle n \rangle, X)}.
\]

The key shows the distributions by color and \( E \) and the Fano factor \( F \) are given for each distribution. The parameters \((N, b) = (500, 0.1)\), and \((a, K) = P_5 = (0.06, 1.3 \times 10^{-3}), P_6 = (0.08, 5 \times 10^{-4}), P_7 = (0.09, 2 \times 10^{-4}), P_8 = (0.099, 2 \times 10^{-5})\). The probabilities of finding up to 400 proteins (or more than 400) for curves \( P_5, P_6, P_7, \) and \( P_8 \), are, respectively, approximately 0.42 (or 0.58), 0.22 (or 0.78), 0.11 (or 0.89), 0.01 (or 0.99).

FIG. 3. (A) and (B) show probability distributions obtained with \( z_0^+ \) while \( z_0^- \) was used to construct graph C. Approximate values of \( z_0^+ \) were obtained from Eq. 14. Graph A has \((a, z_0^+)\) in \( L_1 = (0.07, 0.99), L_2 = (0.099, 0.99), \) and \( L_3 = (0.09, 0.99)\). Graph B has \((a, z_0^+)\) in \( L_4 = (6, 0.71), L_5 = (15, 0.86), \) and \( L_6 = (24, 0.99)\). Graph C has \((a, z_0^-)\) in \( L_7 = (6, 0.35), L_8 = (15, 0.29), L_9 = (100, 0.13), \) and \( L_{10} = (150, 0.10)\).
A third type of splitting arises from the following. The parameter $a$ is the eigenvalue of the Cartan operator and gives the OFF to ON transition rate (see Eq. (12)). The action of the ladder operators on the probability generating function $\phi_{b,a}$ changes the value of $a$ by one (Eq. (13)) and connects probability distributions in which $b$ values are the same and $a$ values differ by an integer. The action of the raising operator changes $a = \frac{f}{\rho}$ to $a' = a + 1 \rightarrow \frac{f'}{\rho'} = 1 + \frac{f}{\rho}$, and the action of $E_-$ is constructed by analogy. Let us assume that the action of $E_+$ only changes $f$, hence $\rho' = \rho$ and $f' = f + \rho$. $b$ remains unchanged under the action of $E_+$, hence the remaining constants $N$ and $z_0$ change. For a fixed value of $z_0$ one has $N \rightarrow N - \frac{1}{1-z_0}$. For a fixed value of $N$ we consider that $z_0^\pm \rightarrow z_0^\pm + \Delta z_0^\pm$ with 
$$
\Delta z_0^\pm = \pm (1 + \frac{1}{2N}) \sqrt{1 - \frac{4bN}{(N+a+1)^2}} + \sqrt{1 - \frac{4bN}{(N+a)^2}}.
$$
The increment of $z_0^+$ (or $z_0^-$) corresponds to an decrease (or increase) of the value of $h$ that implies an increase of the mean protein number (see FIG 3B).

The dynamics of the gene expression process may have two distinct characteristics, depending on the value of $b$. For $b \gg 1$ the dominant decay rate to equilibrium is $\rho$ and the changes of the value of $h$ are not sufficient to cause changes in the time for the system to approach equilibrium, $b(\rho + h)$. This regime coincides with a unimodal probability distribution and the action of the raising operator on the generating functions causes the mode of its probability distribution to be displaced to the right. For the case of $b(\rho + h) \ll \rho$ (or $b \ll z_0$) we have that $b(\rho + h)$ is the dominant decay rate and the increase (or decrease) of $h$ corresponds to the system reaching equilibrium earlier (or later). This regime is characterized by probability distributions that may become bimodal and the action of the raising operator corresponds to an increase of the maximum probability of finding $n$ equal to the higher mode (see FIGs 3A and 2B).

The regime with bimodal distributions has important experimental and theoretical consequences. In this regime, most of the proteins synthesized by the gene during the ON state are degraded before it switches back to the OFF state, and the remaining proteins degrade before the gene switches ON, giving rise to bimodal distributions of $n$ which have been experimentally observed [10, 11]. In that case, the assumptions underlying the Langevin approach fail [12] because the number of proteins $X_L$ at the steady state regime of the Langevin equation is governed by distributions that are Gaussian around $X$. The probability distributions in this case and the breakdown of the Langevin regime are shown in FIG 2B.
We began our treatment by considering the macroscopic system because the master equation solution applies to cases with any number of molecules. This point is demonstrated by FIG 2A, which shows that increasing the equilibrium binding affinity \( K = \frac{h}{f} \) reduces the deterministic equilibrium concentration, as expected. For fixed \( K \), reducing \( f \) requires reducing \( h \). FIG 2A shows that there is a symmetry breaking as the average protein number in the deterministic model splits from that given by the stochastic model, and moreover that the average number of proteins in the stochastic model is a function of \( f \) even when \( K \) is held constant, behavior never seen in the deterministic model. Although the deterministic correspondence principle holds for small numbers of molecules (\( \approx 10 \)), correspondence is lost at one repressor molecule per cell, as discussed above.

The kinetic symmetries fully manifest themselves in the macroscopic case. The invariant of the algebra, \( b = \frac{L}{\rho} + \frac{hk}{(\rho+h)^2} \), is the ratio between the switching rate and the protein removal rate. Since the invariant is quadratic in \( h \), there exist two kinetic regimes for the same value of \( b \). The first has protein removal predominantly because of protein destruction (for example, when \( \rho \gg h \)) while protein binding prevails in the second. These regimes are macroscopically indistinguishable in the presence of a thermodynamically large number of operator sites. This fully macroscopic picture in fact never occurs in a biological system, because the molecular number of operator sites per cell is small. In the “semi-macroscopic” case of many protein molecules and a small number of operator sites, corresponding to the \( z^+ \) branch in FIG 1A,B, protein removal takes place primarily by first order decay. This super-Fano regime approaches the solutions of a near equilibrium thermodynamic system as molecular number increases. In the \( z^- \) branch, protein removal takes place primarily by binding, the operator becomes strongly repressed, and sub-Fano behavior results, a situation we have discussed in detail elsewhere [9].

A further symmetry breaking manifests itself for certain values of \( b \) with respect to the protein number distribution when the number of operators is small. For the case of \( b < 1 \) the gene switching is slow in comparison with the protein removal rate, hence the probability distributions for the protein number when the gene is ON (or OFF) are split, and bimodal probability distributions are observed (see FIGs 3A and 2B). When the operator number is large, these differences in ON and OFF states would average out in the reaction mixture and become unobservable. Here the actual biological regime of small gene number per cell is experimentally significant because it permits direct observation of stochastic switching.
between ON and OFF in living cells \[10\]. When the gene switching is fast in comparison with protein removal rate \((b > 1)\) the distributions are unimodal and the existence of the two gene states cannot be established by the measurement of protein numbers, even with low gene copy number (see FIG \[3\]).

In conclusion, we have made use of symmetries described by a Lie algebra to fully characterize the behavior of a self-repressing gene. Because the exact solutions represent the behavior of the system for any number of reacting molecules and all values of kinetic constants, we interpret the deviation from deterministic behavior, the splitting of the two branches of \(z_0\), and the emergence of bimodal protein distributions as different types of symmetry breaking. The role the symmetries play in this analysis differs from how they are used in quantum problems, where, for example, energy levels are invariant. In both physical and biological problems, they are important as an aid to understanding natural phenomena. Deeper insight into the role of symmetries in biology will come from analyzing other cases where they are useful. We are aware of applications to the evolution of the genetic code \[13\] and to phylogenetic branching processes \[14\], but no other cases where kinetic symmetries have been used in an analysis of stochastic gene expression.

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