RESEARCH COMMUNICATION

Highly sensitive control of transcriptional activity by factor heterodimerization

Stéphane SWILLENS* and Isabelle PIRSON

Institut de Recherche Interdisciplinaire, Faculté de Médecine, Université Libre de Bruxelles, Campus Erasme, route de Lennik, 808, B-1070 Brussels, Belgium

Several molecular mechanisms have been proposed to explain highly sensitive controls of cellular functions by effector molecules. Here we study an equilibrium model describing the regulation of transcriptional activity through the hetero-

dimerization of transcription factors. We demonstrate that this model involves a new type of biochemical control which accounts for a very high sensitivity.

INTRODUCTION

The sensitivity of a biological response to variations in the level of the stimulus can be characterized by referring to the sensitivity exhibited by a Michaelian (or hyperbolic) response. Those responses which are more sensitive than the hyperbolic response have been called ultrasensitive, and are generally described by a sigmoidal relationship between the effect and the level of the stimulus [1]. Ultrasensitivity can be generated by an equilibrium mechanism involving a positive co-operativity between the subunits of an oligomeric protein [2] or a transition between the low- and high-affinity conformations of the subunits [3,4]. For these models, the steepness of the response curve is limited by the number of subunits. Such a limitation is not encountered in non-equilibrium models, as for instance in the case of a reversible covalent modification system in which the modifying enzymes operate outside the boundaries of first-order kinetics [5]. This non-equilibrium model may generate very steep response curves.

A distinguishing feature of several families of eukaryotic transcription factors is that specific DNA sequence motifs present in promoter regions are recognized by homodimeric or heterodimeric forms of the family members. The different factor pairs may confer diverse signalling pathways on the control of transcriptional activity [6]. In this paper, we theoretically analyse the behaviour of a model suggested by a recently published reaction scheme describing the system involved in c-Myc-stimulated transcriptional activity [7]. When an adequate combination of the equilibrium constants is used, the proposed model displays a pronounced ultrasensitivity, resulting from the interplay between activating and inhibiting heterodimers which compete for the same recognition DNA sequence or for overlapping DNA binding sites. Thus, contrary to other equilibrium models, this model is able to explain a very abrupt transcriptional response induced by a moderate change in the concentration of a stimulating factor. Such behaviour could be physiologically important when a certain cell function must appear or disappear in response to an intracellular or extracellular signal. In this case, the cell must work according to a binary mode, and thus may use such an ultrasensitive transcriptional control to carry out the decision to go into a new cellular program or state.

RATIONALE

The model is based on the reaction scheme [7] describing c-Myc-mediated transcriptional activation; this involves three factors: Myc, Max and Mad (Figure 1a). For the sake of simplicity, the homodimer Max–Max was not taken into account because the heterodimers would be favoured over the homodimer, and phosphorylation of Max *in vitro* as well as *in vivo* inhibits the repressional activity of the homodimer [7,8]. The model thus consists of the reversible coupling of a central component C with two functionally different heterodimeric partners, a positive component P and a negative component N, leading to an activator A and a repressor R respectively. Both heterodimers compete for the same recognition DNA sequence, but only the activator A is capable of activating the transcription process. As shown in Figure 1(b), the reversible reactions are characterized by their respective equilibrium dissociation constants K_p , K_n , K_a and K_r . The concentrations of the different monomers and heterodimers must obey the following equilibrium equations:

$$K_p = \frac{[C][P]}{[A]} \quad (1)$$

$$K_n = \frac{[C][N]}{[R]} \quad (2)$$

The fractional transcriptional activity f is defined as the fraction of time during which the DNA site is occupied by the activator A. This site is also recognized by the inactive heterodimer R, which thus acts as a repressor of transcription. According to the general equation of competitive inhibition, one gets:

$$f = \frac{[A]}{[A] + K_n \left(1 + \frac{[R]}{K_r} \right)} \quad (3)$$

Assuming that the concentration of the DNA recognition site is negligible compared with that of the protein components of the system, the total concentrations of the monomers C, P and N are given by:

$$[C]_{\text{tot.}} = [C] + [A] + [R] \quad (4)$$

$$[P]_{\text{tot.}} = [P] + [A] \quad (5)$$

$$[N]_{\text{tot.}} = [N] + [R] \quad (6)$$

* To whom correspondence should be addressed.

Figure 1 Theoretical model describing heterodimerization of transcription factors

(a) c-Myc-mediated transcription system as described in [7]. CKII refers to casein kinase II, which phosphorylates Max. (b) A central component C dimerizes either with a positive component P or with a negative component N, leading to the formation of the activator dimer A or the repressor dimer R respectively. Both heterodimers recognize the same DNA target site, but only the activator is capable of stimulating transcriptional activity. If the model refers to the c-Myc system, the different components are identified as follows: P, Myc; C, Max; N, Mad; A, Myc-Max dimer; R, Max-Mad dimer. The DNA site refers to the core sequence CACGTG.

The simulation aimed to show how the fractional activity f varies in response to an increase in the concentration of the positive component P. It has been shown that the half-life of the c-Myc protein is unaffected by dimerization with Max [8]. Thus the relative rates of protein synthesis and degradation control the total concentration of c-Myc rather than its free concentration. Therefore the simulation will consider the total protein concentrations as the independent variables of the system.

In order to compute f as a function of $[P]_{tot.}$, the numerical computation strategy considers that fixed values of $[C]_{tot.}$ and $[N]_{tot.}$ and varying values of $[P]$ are given. Using eqns. (1), (2) and (6), eqn. (4) becomes:

$$[C]^2 + [C] \left(K_n + \frac{[N]_{tot.} - [C]_{tot.}}{1 + \frac{[P]}{K_p}} \right) - \frac{K_n [C]_{tot.}}{1 + \frac{[P]}{K_p}} = 0 \quad (7)$$

which allows the computation of $[C]$. Using eqns. (1), (2) and (6), one obtains:

$$[A] = \frac{[C][P]}{K_p} \quad (8)$$

$$[R] = \frac{[N]_{tot.}[C]}{K_n + [C]} \quad (9)$$

Finally, f and $[P]_{tot.}$ can be computed by introducing the values of $[A]$ and $[R]$ into eqns. (3) and (5). All of the numerical simulations use the same arbitrary units for component concentrations and equilibrium dissociation constants.

Two indexes can be proposed to characterize the ultrasensitivity: the ratio ρ of the total concentrations of P leading to 90 % and 10 % of the maximal response respectively, and the Hill coefficient h that occurs at the half-maximal response. A highly ultrasensitive response is characterized by $\rho \ll 81$ and $h \gg 1$.

RESULTS AND DISCUSSION

The stimulation of transcriptional activity by the positive component P or its repression by the negative component N are both the result of a cascade of two events, consisting of heterodimerization with the central component C followed by coupling of the dimer with the DNA recognition site. Thus the potency of each heterodimeric partner of C in stimulating or repressing transcriptional activity is determined by its ability to associate with C and with the DNA site. Different theoretical situations can be considered on the basis of the relative binding affinities of the components. Preliminary simulations suggested that ultrasensitivity can be obtained if C recognizes P better than it recognizes N ($K_p < K_n$), and if DNA transcription is more easily repressed by R than activated by A ($K_a > K_r$). In fact, a 100-fold difference between K_n and K_p , and between K_a and K_r , is sufficient to produce a highly ultrasensitive response curve characterized by a ρ value equal to 2.1 (curve a in Figure 2). Thus a 2-fold increase in $[P]_{tot.}$ is sufficient to switch f from about 10 % to 90 % of the maximal response. The Hill coefficient h calculated at mid-saturation is equal to 10, which classically corresponds to a phenomenon involving highly positive co-operativity. The ultrasensitivity may be even higher for lower values of K_p . When K_p is 10 or 100 times lower, h increases up to 50 or 94, and ρ decreases to 1.2 or 1.1 respectively (curves b and c in Figure 2). That means that a 20 % or 10 % variation in $[P]_{tot.}$ increases f from 10 % to 90 % of the maximal response in these two respective cases. On the contrary, the ultrasensitivity completely vanishes when P and N bind to C with similar affinities (curve d in Figure 2). In this case, the system displays a Hill coefficient h equal to 1, characteristic of a hyperbolic response.

The high ultrasensitivity obtained when $K_p < K_n$ and $K_a > K_r$ may be explained as follows. Because of the postulated relative affinities, the P component may easily displace the N component from the C component. However, as long as a certain amount of the repressor dimer R subsists, the DNA target cannot be trapped by the activator dimer A, which exhibits a lower affinity. Only when the majority of the C molecules have been sequestered by P does the DNA then become available to the entire pool of the dimer A. Hence a near-maximal transcriptional response is abruptly induced. Thus the existence of high ultrasensitivity is

Figure 2 Simulation of the model with $K_p < K_n$ and $K_s > K_r$.

The fractional transcriptional activity f is plotted as a function of the total concentration of the positive component P for different values of K_p : 0.001 (a), 0.0001 (b), 0.00001 (c) and 0.1 (d). The other parameter values are $K_n = 0.1$, $K_s = 0.1$, $K_r = 0.001$, $[C]_{tot} = 100$ and $[N]_{tot} = 10$. Inset: Hill plot of the four curves.

Figure 3 Effect of a 2-fold variation in the total concentration of the negative component N

The parameters are the same as for curve a of Figure 2, except that $[N]_{tot}$ is equal to 10 (a), 5 (b) and 20 (c).

the consequence of the 'double blocking' action of the negative component N . Indeed, both the sequestrations of C by N and of the DNA sites by the dimer R thus formed are required to produce a very steep response curve. The simulation may show that other models accounting for only one type of sequestration or for two types of sequestration, achieved by two different inhibiting chelators, were unable to provide such highly sensitive controls of transcriptional activity.

The next simulations show how the response depends on the total concentrations of the two other components, N and C . Figure 3 shows that the response is not very sensitive to a moderate variation in $[N]_{tot}$. An increase of the negative component N leads to a modest inhibition of the response both below and above the threshold value for $[P]_{tot}$. Thus, under the

Figure 4 Effect of a 2-fold variation in the total concentration of the central component C

The parameters are the same as for curve a of Figure 2, except that $[C]_{tot}$ is equal to 100 (a), 50 (b) and 200 (c).

conditions considered, the system is much less sensitive to the negative component than to the positive component. In contrast, Figure 4 shows that the central component is more effective, as it efficiently displaces the threshold value for $[P]_{tot}$: the P component becomes more potent as the total concentration of C decreases.

The proposed simulations considered the case where the response is more sensitive to the positive component than to the negative component. However, because of the symmetrical structure of the model, the reverse situation can be produced by inverting the relationship between the equilibrium constants, i.e. $K_p > K_n$ and $K_s < K_r$. In this case, the transcriptional response is controlled over a wide range of total concentrations of P , which could be of physiological interest if the cell has to adjust the transcription rate quantitatively and thus tune accurately the cell machinery according to current metabolic requirements (results not shown).

The simulation results repeatedly refer to total factor concentrations as independent variables. This choice was made because the cell apparently controls the total amount of c-Myc rather than its free concentration [8]. However, assuming that the P -mediated transcriptional activation requires a burst of P production, one may prefer to express the transcriptional response induced by a certain P concentration as a function of the time required to generate this concentration. This alternative kinetic view could be more realistic for those systems in which the cell controls the free concentration of the factor instead of its total concentration. In this case, the threshold values describing critical total concentrations should be interpreted in terms of delay values or time lags in the kinetics of the triggering of transcription.

When we started this study, we were fully aware that the actual Myc system is certainly more complex than the scheme and the model presented in Figure 1. On the other hand, it is not known if the control of transcriptional activity by c-Myc displays ultrasensitivity. Thus it must be emphasized that the model has not been developed with the aim of describing the actual quantitative role of c-Myc in the regulation of transcription. The objective was only to show unexpected properties of factor dimerization when the heterodimers competitively bind to the

same DNA site or to overlapping sites. However, one may wonder if transcription factors of the c-Myc system take advantage of their ability to form heterodimers. This could be the case, providing that the relative affinities at the levels of dimerization and DNA binding are adequate. For instance, the transcriptional control could be ultrasensitive to a variation of c-Myc if Myc-Max is notably more stable than Max-Mad ($K_p < K_n$) and if Max-Mad has a higher binding affinity than Myc-Max for the DNA site ($K_a > K_r$). Up to now only *in vitro* data have been obtained, and these suggest that Myc and Mad bind to Max equally well [7]. If this corresponds to the *in vivo* situation, the system described by the model could not display any ultrasensitive control. However, it must be stressed that one or other binding affinity could be changed through an allosteric control or a covalent modification of some factors, and thus the relative affinities *in vivo* could vary depending on the state of the cell. For instance, it has been reported that the kinetic properties of binding of Myc-Max and Max-Max to DNA depend on the phosphorylation state of *in vivo* phosphorylation sites in Max [9]. Another complexity of this system results from the observed inverse correlation between the expression of the genes for Myc and Mad [10]. If variations in the concentrations of Myc and Mad are indeed inversely related, transcriptional activity could be theoretically controlled with a higher sensitivity, since a decrease in the concentration of the N component would increase the potency of the P component.

In conclusion, although it is premature to predict the quan-

titative behaviour of the Myc system by simulating the model described here, we might not disregard potentially appealing features resulting from the intricate dimerization scheme, which could perhaps apply to other factor families. From a purely theoretical point of view, the proposed double blocking concept would be the first example of an equilibrium model that is able to generate highly ultrasensitive responses without invoking the existence of a multimeric protein composed of a very high number of subunits.

We thank Dr. J. E. Dumont for his support in this work, which was performed under a contract with the Ministère de la Politique Scientifique (P.A.I.). I.P. is a research fellow of the Fonds National de la Recherche Scientifique (F.N.R.S. TELEVIE).

REFERENCES

- 1 Koshland, D. E., Jr., Goldbeter, A. and Stock, J. B. (1982) *Science* **217**, 220–225
- 2 Adair, G. S. (1925) *J. Biol. Chem.* **63**, 529–545
- 3 Monod, J., Wyman, J. and Changeux, J. P. (1965) *J. Mol. Biol.* **12**, 88–118
- 4 Koshland, D. E., Jr., Nemethy, G. and Filmer, D. (1966) *Biochemistry* **5**, 365–385
- 5 Goldbeter, A. and Koshland, D. E., Jr. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6840–6844
- 6 Lee, K. A. (1992) *J. Cell Sci.* **103**, 9–14
- 7 Ayer, D. E., Kretzner, L. and Eisenman, R. N. (1993) *Cell* **72**, 211–222
- 8 Blackwood, E. M., Lüscher, B. and Eisenman, R. N. (1992) *Genes Dev.* **6**, 71–80
- 9 Bousset, K., Henriksson, M., Lüscher-Firzlaff, J. M., Litchfield, D. W. and Lüscher, B. (1993) *Oncogene* **8**, 3211–3220
- 10 Larsson, L. G., Pettersson, M., Öberg, F., Nilsson, K. and Lüscher, B. (1994) *Oncogene* **9**, 1247–1252