In vivo repressor kinetics exclude simple operator occupancy model for gene regulation

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Models of gene regulation *via* repressing transcription factors commonly assume that the degree of repression is determined solely by the equilibrium binding-constant of the repressor. Whether this assumption holds *in vivo* is, however, untested. To test its validity we have in a direct manner measured both spontaneous dissociation and the association time for *lac*-repressor in *E. coli* to bind its operator. We find that a simple equilibrium model based on these binding and unbinding times does not explain the degree of repression observed *in vivo*. We instead suggest that, for example, a non-equilibrium model better explain the *in vivo* situation

I. INTRODUCTION

TRANSCRIPTION FACTORS such as the *lac*-repressor in *E. coli* are key players in gene regulation. These repressors inhibit transcription initiation by binding to sequence specific sites on the chromosome [1]. A common way of modeling regulation of transcription initiation by repressors is by assuming that transcription initiation is inhibited when the repressor is bound to its operator, but possible when the repressor is not bound [2]. In this description the ratio of expression in the absence and the presence of repressor, *i.e* the repression ratio (RR), is solely given by the fraction of time the operator is free from repressor such that

$$RR = \frac{\tau_{on} + \tau_{off}}{\tau_{on}} = 1 + \frac{[TF]}{K_D}.$$

Here τ_{on} is the time it takes for the repressor to find and bind its operator, τ_{off} is the time the repressor stays bound to its operator and K_D is the corresponding equilibrium binding-constant for the repressor binding to its operator.

To test whether the above model of the repression ratio holds, both in terms of form and definitions of included parameters, we recently measured the association and dissociation rates for fluorescently labeled LacI dimers and compared to repression ratios based on protein expressions levels, *i.e* the ratio of protein concentrations with and without inducer [3].

II. IN VIVO KINETIC MEASUREMENTS

To measure the time LacI stays bound to its operator site an *in vivo* version of a chase experiment was developed. Here a fluorescently labeled LacI bound to its operator is upon its spontaneous dissociation replaced by a non-fluorescent LacI present in excess, and bound fluorescent LacI is detected as diffraction limited spots in wide field microscopy. Association of LacI to its operator is measured following appearance of operator bound LacI after addition of inducer. Association and dissociation times are measured for two operators, the natural O_1 and the synthetic O_{sym}, which gives a higher repression ratio.

III. MODELS OF TRANSCRIPTION INTIATION

Using the simple occupancy model above, the measured kinetic binding times can explain the observed repression ratio in the case of the O_1 operator but not in the case of the stronger O_{sym} operator in which the repression rate based on kinetic data is too small. To account for this discrepancy we constructed more complex models, which explicitly include the binding of RNA polymerase (RNAP). A cooperative equilibrium model can explain the in vivo repression given that LacI affects the equilibrium binding-constant of RNAP in the case of O_1 but not in the case of O_{sym}. It is, however, possible to construct a non-equilibrium model, in which transcription drives the LacI binding out of equilibrium, that uses the same reactions but does not require sequence-specific cooperativity between LacI and RNAP to explain the data. One such non-equilibrium model is the case where RNAP and LacI do show cooperativity in binding, but at the same level both the O_1 and the O_{sym} case. The reason this model well explains the data is that O_1 is further away from equilibrium compared to O_{sym}. Another possible non-equilibrium model is the case where only the turnover-rates of binding and unbinding, but not the equilibrium constants, are changed by RNAP binding to the operon.

IV. CONCLUSION

Based on the in vivo kinetic data we find that the simple equilibrium occupancy model is not enough to predict gene repression. Here we instead suggest a simple non-equilibrium model to bridge the inconsistencies.

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