To the next level of transcription: influence of the accessible genome size

Amanda van der Sijs, under supervision of Willem Kegel and Gert Folkers

Transcription is the process by which a gene segment on DNA is copied into messenger RNA. Several proteins are involved in transcription: RNA polymerase, which reads the DNA and converts that into RNA, and transcription factors, which either activate or repress the binding of RNA polymerase to DNA. A lot of progress has been made on the direct influence of transcription factors by binding to promoter regions on the genes of interest. However, these proteins also bind to other sequences of DNA and thus, the total amount of DNA available for these proteins to bind to, competes with the binding to promoter regions (Figure 1).[1]

Somewhat surprisingly, this 'next level' of transcription regulation has not been systematically addressed yet. Indeed, several processes in the cell are expected to lead to significant variations in the amount of accessible DNA to the proteins involved in transcription regulation. This affects the chemical potential of these proteins, which in turn influences the fraction of bound proteins to promoter and operator regions, which ultimately influence overall transcription rates. Such behavior follows from the basic statistical thermodynamics for simple genetic architectures (Figure 2).[2]

In this project we quantitatively investigate how non-specific DNA influences transcription using an experimental, theoretical and, in collaboration with the group of Jocelyne Vreede (UvA), a computational approach.

To determine the effect of the number of non-specific base-pairs on the transcription rate, transcription rates will be monitored using a Lac promoter and operator/repressor in a simple repression architecture. As reporter gene we use a 'broccoli' construct, mRNA aptamers that fluoresce upon binding to an appropriate chromophore (here: DFHBI-1T) (Figure 3).[3]

We will systematically vary the accessible amount of non-regulatory DNA in in vitro experiments and monitor the transcription rates. In particular, we address how the degree of wrapping and coverage of the genome by DNA structuring factors influences the transcription rates.

If successful, this work will pin down the regulatory role of the accessible genome size in biological cells and provides a next level of transcription regulation, that is, regulation beyond the direct interactions between operator regions and transcription factors.

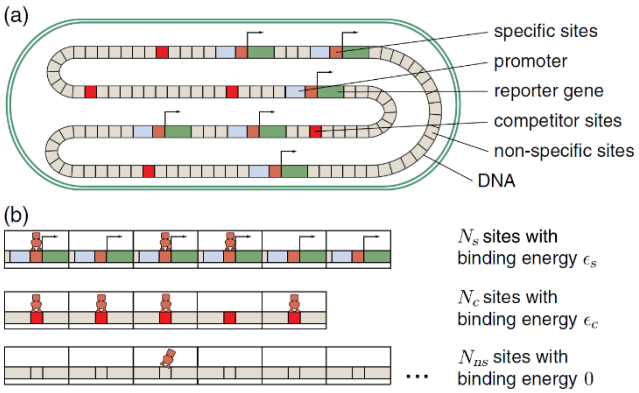

Figure 1. (a) Schematic of chromosome when viewed as a lattice of possible binding sites that can be occupied (or not) by a repressor. Within the cell are multiple identical, regulated promoters (that produce a measurable gene product), "competitor sites" that bind the repressor stronger than a nonspecific interaction but do not regulate a gene, and nonspecific sites that each bind the repressor weakly. (b) In the grand canonical framework, each of these types of binding sites is treated as a lattice of possible binding sites, characterized by the number of sites in the lattice N and the energy with which each site binds the transcription factor ϵ, with a chemical potential responsible for maintaining balance between the number of molecules bound one each lattice.[1]

$$\langle P_s \rangle = \lambda_p \frac{\partial \ln \Xi_s}{\partial \lambda_p} = N_s \frac{\lambda_p e^{-\beta \epsilon_p}}{1 + \lambda_p e^{-\beta \epsilon_p} + \lambda_r e^{-\beta \epsilon_s}}$$

Figure 2. The average number of RNAP molecules adsorbed onto the NS promoter sites. The fugacities are $\lambda p = e^{\beta \mu p}$, where μ_p is the chemical potential of the RNAP, and $\lambda r = e^{\beta \mu r}$ where μ_r is the chemical potential of the repressor molecule.[1]

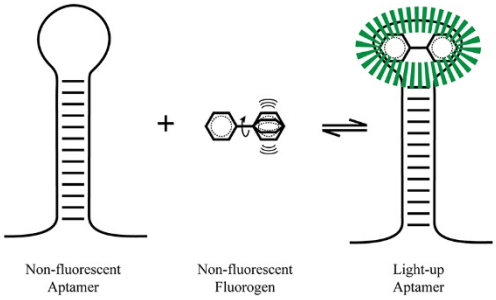

Figure 3. Transcription of the 'broccoli' DNA yields RNA that fluoresces upon binding a dye.[3]

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3. Ouellet, Jonathan. “RNA Fluorescence with Light-Up Aptamers.” *Frontiers in Chemistry* 4 (2016). https://doi.org/10.3389/fchem.2016.00029.