Regulation of NF-κB responsive genes in a single cell

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1 Experiment and problem formulation.

Nuclear factor κB (NF-κB) regulates numerous genes important for pathogen or cytokine inflammation, immune response, cell proliferation and survival. In resting cells it remains inactive in cytoplasm, bond to its inhibitor IκBα. In response to extracellular signals such as TNF, IκBα is destroyed, NF-κB enters the nucleus, binds to specific regulatory sites and triggers gene transcription. Our observations [1] show (Fig. 1) that in HeLa cells, the NF-κB responsive genes can be grouped into 3 characteristic classes: early (such as IκBα, A20 or IL8) for which the amount of mRNA transcript has its maximum at about 1 hour, intermediate (such as NF-κB1 or TNFAIP2) with the maximum at 3 hours and late (such as NAF1 or NF-κB2) with the maximum at about 6 hours. In contrast to some other cell lines, in HeLa cells NF-κB is not effectively lead out of the nucleus by the newly synthesized IκBα, but rather, after entering the nucleus at 15 min from the beginning of TNF stimulation, it remains there for at least 6 hours [1]. This implies that some other cofactors are needed to initiate and terminate expression of genes belonging to these 3 groups. The aim of this work is to propose the mechanisms of gene regulation at a single cell level able to generate these 3 characteristic classes of expression profiles involving a small number of co-regulators. We will not try to identify these co-regulators, which at this point should be understood in broad sense as activating (e.g. histone acetylation) or repressing events, not necessarily connected with DNA protein binding.

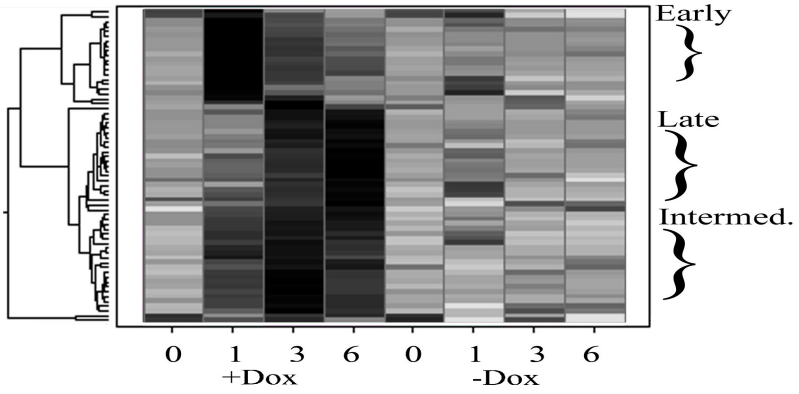

Figure 1: Kinetics of NF-κB-dependent gene expression in HeLa cells [1]. Data represent the mean of three independent time courses analyzed by high-density microarrays. In this experiment the NF-κB nuclear translocation is enabled by culturing cells in the presence of doxycycline (Dox). The expression profiles for selected genes of each group where confirmed by Northern blots.

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2 Model and results.

The observed profiles of expression may be explained by assuming that all 3 classes of genes are regulated by at most 2 co-activators (not including NF-κB) and 1 repressor. We assume that: (1) Each gene has two potentially active alleles, and the activation and repression of these alleles proceed independently. (2) Expression of any early gene is initiated by NF-κB binding, while to initiate expression of an intermediate gene additionally one co-activator is needed, and to initiate expression of late genes two co-activators are needed in addition to NF-κB. (3) Transcription of all genes is terminated by a repressor. (4) All genes have the same mRNA degradation halftime equal to 30 min. (5) Binding of activators and repressor occur in a stochastic way, with binding halftimes (chosen to fit experimental expression profiles) of 10min, 70min, and 4 hours, respectively, for activators and of 70min for the inhibitor.

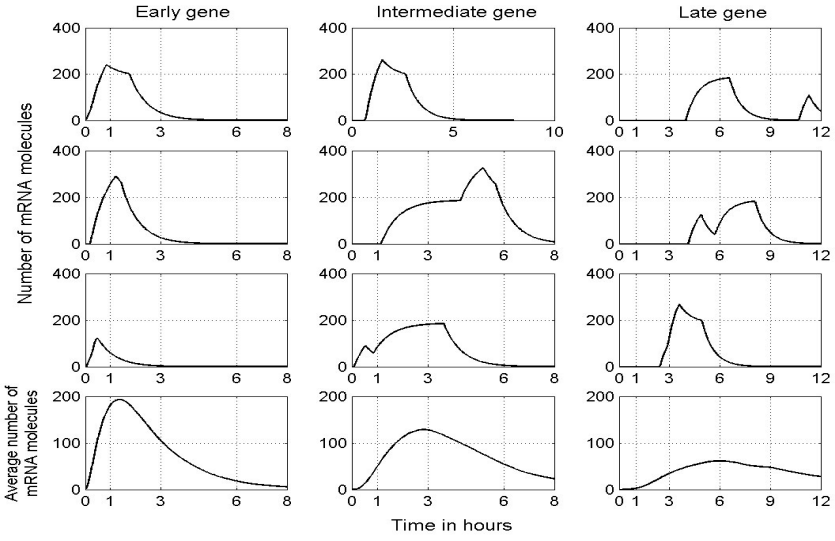

Figure 2: The mRNA profiles for early, intermediate and late genes. First 3 rows show mRNA profiles in single cells, while the profiles in last row result from averaging over population of 1000 cells. These latter profiles should be compared to experimental data in Fig. 1. The kinks visible on single cell profiles correspond to initiation or termination of expression in any of two homologous copies of the gene. The difference among single cell profiles is larger for late genes and as a result the averaged expression profile is broader, what is well confirmed in Northern blot data.

We have shown that the three classes of profiles can be explained by a relatively small number of regulatory factors. Moreover, our analysis shows that, especially for late genes, the single cell mRNA profiles may be very different from the averaged profile. The single cell experiments testing this hypothesis are currently underway.

References

[1] Tian, B., Brasier, A. R., unpublished data