MANIPULATING PHENOTYPES BY EPIGENETIC MECHANISM

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For many years, the plasticity and variation of phenotypes observed in CHO cell lines was attributed to genomic variation. However, while individual mutations of single genes may certainly contribute to a defined phenotype, it typically is the adaptation of the expression pattern of multiple genes which together then modulate and define cellular behavior. Such changes in the transcription pattern are defined by several layers of epigenetic regulation that act on short term and long term, serving both as rapid response mechanisms and as cellular "memory"[1, 2]. These include differential DNA-methylation, predominantly in promoter regions, but also in regulatory regions of the genome. These co-operate and are co-regulated with modifications of histones which change the state of chromatin and thus the accessibility for the transcriptional machinery. On top of these, there are interactions between specific genomic regions and triplex-forming long-non-coding RNAs that can both up- or downregulate transcription by attracting or blocking off transcription factors. The later can serve as very rapid and very strong regulators of transcription.

Such detailed understanding of the underlying mechanisms can be used to advantage to enhance our control over phenotypes both by specifically altering the expression level of individual genes (to the degree of turning them ON or OFF[3]) and by altering the global transcriptome to achieve enhanced cellular performance. Likewise, directed evolution and adaptation protocols also result in a new transcriptome defined by epigenic memory that lays down altered cellular behavior[1]. Ultimately, these tools offer new possibilities for metabolic or cellular engineering, which have the advantage of being fully reversible and dosable, as no changes in the genome sequence are required. Such epigenetic control mechanisms could be used in two directions: i) to increase the phenotypic diversity within a population, for instance during cell line development, to enable isolation of rare variants with superior properties; and ii) to stabilize an already selected phenotype such that more reproducible process outcomes are achieved.

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