WHAT´S IN A PHENOTYPE?

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The large diversity in phenotypes that is found in CHO cells and their subclones has been largely attributed to genomic variance and selection for specific mutations that control such phenotypes. Only lately has it become clear that a major player with respect to diversity actually is epigenetic regulation of the precise level of expression of all transcribed genes in a cell line. Several epigenetic mechanisms have been shown to control different types of responses, including i) short term adaptation of the expression level of individual genes, for instance in response to nutrient limitation, are controlled by specific histone marks; ii) very strong regulation in response to environmental signals is accomplished by long-non-coding RNAs that interact with the promoters of target genes via triplex formation[1], and iii) ON/OFF switches that act via DNA-methylation in promoter regions[2] and enable a yes or no decision that has no impact on relative expression strength. DNA-methylation in general, not just in promoter regions, but across the entire genome plays a major role in controlling gene expression and is inheritable, thus serves as the long term memory of cells. It enables new gene expression patterns for instance after adaptation to new culture conditions or establishment of favorable phenotypes during cell line development and can be manipulated to permanently activate or silence expression of specific genes[3]. Manipulation of histone marks on the other hand allows for transient changes in gene expression levels. That the different mechanisms of epigenetic control are linked and work together was demonstrated by the fact that after changing the DNA methylation pattern of promoters, the respectively required histone marks were autonomously added by the cells. Understanding these mechanisms enables us to manipulate cells to obtain favorable phenotypes, both in a targeted[3] and a random way. The downregulation of enzymes that remove or transfer DNA-methylation was shown to result in an increase in diversity of the population as measured by the coefficient of variation for instance of protein secretion, thus enhancing the percentage of outliers present and the likelihood of selecting a producer with higher maximum productivity. Apart from such researcher driven approaches, there are strong indications that the cells themselves, when exposed to unfavorable or stressful conditions, actively promote the reprogramming of their methylome so as to generate new transcriptome patterns that enable them to handle the new conditions. Unwittingly, this has been taken advantage of during cell line development campaigns, as i) our screen of chemicals that are known or predicted to interfere with DNA-methylation showed that both MSX and MTX[4] increase population diversity, irrespective of gene amplification and ii) the process of subcloning itself has a similar effect. Out of a panel of 36 subclones with a range of phenotypes (with respect to growth rate, IVCD and qP), 6 were picked that cover this range and bisulfite-sequenced. Each subclone had a large number of differentially methylated regions (DMRs) compared to the originator cell line, the majority of which were unique to only one subclone, with very little overlap. Together with data from single cell transcriptome analysis that show that the diversity within a pool of cells that are grown under the same culture conditions is very low, this would support the hypothesis that the diversity of phenotypes observed is not due to selection of variants that were previously present, but that the cells actively achieve diversification by unknown mechanisms. Such active reprogramming by cells would also account for so far unexplained process variations, or phenotypic drift in long term cultures. A more detailed understanding of the underlying mechanism will allow to obtain better control of phenotypes both for cell line engineering and the stabilization of phenotypic properties.

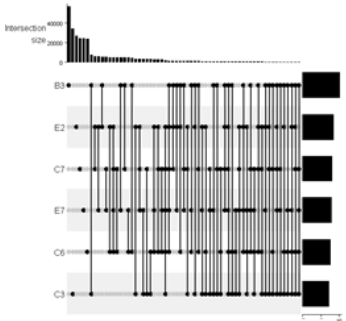

Figure: Distribution of DMRs in subclones

[1] Hernandez et al. (2019) Biotechn. Bioeng, 116:3:677 https://doi.org/10.1002/bit.26891
[2] Feichtinger et al. (2016) Biotechn. Bioeng. 113:10:2241-2253
[3] Marx et al. (2018) Biotechn J 13:10, 1700217 https://doi.org/10.1002/biot.201700217

[4] Vishwanathan et al. (2014) Biotechn. Bioeng. 111:3:518 https://doi.org/10.1002/bit.25117