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To clone or not to clone? – Wrong question! An investigation on genome heterogeneity and stability and on what controls cell behavior

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An investigation on genome heterogeneity and stability and on what controls cell behavior

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The most striking characteristic of CHO cells is their adaptability, which enables efficient production of proteins as well as growth under a variety of culture conditions, but also results in genomic and phenotypic instability. Potential causes include i) the high number of chromosomal rearrangements, including variation in chromosome numbers observed in CHO or any other rapidly growing cell line; ii) mutations including SNPs or InDels that change the activity or function of enzymes; iii) epigenetic changes that alter the gene expression pattern of a cell without impacting the genome sequence itself. To understand the relative contribution of these towards phenotype evolution, full genome sequences and methylomes of 6 related cell lines were analysed for changes in genome sequence and in DNA-methylation patterns. In addition, histone modifications and DNA-methylation patterns at several time points of a batch culture were determined. Finally, different methods to assess genomic stability over time were tested, including the distribution and spread of chromosome counts per cell in a population, and the analysis of large scale rearrangements by chromosome painting and amplified fragment length polymorphism (AFLP).

In summary, our results reveal the following picture:

- On the epigenetic level, short term adaptation of gene expression patterns to alterations in the environment (such as changes in nutrient availability or waste concentrations during a process) are predominantly controlled by modifications of histones and resulting changes in chromatin states.
- Long term adaptation to altered culture conditions, such as the transition from adherent to suspension culture, adaptation to different media or selection of specific phenotypes, are controlled by more stable changes in DNA-methylation patterns which are largely inherited by daughter cells as long as conditions remain constant.
- Genomic variants including SNPs, InDels, translocations, copy number variation and inversions, occur (and disappear) on a continuous basis, even over time. These variants happen on a random basis, they may contribute to phenotype if they provide a growth advantage, however, due to their continuous occurrence they are difficult to stabilize and/or control and may well be unavoidable.
- The majority of SNPs (99%) have no impact on coding sequence.
- All populations analysed, whether subclone or pool, contained a comparable absolute number of variants and at a similar frequency distribution within the population. The effect of subcloning on genome homogeneity is thus lost by the time cells are expanded to sufficient numbers for an MCB.
- Due to the variation present in each population, methods to assess genomic identity or stability are severely hampered by background noise, making the use of AFLP and probably other methods such as STR or DNA fingerprinting difficult. Nevertheless, genomic changes can be followed and semi-quantified by these methods in combination with rigid statistic tests.
- Counting the number of chromosomes per nucleus reveals a large spread in numbers, with typically only 30-50% of cells forming a peak at a dominant chromosome count. Again, cells with aberrant chromosome counts appear and disappear on a continuous basis, as subcloning does not lead to more homogenous count distributions. Over time, chromosome counts become more divergent, frequently near-tetraploid counts appear.
- Chromosome painting reveals frequent, large scale rearrangements, with aberrant chromosomes present from start, and/or appearing and disappearing over time.

In view of these results, the question arises whether subcloning is a suitable step to ensure genomic homogeneity and stability and whether, rather than proving that a clone is actually derived from a single cell, efforts should not be directed towards developing tools and methods that enable reliable and rapid characterization of subclones/cell lines in terms of homogeneous behavior rather than genome. While subcloning may be required for assurance of a single gene integration site or for selection of specific behavior that may be energetically more demanding and thus requires protection from outgrowth by faster growing cells, the common expectation that subclones are genomically homogenous needs to be challenged for a rapidly growing mammalian cell line.