The cell cycle as a process parameter in mammalian cell culture

Physiological synchronisation utilising elutriation and optimised read outs

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Mammalian Cell Synchronisation¹,²

Cell Synchronisation is performed by Counter flow Elutriation¹, a selective method to sort living cells based on their size:
- Mammalian cells grow throughout the cell cycle (CC)
- Selection by size leads to a separation of cells in different CC-phases
- Increasing in size: G0, G2 & M phase

Relevance & classic determination of CC-Distribution by PI staining & FACS

Differences in the metabolic behaviour of cells in different cycle phases have frequently been reported.
Our studies are the first:
- Systematic approach to quantify & model these effects
- Under near-physiological conditions
- Without the perturbations of whole culture synchronisation methods (i.e. chemical)

Synchronous cultures characteristics:
- Narrow size distributions
- Synchronised cell growth
- (Nearly) uniform DNA patterns³

Determining the Cell Cycle state:

Synchronized cultures:

Cell Cycle distributions vs. growth rate μ:

Monitoring synchronized cultures:

The FUCCI-System⁶

- The FUCCI²⁵ is Fluorescent Ubiquitination-based Cell Cycle Indicator System
- For real time cell cycle analysis
- Two different fluorescence conjugated fusion proteins
  - G1: mKO2-HDcT(30/120)
  - S/G2/M: mVenus-HGemini(1/110) in Cell line C+M or (1/60) in cell line C+N
- Expressed under the control of the mammalian cell cycle without influencing it
- Achieved by deletion studies of cell cycle dependent proteins
- Possible using a broad range of fluorescence methods (specificity provided)

Adaptation for current project:
- Was available with i.e. HeLa, COS, Zebrafish, Drosophila and Mouse lines
- Not with the most commonly used producer cell line: CHO
- We used a lentiviral vector system to create the stable cell lines CHO-K1 FUCCI C+M as well as CHO-K1 FUCCI C+N

References: