

P017 Flow cytometric detection and analysis of human cultured cells in cytokinesis

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Cytokinesis is an essential process by which the mother cell cytoplasm is divided between the two daughter cells. While the development of live cell imaging techniques has provided the means to monitor cytokinesis in cultured mammalian cells, a direct biochemical approach has been hampered by the lack of an easy method to sort cells undergoing cytokinesis. We have recently described a flow cytometric method to detect and sort HeLa cells in cytokinesis from a pre-synchronised cell culture. The sorted population is highly enriched for cells in cytokinesis and shows a minimal contamination with late mitosis and G1 cells. Moreover, a sufficient amount of cells can be collected to be further analysed by immunofluorescence and immunoblotting. A number of regulatory proteins are sequentially dephosphorylated and degraded during mitotic exit and cytokinesis. The mitotic protein Cyclin B1 starts to be degraded as soon as cells enter anaphase, while degradation of the passenger protein Aurora-B occurs later during cytokinesis and G1. The mitotic phosphoepitope MPM-2 progressively disappears during anaphase. Using these proteins as markers, we will show how flow cytometry can be used to accurately follow up mitotic exit and cytokinesis.