Time-lapse Proliferating Cell Nuclear Antigen (PCNA)

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Tuesday and Wednesday we use the same type of cells with the same transgenic proteins. For this reason you can get the description of the confocal setup and setting up a time-lapse experiment in the file "Confocal Microscope ".

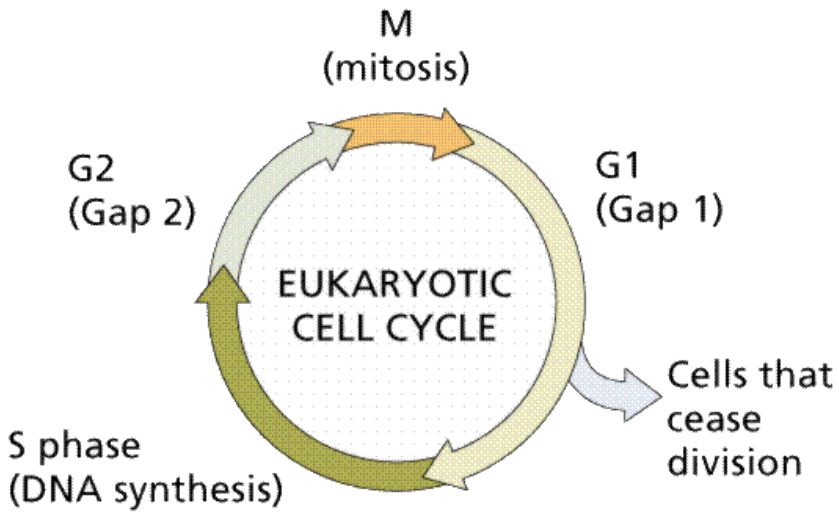
INTRODUCTION:

DNA Replication

DNA replication and gene transcription have in common that both processes act on DNA, which requires the recruitment of chromatin remodelers to the active sites. These active sites can be visualized by confocal microscopy as foci or speckles, allowing us to count the number, the shape and the size of those structure at any stage of replication or transcription (this will be done in the time lapse imaging practical). In this practical you will either measure the binding kinetics of PCNA in individual foci or study the DNA binding by ARs using fluorescence recovery after photobleaching (FRAP)[1]. In both studies active and inactive proteins are compared, which is essential for proper interpretation of the results.

Role of PCNA in DNA replication

DNA replication occurs during the S-phase of the cell cycle (Figure 3). Proliferating cell nuclear antigen (PCNA) is a clamp that supports DNA replication by binding as a trimer around the DNA and serve as a scaffold. Many other supportive proteins, including chromatin remodeling factors, interact with DNA-bound PCNA to further support proper replication. Similar to the androgen receptor PCNA that binds to DNA during replication accumulates in tiny structures, termed PCNA foci that can be visualized by confocal microscopy (Figure 4). Replication starts in euchromatin in early S-phase. In mid S-phase heterochromatin (mostly near the nuclear membrane and the nucleoli) is replicated. Finally in late S-phase, a few large PCNA foci remain. It is currently not clear what the function of those larger foci is. Thus, PCNA foci change during progression through S-phase both in size and number as will be studied in the time lapse imaging practical.



[1] note that in one of the other practicals FRAP will also be applied to focal adhesions, little structure at the cell membrane that are involved in cell migration; this is described in another protocol

Figure 1. The cell cycle.

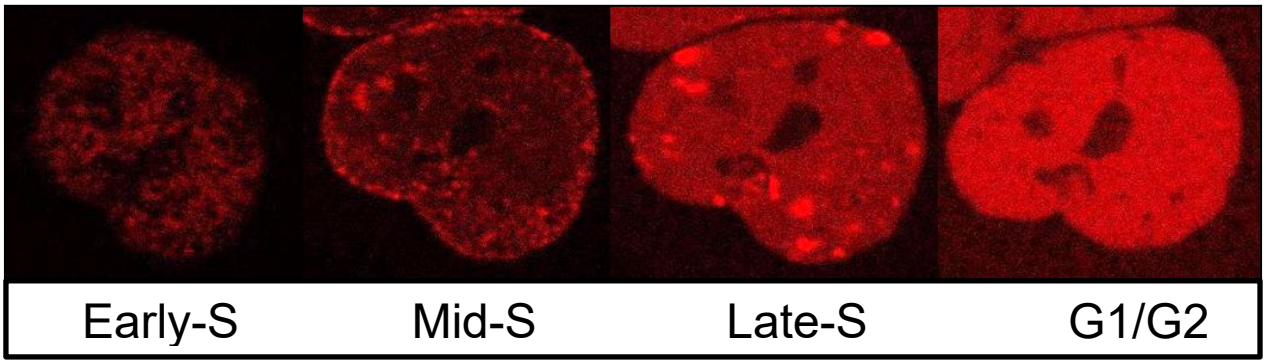


Figure 2. Confocal images of a cell expressing mCherry-tagged PCNA during the subsequent stages of S-phase an in G1 or G2 phase.

Leica microscope:

1. For time-lapse imaging, experimental parameters are required, including exposure time, number of fields, and the number of repeats should be adjusted as needed.
2. Mark different position in the stage and follow the cells overnight.

Zeiss microscope:

1. Activate Z, time and positions.
2. Define the overnight settings: Laser power, PMTgain, exposure time, time-interval, number of fields, number of repeats, autofocus etc.
3. Mark different positions and follow the cells overnight.

Practicum PCNA:

1. The cells are already mounted and transferred to a heated (37°C) stage of the microscopic, under 5% CO2 conditions throughout image acquisition.
2. Find the cells expressing PCNA-mCherry and configure all the imaging parameters desired to acquire images, including z-stack settings, scan speed, laser power, gain, offset, frame averaging, and resolution.

Data Analysis PCNA:

1. Calculate the percentage of dividing cells and use this to make an estimation of the time needed for a complete cell cycle.
2. Monitor cells which enter a certain cell cycle stage (G1/G2, early-S, mid-S, late-S) and use all these time-intervals to calculate the length of the different stages in the cell cycle (G1,S,G2,mitosis). For this you need to find cells with 3 different stages, for instance G1, early-S, late-S, which gives you the early-S length. Do this for all stages.