

## 2.2 Methods for studying apoptosis in individual cells

A number of methods have now been developed to study apoptosis in individual cells. In the following sections, we will describe details of several of these apoptosis assays.

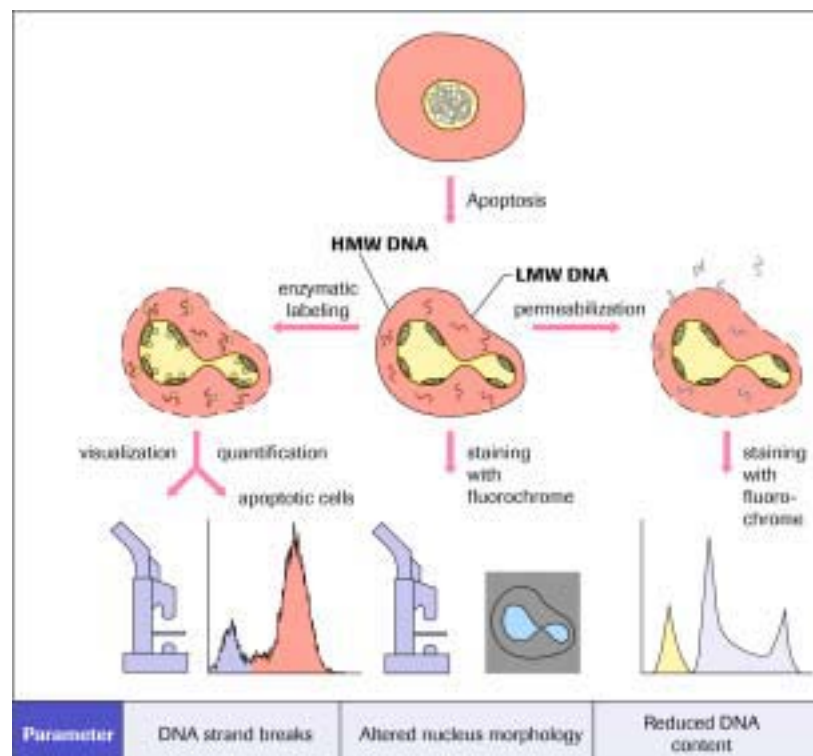
We focus on two key apoptotic events in the cell:

① DNA fragmentation used to study death in cell populations may also be used to study death in individual cells. As described in Section A 2.1.1, DNA cleavage is a hallmark for apoptosis, and assays which measure prelytic DNA fragmentation are especially attractive for the determination of apoptotic cell death.

The methods used to assess DNA strand breaks are based on labeling/staining the cellular DNA. The labeled/stained DNA is subsequently analyzed by flow cytometry, fluorescence microscopy or light microscopy (Figure 20). In general, two different labeling methods may be used to identify DNA in apoptotic cells:

- ▶ **Enzymatic labeling:** Cellular DNA is labeled with modified nucleotides (*e.g.*, biotin-dUTP, DIG-dUTP, fluorescein-dUTP) using exogenous enzymes (*e.g.*, terminal transferase, DNA polymerase). This labeling detects extensive DNA strand breaks. Enzymatic labeling is discussed in detail below (Section A 2.2.1 of this guide).
- ▶ **Staining with fluorochromes:** Cellular DNA is stained with fluorescent DNA-binding dyes (DNA-fluorochromes) capable of intercalating into DNA. Upon binding to DNA these dyes become highly fluorescent. Apoptotic cells are binding less dye molecules, since they characteristically lose DNA during the staining process (described in Section A 2.2.3 of this guide).

② In addition, individual cell death may be studied by assays that measure alterations in plasma membranes (alterations in the asymmetry or permeability of individual cell membranes, which occur as the membrane shrinks and becomes increasingly convoluted.) For instance, during apoptosis, phosphatidylserine translocates from the cytoplasmic side of the membrane to the extracellular side and can be detected with Annexin-V (described in Section A 2.2.2 of this guide).



▲ Figure 20: Schematic illustration of the two basic principles for detecting DNA fragmentation in single cells.