## 5.1 Viruses kill cells by different mechanisms

Welcome to the first video of this section. It deals on the detection of the biological effects caused by the viral infection. The first effect of which we'll talk about is cell death.

Cell death plays a crucial role in several diseases. But have you ever stopped to think that it is also an essential and normal phenomenon to keep the physiological balance or homeostasis? In general, cells die by **apoptosis** (also called programmed cell death) or **necrosis**.

Apoptosis is triggered by normal processes for the body and is normally a beneficial process, while necrosis is triggered by external factors, such as trauma or infectious diseases, and is harmful.

The process is different in both types of cell death. Apoptosis begins with the emergence of a few bulges in the membrane, called "blebs". The cell shrinks and there is nuclear collapse, with fragmentation of the nucleus and of the DNA, and chromatin condensation. Subsequently, the cell decomposes into the so-called apoptotic bodies that are easily phagocytosed by phagocytic cells. It is a "clean" process, in which the cells does not emit "distress signals" and there is no inflammation. On the contrary, in necrosis the plasma membrane is broken down, and metabolic changes occur that make the cell swell and break. This attracts phagocytic cells and there is swelling. If it is not controlled, it can put an end to the life of the individual. This cellular necrosis is harmful.

How to distinguish apoptosis and necrosis produced by the virus? There are many ways, but we're going to talk about two of them. The first is using two fluorescent reagents. One of them, green, which is called annexin, adheres to certain molecules that are only on the surface of the membranes of apoptotic cells. The other, yellow-orange, propidium iodide, penetrates the damaged cell membranes to be intercalated between the two strands of DNA. After adding both reagents to cells, they are analysed with the flow cytometer that we have already seen, which counts the cells. It distributes them in the four quadrants of a graph depending on the intensity of green and orange. In this way, living cells are not stained with any of the two dyes and they are placed in quadrant A. At the beginning of apoptosis the cells are stained with Annexin but they do not let propidium iodide pass, and they are placed in the quadrant B. In the final stages of apoptosis this reagent can penetrate, and the cells are stained both orange and green, and they are placed in the quadrant C. Lastly, necrotic cells are not stained by Annexin but they are by propidium iodide, and are classified in the quadrant D. The flow cytometer counts the number of cells there are in each quadrant, and it calculates its percentage of the total. This way we can determine what predominates: apoptosis or necrosis. In the additional information there are exercises for you to solve.

The other system to detect apoptosis is through a technique called TUNNEL. We have already mentioned that during apoptosis the DNA is cut, generating many fragments. An enzyme called TdT adds dNTPs to the end of the DNA fragments to try to repair it. Well, if we add dUTP marked with some other molecule to the medium where the cells are, this is incorporated into the new DNA strand. Subsequently, the reaction is evaluated depending on the labelling that we have used for the dUTP. In this way, we can see the fragments of DNA inside the nuclei of the apoptotic cells. The blue color that you see in this picture is due to another reagent which is added to view the nuclei themselves.

We hope that it has become clear with this video, that there are two types of cell death: a physiological, natural and scheduled one, called apoptosis; and one that can be detrimental to the organism, called cellular necrosis. It is important to differentiate one from the other to assess the pathogenic mechanisms of viruses.

Thank you very much for your attention. Oh, and remember to do the exercises.