Technical tips
Session 6

Synchronization with double-thymidine treatment: Cells in a culture are usually at different stages of the cell cycle (unsynchronized). The addition of nitrogen base in excess, such as thymidine, blocks DNA replication. Eventually, all the cells will reach this same point of the cell cycle and stop on it.

Radio-resistant DNA synthesis: any condition that make the cells insensitive to radiation so the DNA repair machinery cannot respond by stopping DNA replication in order to repair the damaged DNA.

Reverse transcriptases: Enzymes that function as a RNA-dependent DNA polymerase. They are encoded by retroviruses, where they copy the viral RNA genome into DNA prior to its integration into host cells.
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Reverse transcriptases have two activities:

- **DNA polymerase activity:** In the retroviral life cycle, reverse transcriptase copies only RNA, but, as used in the laboratory, it will transcribe both single-stranded RNA and single-stranded DNA templates with essentially equivalent efficiency. In both cases, an RNA or DNA primer is required to initiate synthesis.

- **RNase H activity:** RNase H is a ribonuclease that degrades the RNA from RNA-DNA hybrids, such as are formed during reverse transcription of an RNA template. This enzyme functions as both an endonuclease and exonuclease in hydrolyzing its target.

All retroviruses have a reverse transcriptase, but the enzymes that are available commercially are derived from one of two retroviruses, either by purification from the virus or expression in E. coli:

- **Moloney murine leukemia virus**: a single polypeptide
- **Avian myeloblastosis virus**: composed of two peptide chains

Both enzymes have the same fundamental activities, but differ in a number of characteristics, including temperature and pH optima. Most importantly, the murine leukemia virus enzyme has very weak RNase H activity compared to the avian myeloblastosis enzyme, which makes it the clear choice when trying to synthesize complementary DNAs for long messenger RNAs.

Reverse transcriptase is used, as you might expect, to copy RNA into DNA. This task is integral to cloning complementary DNAs (cDNAs), which are DNA copies of mature messenger RNAs. Cloning cDNAs is discussed elsewhere in more depth, but the technique is usually initiated by mixing short (12-18 base) polymers of thymidine (oligo dT) with messenger RNA such that they anneal to the RNA's polyadenylate tail. Reverse transcriptase is then added and uses the oligo dT as a primer to synthesize so-called first-strand cDNA.

Another common use for reverse transcriptase is to generate DNA copies of RNAs prior to amplifying that DNA by polymerase chain reaction (PCR). Reverse transcription PCR, usually called simply RTPCR, is a useful tool for such things as cloning cDNAs, diagnosing microbial diseases rapidly and a myriad of other applications. In most cases, standard preparations of reverse transcriptase are used for RTPCR, but mutated forms with relatively high thermal stability have been developed to facilitate the process.

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Lactacystin: Antibiotic that covalently modifies all the proteasome's catalytic β-subunits. Lactacystin and its more potent derivative **beta-lactone** inhibit proteolysis.

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Gleason score: Is a system of grading prostate cancer. The Gleason grading system assigns a grade to each of the two largest areas of cancer in the tissue samples. Grades range from 1 to 5 (see illustration below), with 1 being the least aggressive and 5 the most aggressive. Grade 3 tumors, for example, seldom have metastases, but metastases are common with grade 4 or grade 5.

The two grades are then added together to produce a Gleason score. A score of 2 to 4 is considered low grade; 5 through 7, intermediate grade; and 8 through 10, high grade. A tumor with a low Gleason score typically grows slowly enough that it may not pose a significant threat to the patient in his lifetime.

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