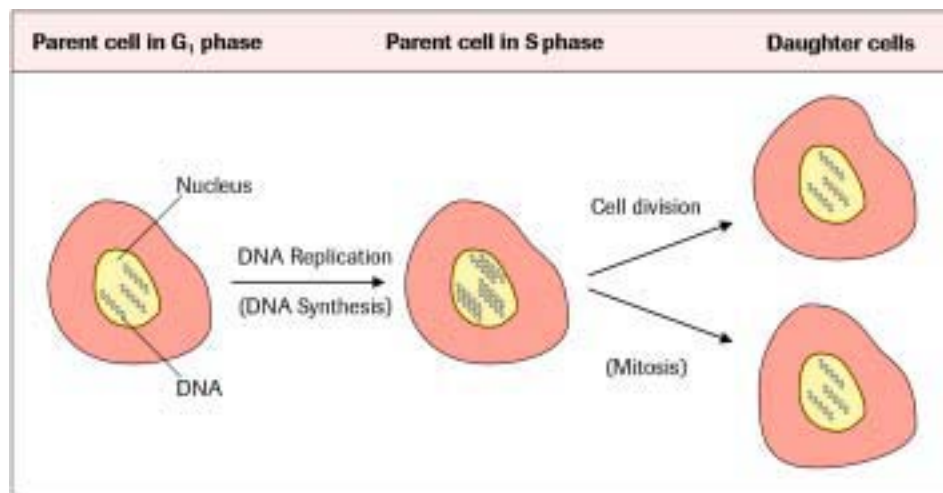


### 2.1.2 Assays that measure DNA synthesis

During cell proliferation the DNA has to be replicated before the cell is divided into two daughter cells.

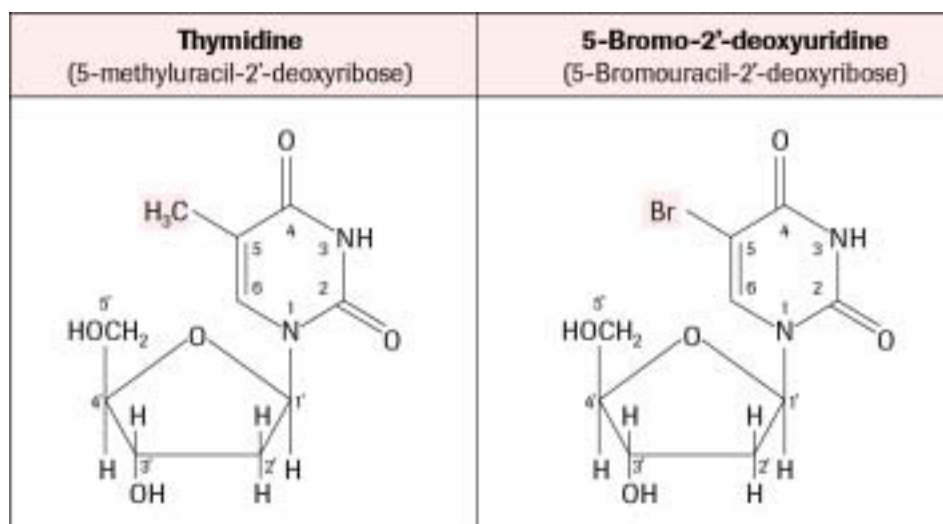
This close association between DNA synthesis and cell doubling (Figure 60) makes the measurement of DNA synthesis very attractive for assessing cell proliferation. If labeled DNA precursors are added to the cell culture, cells that are about to divide incorporate the labeled nucleotide into their DNA. Traditionally, those assays involve the use of radiolabeled nucleosides, particularly tritiated thymidine ( $[^3\text{H}]\text{-TdR}$ ). The amount of  $[^3\text{H}]\text{-TdR}$  incorporated into the cellular DNA is quantitated by liquid scintillation counting (LSC)<sup>63, 70</sup>.



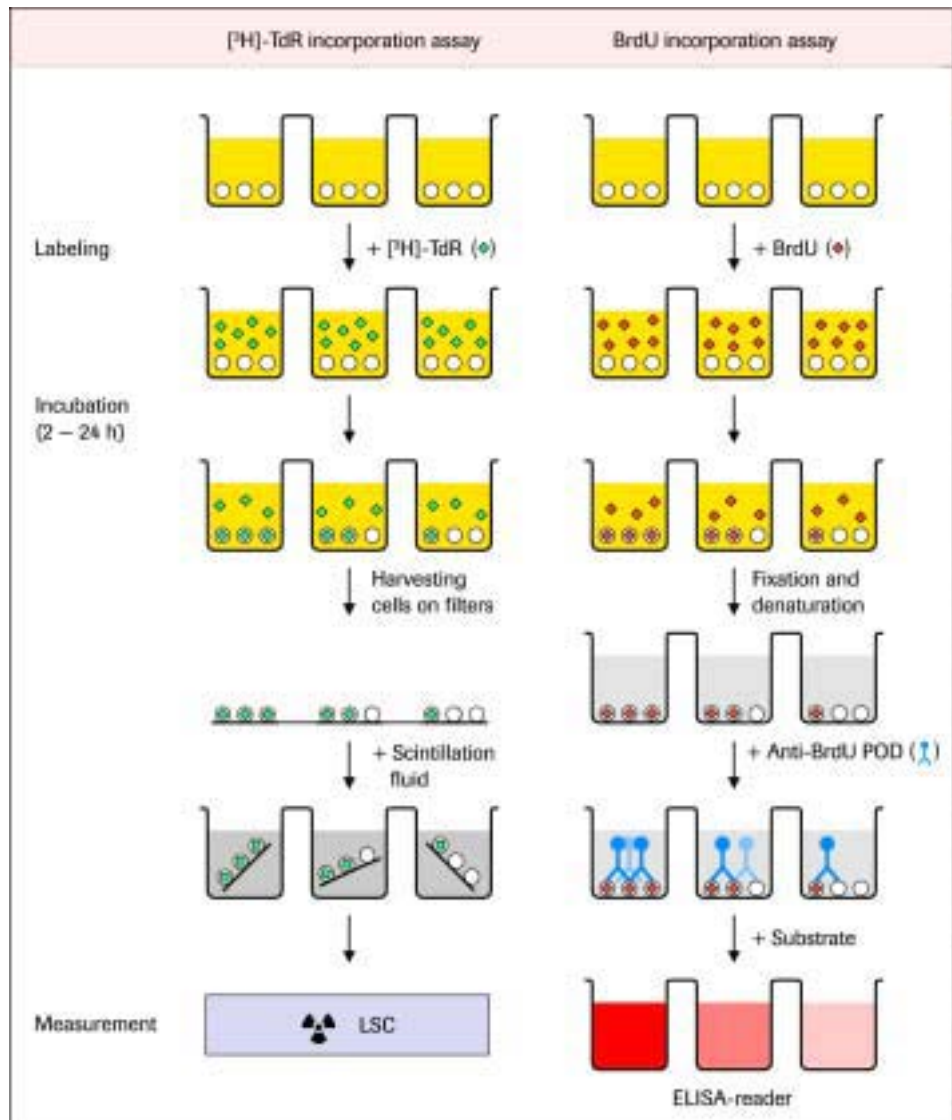
▲ Figure 60: Cell proliferation, a close association between DNA synthesis and cell doubling.

Experiments have shown that the thymidine analogue 5-bromo-2'-deoxy-uridine (BrdU) is incorporated into cellular DNA like thymidine (Figure 61). The incorporated BrdU could be detected by a quantitative cellular enzyme immunoassay using monoclonal antibodies directed against BrdU<sup>64</sup>. The use of BrdU for such proliferation assays circumvents the disadvantages associated with the radioactive compound  $[^3\text{H}]\text{-TdR}$ .

The first report of this technique involved the extraction and partial purification of DNA from BrdU-labeled proliferating cells, followed by an enzyme immunoassay in a separate assay<sup>71</sup>. Because this method was relatively laborious, the entire BrdU-based procedure was adapted to a 96 well microplate<sup>72</sup>. This adaptation required no harvesting of the cells; the complete assay from the start of the microculture to data analysis by an ELISA plate reader was performed in the same microplate (Figure 62).



▲ Figure 61: Molecular structure of thymidine and BrdU.



▲ Figure 62: Measurement of DNA synthesis using modified nucleotides [<sup>3</sup>H]-TdR and BrdU.

Roche Applied Science offers three kits that use the convenient BrdU-based assay and the microplate format. The BrdU Labeling and Detection Kit III is a first generation assay. The colorimetric and chemiluminescence Cell Proliferation ELISAs, are second generation assays that offer fewer steps, a faster assay, and greater sensitivity than the first generation assay (Table 14). These three kits are described on the following pages.

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