Measuring the uptake of neutral red by a cell culture after incubation with a candidate compound will provide insight into the candidate compound's impact on cell viability. Compounds that are cytotoxic will decrease the absorption of neutral red as compared to cells grown in the absence of the compounds.

Although each of these assays can be used to identify cytotoxic compounds at a very early stage of a program, they do not provide information on the biochemical causes of the cytotoxicity. In order to determine the root cause, additional studies would be required. The value of gaining an understanding of the mechanistic underpinnings of cytotoxicity of a particular candidate compound should be carefully assessed.

CARCINOGENICITY, GENOTOXICITY, AND MUTAGENICITY

The interrelated concepts of carcinogenicity,[23] genotoxicity,[24] and mutagenicity[25] are exceptionally important in the identification of novel therapeutics. In general, carcinogenic compounds cause cancer through a variety of different mechanisms such as alteration in cellular metabolism or DNA damage that cause uncontrolled proliferation of malignant cells. Compounds that are genotoxic damage the genetic information within a cell. Changes to DNA created by genotoxic compounds can be in the form of single strand DNA breaks, double stranded DNA breaks, or mutation of the DNA. In some cases, genotoxicity leads to apopotosis (programmed cell death), but it can also lead to the formation of malignant cells (cancer). Mutagenicity is a subset of genotoxicity in which the DNA is mutated. In this case, damage caused by a genotoxin is improperly repaired, permanently altering the DNA. Compounds with these properties represent a potential risk to patient health. While there are some marketed drugs that possess these attributes, modern drug discovery programs actively eliminate suspect candidates through a variety of *in vitro* screening methods.

The Ames assay is one of the most widely employed methods of identifying potential carcinogenic compounds (Figure 8.10). Originally described by Bruce Ames in the early 1970s,[26] this test is specifically designed to identify compounds that have mutagenic properties. Although not all mutagens are carcinogenic, a positive signal in an Ames assay is viewed as an indication of high risk. Compounds that are "Ames positive" are rarely progressed further. In practice, the assay monitors the growth of a specially designed bacterial strain, usually *Salmonella typhimurium*, which cannot grow in the absence of histidine. Mutations in the genes that control the histidine synthesis prevent the bacteria from producing histidine on their own, so they will only grow when histidine is added to the growth media. The bacteria are grown in the presence of a test compound and a limited supply of histidinde. Once the histidine supply