in the metaphase portion of the cell cycle. The observation of gross changes in chromosomal structure serves as an indicator that significant DNA damage/mutation has occurred and deleterious consequences are likely (e.g., cancer, genetic diseases). In practice, a cell culture is incubated for 3 h with a candidate compound and then a compound capable of arresting the cell cycle at metaphase (e.g., colcemid) is added. At this stage of mitosis, the condensed and highly coiled chromosomes are aligned in the middle of the cell. The cells are then fixed and microscopically examined to assess for chromosomal aberrations. Compounds that form metabolites capable of causing chromosomal aberrations can also be identified by performing the experiments in the presence of rat liver extract (specifically the S9 fraction).

As discussed above, some genotoxic compounds damage DNA by causing strand breaks. Compounds that cause this kind of DNA damage can be identified using a single-cell gel electrophoresis (SCGE) method referred to as the Comet assay (Figure 8.13).[29] Originally developed by

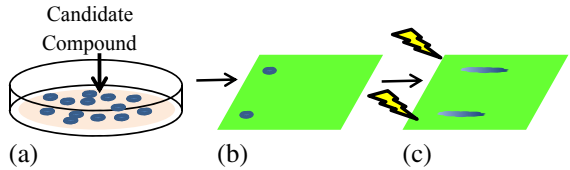

FIGURE 8.13 The Comet assay can detect compounds that cause DNA strand breaks. (a) Cells are incubated with a candidate compound for a defined time period. (b) A single cell is embedded in a gel electrophoresis matrix (agarose matrix) lysed, and then (c) an electric field is applied across the gel. If the candidate compound causes DNA strand breaks, staining of the gel upon completion of the experiment will produce a comet-shaped image as a result of differential rates of migration of the full DNA as compared to the DNA fragments produced by strand breaks.

Peter Cook,[30] this assay takes advantage of differences in migration rates of DNA strands in an electrophoresis gel. DNA fragments created by strand breaks and relaxed chromatin migrate through electrophoresis gel at a faster rate as compared to unchanged DNA. Upon staining and visualization, compounds that are capable of inducing DNA strand breaks will produce an image that resembles the tailing of a comet, which is the rational for the name of the assay. Typically, a cell culture is treated with a test compound for a sustained time period, and then single cells are embedded in an agarose matrix on a microscope slide. Lysis of the cells under mildly basic conditions releases the DNA and this is followed by the application of an electric field across the gel. This causes the released DNA to migrate through the gel at a rate based on their size. The presence of a comet shape upon visualization indicates a positive result in the Comet assay and suggests that the compound in question has genotoxic properties.