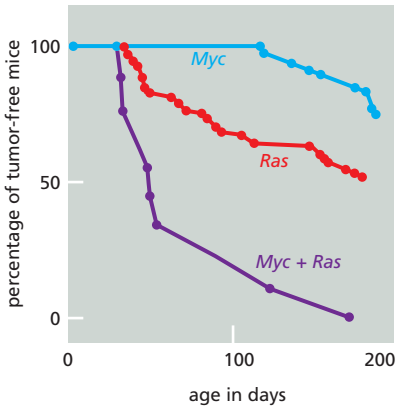

Figure 20–29 Oncogene collaboration in transgenic mice. The graphs show the incidence of tumors in three types of transgenic mouse strains, one carrying a *Myc* oncogene, one carrying a *Ras* oncogene, and one carrying both oncogenes. For these experiments, two lines of transgenic mice were first generated. One carries an inserted copy of an oncogene created by fusing the proto-oncogene *Myc* with the mouse mammary tumor virus regulatory DNA (which then drives *Myc* overexpression in the mammary gland). The other line carries an inserted copy of the *Ras* oncogene under control of the same regulatory element. Both strains of mice develop tumors much more frequently than normal, most often in the mammary or salivary glands. Mice that carry both oncogenes together are obtained by crossing the two strains. These hybrids develop tumors at a far higher rate still, much greater than the sum of the rates for the two oncogenes separately. Nevertheless, the tumors arise only after a delay and only from a small proportion of the cells in the tissues where the two genes are expressed. Further accidental changes, in addition to the two oncogenes, are apparently required for the development of cancer. (After E. Sinn et al., *Cell* 49:465–475, 1987. With permission from Elsevier.)

Transgenic mouse studies confirm, for example, that a single oncogene is generally not enough to turn a normal cell into a cancer cell. Thus, in mice engineered to express a *Myc* or *Ras* oncogenic transgene, some of the tissues that express the oncogene may show enhanced cell proliferation, and, over time, occasional cells will undergo further changes to give rise to cancers. Most cells expressing the oncogene, however, do not give rise to cancers. Nevertheless, from the point of view of the whole animal, the inherited oncogene is a serious menace because it creates a high risk that a cancer will arise somewhere in the body. Mice that express both *Myc* and *Ras* oncogenes (bred by mating a transgenic mouse carrying a *Myc* oncogene with one carrying a *Ras* oncogene) develop cancers earlier and at a much higher rate than either parental strain (**Figure 20–29**); but, again, the cancers originate as scattered, isolated tumors among noncancerous cells. Thus, even cells expressing these two oncogenes must undergo further, randomly generated changes to become cancerous. This strongly suggests that multiple mutations are required for tumorigenesis, as supported by a great deal of other evidence discussed earlier. Experiments using mice with deletions of tumor suppressor genes lead to similar conclusions.

Cancers Become More and More Heterogeneous as They Progress

From simple histology, looking at stained tissue sections, it is clear that some tumors contain distinct sectors, all clearly cancerous, but differing in appearance because they differ genetically: the cancer cell population is heterogeneous. Evidently, within the initial clone of cancerous cells, additional mutations have arisen and thrived, creating diverse subclones. Today, the ability to analyze cancer genomes lets us look much deeper into the process.

One approach involves taking samples from different regions of a primary tumor and from the metastases that it has spawned. With modern methods, it is even possible to take representative single cells and analyze their genomes. Such studies reveal a classic picture of Darwinian evolution, occurring on a time scale of months or years rather than millions of years, but governed by the same rules of natural selection (**Figure 20–30**).

One such investigation compared the genomes of 100 individual cells from different regions of a primary tumor of the breast. A large fraction—just over half—of the chosen cells was genetically normal or nearly so: these were connective-tissue cells and other cell types, such as those of the immune system, that were mixed up with the cancer cells. The cancer cells themselves were distinguished by their severely disrupted genomes. The detailed pattern of gene deletions and amplifications in each such cell revealed how closely it was related to the others, and from this data one could draw up a family tree (Figure 20–30B). In this case, three main branches of the tree were seen; that is, the cancer consisted of three major