The sum of the genetic construction of an organism constitutes its *genotype*. The characteristics expressed by a cell constitute its *phenotype*. The phenotypic response of a culture may change reversibly with alterations in environmental conditions, whereas the genotype is constant irrespective of the environment. A *mutation* is a genotypic change and is irreversible. A whole culture undergoes a phenotypic response, whereas only a rare individual will undergo a genotypic change. For example, if a culture changes in color from white to green when oxygen levels fall and then changes from green to white upon an increase in dissolved oxygen, the change would be phenotypic. Now consider an alternative experiment where white cells were removed from a culture and placed on a plate (a small circular dish filled with nutrients solidified with agar) and allowed to grow into separate colonies. If one colony, but not the others, turned green and if cells obtained from the green colony remain green when cultured under the original conditions, it would be evidence for a genotypic change. In this case, the white cells would be the *wild type* and the green cells the *mutants*.

Let us consider what mechanisms may lead to genotypic change.

8.2.1. How Mutations Occur

Most mutations occur due to mistakes in DNA synthesis. Some examples are shown in Fig. 8.1. One common form is a *point mutation*. A point mutation results from the change of a single base (for example, cytosine instead of thymine). Some point mutations are *silent mutations* because the altered codon still codes for the same amino acid (e.g., UCU and UCA both code for serine). Even if the point mutation causes the substitution of a different amino acid, it may or may not alter protein activity substantially. A change of amino acid near the active site might alter protein activity greatly, whereas the same substitution at another site might not be very critical.

One type of point mutation that usually has a profound effect results in a nonsense or stop codon (e.g., CAA for glutamine to UAA for stop on the *m*-RNA from the altered DNA). This results in the premature termination of translation and an incompletely formed protein.

Generally, *deletion mutations* have profound effects on cellular metabolism. By deleting or adding one or more bases, we can alter the whole composition of a protein, not just a single amino acid. A deletion can shift the *reading frame* when translating the resulting *m*-RNA. This is illustrated in Fig. 8.2.

Additions often take place through *insertion elements* (IS). These elements are about 700 to 1400 base pairs in length; in *E. coli* about five different IS sequences are known and are present on the chromosome. These elements can move on the chromosome from essentially any one site to another. Often they will insert in the middle of a gene, totally destroying its function.

Back mutations or *reversions* are possible. Revertants are cells for which the original wild-type phenotype has been restored. Restoration of a function can occur due to a direct change at the original mutation (e.g., if the original mutation was CAA to UAA, then a second mutation for UAA to CAA restores the original genotype and phenotype). Second-site revertants can occur that restore phenotype (*suppressor mutations*), but not genotype (e.g., a second deletion mutation that restores the gene to the normal reading frame or a mutation in another gene that restores the wild-type phenotype).