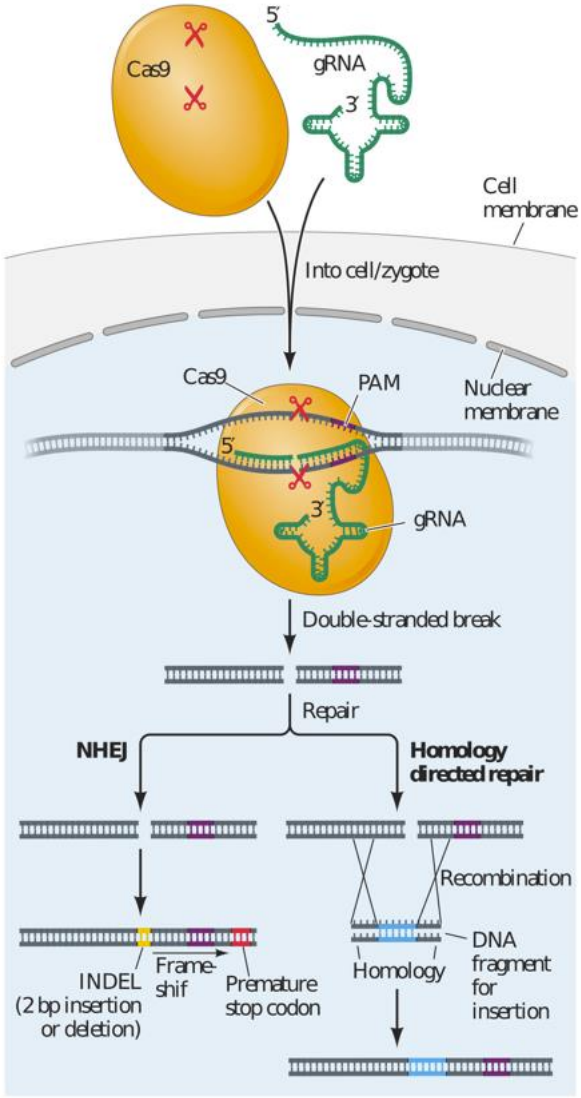
<FURTHER DEVELOPMENT 3.24>

CRISPR/CAS9 Genome Editing



CRISPR/Cas9-mediated gene editing. The CRISPR/Cas9 system is used to cause targeted indel formation or insertional mutagenesis within a gene of interest. A gene-specific guide RNA (gRNA) is designed and introduced into cells together with the nuclease Cas9, for instance by co-injection into a newly fertilized zygote. The gRNA will bind to the genome with complementarity and will recruit Cas9 to this same location to induce a double-stranded break. Non-homologous end joining (NHEJ) is the cell's DNA repair mechanism that often results in small insertions or deletions (approximately 2–30 base pairs; a 2 base-pair insertion is illustrated here), which can cause the establishment of a premature stop codon and potential loss of the protein's function. In addition, plasmids carrying insertions with homology to regions surrounding the gRNA target sites are used to insert known sequences at the double-stranded break. Such methods are being explored as a way to repair mutations. PAM, protospacer adjacent motif.