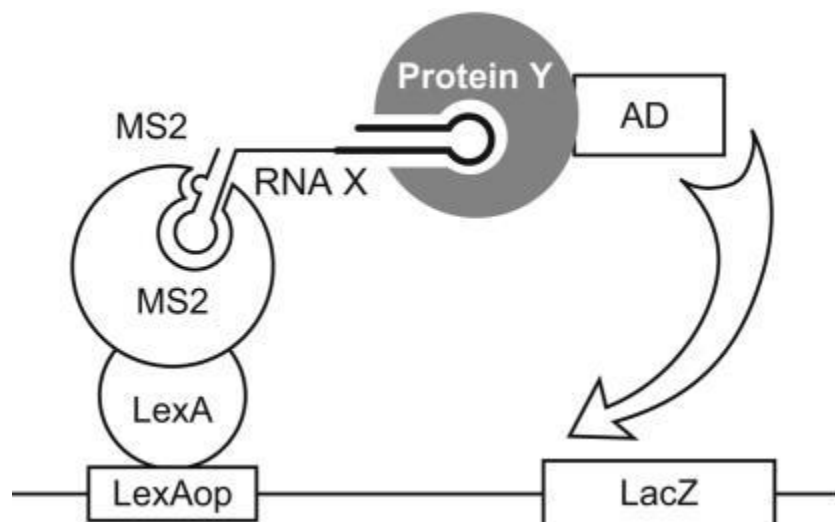


YEAST THREE-HYBRID SYSTEM

The yeast three-hybrid system has become a useful tool in analyzing RNA–protein interactions. An RNA sequence is tested in combination with an RNA-binding protein linked to a transcription activation domain (AD). A productive RNA–protein interaction activates a reporter gene in vivo. In the yeast three-hybrid system, a chimeric protein containing both a DNA- and RNA-binding domain tethers an RNA to the promoter of a reporter gene. Typically, this protein consists of a LexA/MS2 coat protein fusion. A hybrid RNA binds to the MS2 portion via tandem MS2-binding sites. The RNA also contains a sequence of interest, X, which binds to an RNA-binding polypeptide, Y. Y is linked to a transcription activation domain (AD). When the requisite interactions occur, the reporter gene is activated. Typically, the interaction between RNA X and protein Y is monitored by assaying *HIS3* and *LacZ* expression levels.



The concept and the components of the yeast three-hybrid system are shown schematically in Fig. 2. This system utilizes three hybrid molecules to reconstitute transcription of reporter genes in a manner similar to the two-hybrid system. The three hybrid molecules consist of a hybrid fusion protein designated the “hook,” a second hybrid fusion protein designated the “fish,” and a synthetic hybrid ligand designated the “bait.” The hybrid ligand is a covalently linked heterodimer of two small ligands, A and B. If ligand A binds to its receptor fused to a DNA-binding domain (i.e., the “hook”) and ligand B binds to its receptor fused to a transcriptional activation domain (i.e., the “fish”), reporter genes will be activated.

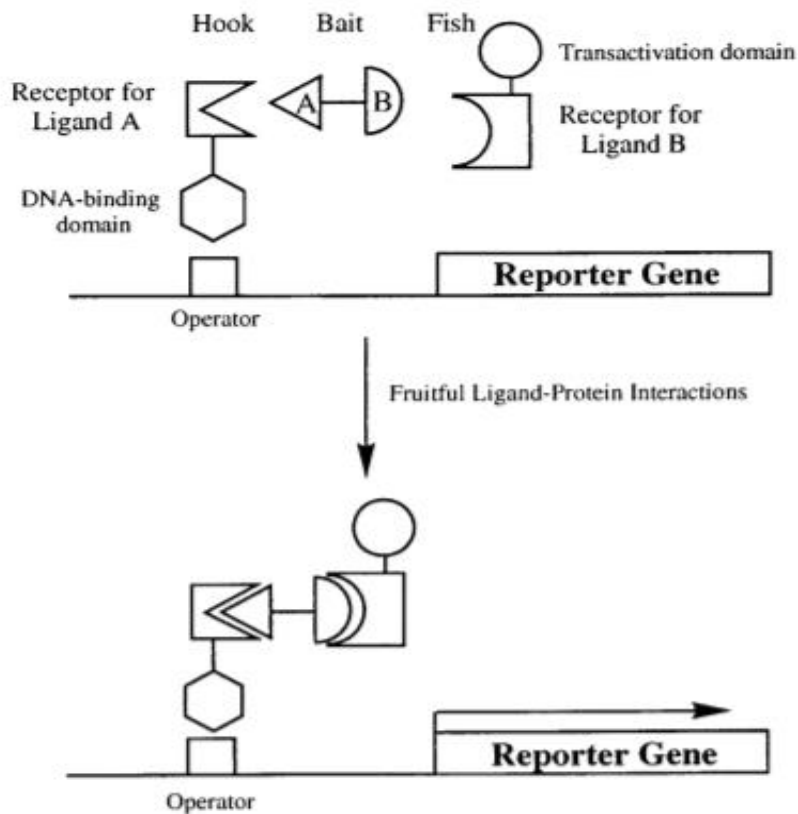


FIG. 2. Diagrammatic representation of the components of the yeast three-hybrid system. The synthetic "bait" hybrid ligand consists of ligand A (triangle) and ligand B (semicircle) connected by a linker. The "hook" fusion protein consists of the receptor for ligand A fused to the DNA-binding domain of a transcription factor. The "fish" fusion protein consists of the receptor for ligand B fused to a transactivation domain of a transcription factor. The relative sizes of the three hybrid molecules are not drawn to scale. The "hook," the "bait," and the "fish" form a trimeric complex, and the resulting proximity of the transactivation domain and the DNA-binding domain leads to activation of the reporter gene.