

The above diagram is a close up of the active site. The two circles in the centre are magnesium ions and magnesium ions are critical for this step to take place. Whilst one metal ion binds both the dNTP and the 3'-hydroxyl group of the primer, the other interacts only with the dNTP. These two metal ions are bridged by the carboxylate groups of 2 aspartate residues in the palm domain of the polymerase, which hold the metal ions in the right proximity and orientation to the substrate. The metal ion bound to the primer activates the 3'-hydroxyl group of the primer, facilitating its attack on the α -photohate group of the dNTP substrate in the active site. In addition, the two metal ions of gether help stabilize the negative charge that accumulates on the penterodicate transition state.

Step 5: a second conformational change occurs by which me pyrophosphate product s released. This conformational change is thought to be the reverse of the first one.

What follows is the dissociation of he couplex (distributive synthesis) or translocation of the substrate to form a new 3' terminus for a new round of incorporation (processive synthesis).