



- a. The receptor can bind to the DNA element it recognizes and activate transcription.
- b. Movie shows that the receptor fused to GFP translocates into the nucleus within seconds after ligand is added to the cell.

A. TFII H distribution on polytene chromosomes

1. Lis lab in Cornell MBG department studies heat-shock genes. These are conserved genes and are useful for studying transcription.
2. Polytene chromosomes in *Drosophila* show characteristic banding patterns using simple phase microscopy.
3. When cells are heat shocked, the loci of heat shock genes will puff up on the polytene chromosomes. This puff is caused by activated transcription in the region.
4. An antibody to TFII H binds at the puff after the heat shock.
5. TFII H has lots of proteins and one is a kinase; it phosphorylates the CTD of RNA Pol II allowing it to transcribe the gene.

B. Protein-protein interactions

1. Mediator is an important protein to mediate signal between chromatin activator and general transcription machinery
2. Mediator is composed of 20 polypeptides.
3. How can we study protein-protein interactions in such a large complex?
4. Isolate Protein X and see what else is present.
5. Fuse Protein X to a small protein that binds a ligand present on a column. These are called GST (glutathione-s-transferase) tagging experiments or GST-pull down assays.
6. Yeast 2-hybrid analysis (Fig 11-39)
 - a. Remember that Gal4 activator has a DNA-binding domain (DBD) and an activation domain (AD).
 - b. Take Gal4-DNA binding domain and attach it to protein X, the protein of which one would like to find all its binding partners.
 - c. Make a library of proteins fused to an activation domain.
 - d. Rig yeast cell so it has a reporter gene with a UAS present upstream.
 - e. If protein X can interact with another protein in the library, then the DNA-binding domain will come in close contact to the activator domain, and transcription can be activated. The Gal4-DBD will bind to the yeast UAS.
 - f. Sometimes this assay reports transcription when the two proteins have just a chance interaction. There are a lot of false positives obtained with this approach.
 - g. Movie shows “bait” protein fused to the DBD and “fish” protein fused to AD. Any fish binding to the bait will activate transcription.