Chromatine Immunoprecipitation (ChIP): This is a powerful in vivo method to quantitate interaction of proteins associated with specific regions of the genome. It involves the immunoprecipitation of protein/DNA complexes that have been stabilized via cross-linking. Intact cells are fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. DNA is then sonicated into small uniform fragments and the DNA/protein complexes are immunoprecipitated using an antibody directed against the DNA-binding protein of interest. Following immunoprecipitation, cross-linking is reversed, proteins are removed by Proteinase K treatment and the DNA is rapidly cleaned up using DNA purification columns. The DNA is then screened to determine which genes/sequences were bound by the protein of interest. The screening can be done using generalized hybridization (Southern blot; it's a very similar technique to that for northern blot -see tech tips for session 3- but it detects DNA instead of RNA) or a more targeted PCR-based approach (you can use specific oligos to amplify a sequence of interest; if that sequence is present in the ChIP sample you will have a PCR product).

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GST/glutathion and His-tag/Niquel-NTA pull-down assays: This method is used when you want to check whether two proteins interact. One of the proteins ('bait' protein) is tagged with GST ("Glutathione S Transferase"), so it has a high affinity for glutathione. In order to tag it with GST you clone its cDNA into a plasmid that will generate a fusion of the protein to GST.

The expression is usually carried out in E. coli. To isolate the bait protein from a cell lysate after expressing it, you incubate the lysate with glutathione cross-linked to agarose ("GST-beads"). This will bind up your protein. You can elute it with excess glutathione and use the purified bait protein in an in vitro binding assay (what is called GST-pull down) to detect protein-protein interactions. This time the bait protein will be put in the presence of another cell lysate (this one now coming from cells of the original organism to which both proteins -the 'bait' and the 'prey' protein- actually belong). Only proteins interacting with the GST-labeled protein ('prey' proteins) will be precipitated when you add again GST-beads and pellet them to separate them from the extract. All samples are then analyzed by SDS-PAGE followed by Western blot (you check the presence of the prey protein in the pull-down with specific antibodies for it and/or because it has a different tag, such as –HA, -myc, etc). A sample of the total lysate containing the prey protein is sometimes utilized as a positive control (demonstrating that this prey protein is actually present in the extract). Others add the purified bait protein as positive control. This is usually called 'input' or 'total'. A negative control results from the exposure of the lysate to GST-beads alone (without bait protein) and the experiment really works if you don't see the prey protein pulled down by the beads alone. Finally, you should see the prey protein "pulled-down" from the lysate by the GST-beads in the presence of the GST-labeled protein or bait (that is indicative that both proteins interact and that's why one can pull down the other).

A variant of this method uses nickel beads (Ni^{2+}-NTA) when the bait protein is tagged with 6X Histidine instead of GST. The rest is essentially the same as above. Ni^{2+}-NTA beads are agarose beads that contain strongly magnetic particles and have metal-chelating nitriloacetic acid (NTA). The beads are precharged with nickel. This way they bind with high specificity, to 6Xhis-tagged proteins. The beads can be separated by using a magnet apparatus. The application of a magnetic field will cause the beads to be held on the sides of the wells/tubes while buffers are exchanged to wash or elute the 6xHis-tagged proteins.

General 'pull-down' technique

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Different types of magnet apparatus

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α-Amanitin: *alpha*-Amanitin is the major toxin of the extremely poisonous toadstools, *Amanita phalloides* (death cap), *A. verna* (destroying angel), *A. virosa*, *A. ocreata*, *A. tenuifolia* and other Amanita species (beware of some mushrooms!). Its primary action is to inhibit RNA polymerase II (in turn interfering with mRNA synthesis). This results in the arrest of protein synthesis and cellular necrosis ultimately leading to severe acute hepatitis (among other symptoms).

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Cisplatin: Cisplatin is believed to kill cancer cells by binding to DNA and interfering with its repair mechanism, eventually leading to cell death. The first step in the process (after the cisplatin molecule penetrates the cell membrane intact) is for a molecule of water to replace one of the chloride ions. The resulting structure can then bind to a single nitrogen on a DNA nucleotide. Then, the second chloride is replaced by another H_2O and the platinum binds to a second nucleotide. Binding studies of cisplatin with DNA have indicated a preference for nitrogen 7 on two adjacent guanines on the same strand. It also binds to adenine and across strands to a lesser extent.

The cisplatin-DNA complex attracts the attention of HMG (high mobility group)-1 and other DNA repair proteins which become irreversibly bound. The resulting distortion to the shape of the DNA prevents effective repair. (The trans isomer of cisplatin is unable to form 1,2 intrastrand links and lacks antineoplastic activity.)

Other antineoplastic agents, such as etoposide, contribute to the platinum-DNA-protein complex and thus synergistically reinforce the activity of cisplatin.

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UREA-PAGE for nucleic acids separation: This is a method to separate molecules of single-stranded nucleic acids such as DNA (ssDNA) according to their size. Urea at high concentration is used to denature double-stranded DNA (dsDNA). The nucleic acids are separated in a polyacrylamide gel but in this case no SDS is added to the samples because the phosphodiester backbone has a negative charge which is proportional exactly to the length of the molecule. Polycrylamide is much better than agarose to both visualize and separate very small fragments of DNA (<100 nucleotides). Sequencing gels are always polyacrylamide gels.