BIO 5099: Molecular Biology for Computer Scientists (et al)

Lecture 25: Biotechnology

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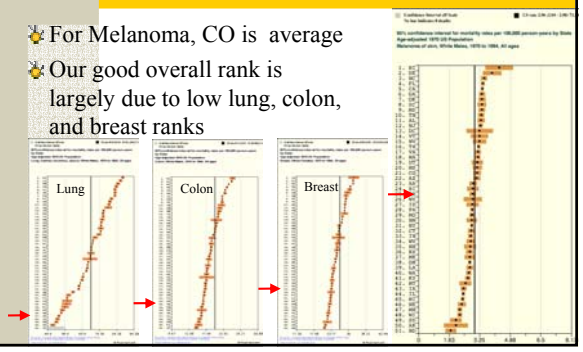
Cancer rates in Colorado

- For Melanoma, CO is average
- Our good overall rank is largely due to low lung, colon, and breast ranks



Catching cancer?

- I mentioned early in the course that some cancers arise as the result of viral infections
  - Chronic Hepatitis B infections cause ~60% of liver cancer. Infecteds have 100x increased risk. Get a HepB vaccine!
- Good news today about a vaccine for another one of these: human papilloma virus (HPV)
  - Clinical trial demonstrated that the vaccine prevents the HPV infection that causes ~ 50% of cervical cancer
  - When it becomes available (not for several years), it will apply to other strains of the virus, protecting against about 70% of cervical cancers. (Also, immunity to genital warts)

Molecular Biotechnology

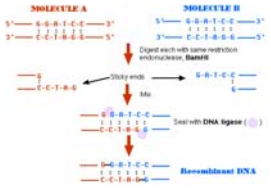
- Genetic Engineering
  - Adding genes to organisms: recombinant DNA
  - Removing genes (or preventing their expression): knockouts and RNAi
  - Challenges and uncertainties in genetic engineering
- Pharmacology: Inventing drugs
- Biological molecules as generic tools
  - Antibodies
  - Evolving enzymes

Adding Genes to Organisms

- *Recombinant* DNA is the product of combining two (or more) separate sources of DNA
  - Usually, the insertion of a foreign gene into an organism.
    - Human insulin in E. coli for drug production
    - Herbicide resistance into crop plants.
- To be successful, a genetic engineer must
  - Insert a coding sequence into a place in the DNA where it will be properly expressed
  - Place the recombinant DNA into an organism so that it is taken up and reproduced along with the rest of the organism's genome.

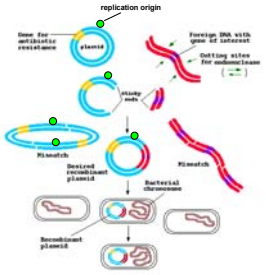
Gene Splicing

- *Restriction endonucleases* cut DNA at particular sequences, leaving "sticky ends"
  - There are hundreds of different restriction enzymes
  - Ensure the cut target and the ends of the to-be-inserted sequence are complementary.
- The sticky ends hybridize, and the nicked molecule is repaired by a DNA ligase.



Splicing a gene into a plasmid

- Recall that *plasmids* are small, circular DNAs in bacteria, separate from the chromosome
  - They are widely used as recombinant DNA vectors. (A *vector* is a way of getting foreign DNA into a cell).
  - Plasmid must have a single origin of replication and the foreign gene(s)
  - Use $CaCl_2$ **to make cells take up the plasmid**



Selecting transformed cells

- Lots of places where things might not work:
  - Plasmid (or foreign gene) hybridizes to itself, or in combinations that are not desired.
  - Only 1 in 10,000 cells take up a plasmid at all
- Need a way to identify the clones (cell lines with identical genomes) that were *transformed*
- *So, also splice in a selectable gene (often antibiotic resistance) into the vector, so we can tell if the foreign gene is being expressed.*
  - *Apply antibiotic, and only cells that took up a properly constituted plasmid will live.*

Splicing a gene into Eukaryotic DNA

- Need different vector to get into genomic DNA
  - Restriction enzymes cut in too many places, so can't use
  - Homologous recombination works for small changes
  - Injecting the gene into a fertilized egg occasionally works
  - Viral vectors (especially retroviridae)
  - Transposon-based vectors (very promising)
- Must control the location of the gene
  - Can't disrupt existing genes
  - Needs to have proper regulatory sequences (& reporters)
- Still challenging, especially in mammals

Gene Knockouts

- Now possible to reliably remove a gene from an organism, called a *knockout*
  - Homologous recombination with nonfunctional version
  - Done to embryonic stem cells in mice, cells are implanted after selection for transformation effectiveness
- More challenging, although sometimes successful is a tissue-specific or developmental time-specific knockout.
  - Antisense DNA, or (apparently more effective) RNA interference techniques prevent expression locally

Genetically modified plants

- Transposons are widespread in plants, and many transgenics have been created.
  - The FDA has been notified of 77 bioengineered foods. Mostly for herbicide resistance, but also adding pesticide genes from Bt (a prokaryote), and knocking out ethylene genes (to prevent ripening without artificial ethylene).
  - USDA says in 2002, 74% of soy and 32% of corn grown in the US will be modified. 2000 survey shows 44% of farms use genetically modified inputs (seeds, grain, etc.).
- GMOs are widespread in US food supply, but controversial in Europe.

Gene Therapy

- Gene therapy is the transfer of nucleic acids into humans (to improve health) by
  - Compensating for defective genes
  - Producing a therapeutic substance
  - Triggering the immune system
- Vectors are particularly difficult in humans
  - Some population of cells is transfected, then implanted
- 494 clinical trials through 9/02; few successes
  - 384 in cancer, 56 for 16 different monogenic traits
  - SCID, one of the few considered successful, now halted

SCID gene therapy

- X-linked Severe Combined Immune Deficiency (SCID; bubble-boy disease) was found to be a defect in a receptor for interleukin-7 (IL-7).
  - IL-7 is the differentiation factor for T-lymphocytes
- Blood stem cells were transfected with normal receptor using a retroviral vector
  - 9 children treated. All responded by developing normal immune responses (e.g. to immunization)
  - Last September (after 3+ years), the trial was halted when one patient developed a proliferating clone of T cells where the vector had inserted itself on chromosome 11

Cloning

- Cloning is the asexual creation of genetically identical organisms
  - Inbreeding accomplishes the same thing sexually (slowly, and with uncertain fixation of alleles).
- Clones have recently been made from adult cells in sheep, cows and mice.
  - Remove nucleus from oocyte, fuse it with an adult cell arrested in G_0 phase of cell cycle (demethylated), stimulate the cells electrically, implant in foster mother
  - Very low success rates (<0.5% for Dolly, 2-3% in mice)

Genome Projects

- Genome project: determining the nucleotide sequence of an entire genome
  - More than 800 genomes complete or in progress, including 17 completed and 180 in progress Eukaryotes
- Many surprises in genomic sequence:
  - Much of the human genome is a few repeated sequences.
  - Still trying to understand the detail of transcriptional control
- Genomic sequence makes engineering easier
  - Can identify flanking sequences to ensure specific recombination

Example Engineered Organisms

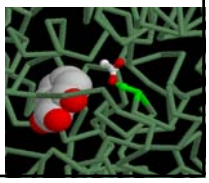
- Prokaryotes produce hundreds of recombinant human proteins, including
  - Human growth factor, insulin, the sepsis drug
  - 130 FDA approved biotechnology drugs and vaccines
- Many novel application areas
  - Environmental remediation (e.g. “oil eating bacteria)
  - Energy production (ethanol, and exotic approaches)
  - Industrial biomaterials (spider silk from goat milk...)
- Transgenic mice used widely in research

Drug Discovery & Invention

- Pharmaceuticals are (mostly) small molecules that interact with the body to improve health
- Traditional process of drug discovery was largely serendipitous (or not!)
- Recently, attempts at “rational drug design”
  - Started with solved protein structures
  - Now involves genomic sequence, gene expression arrays, high throughput chemical synthesis, bioinformatics
- Big changes in the industry, although no dramatic success stories

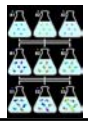
Target Selection

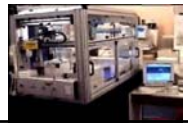
- The first step in finding a new drug is identifying a *target*: the molecule(s) in the body the drug is intended to interact with
  - *Agonist* increases the activity of the target
  - *Antagonists* (or *inhibitors*) decrease activity of the target
- Drug targets are usually membrane-bound receptors.
- Receptor subclasses are important for side effects
  - The cyclooxygenase II story



Lead Identification

- The second step in creating a drug is the identification of a compound, called a *lead*, that binds fairly strongly to the target.
  - Model the atomic structure around the binding site (*pharmacophore*), and find molecules that fit it
  - Test a large and diverse library of drug-like molecules for binding affinity, using combinatoral synthesis and robotic testing.





Optimization

- Once a lead compound is found, then many variations of it are synthesized, looking for
  - Extremely high binding affinity (drug concentrations in interstitial fluid are low)
  - Specificity (avoid side effects)
  - Delivery concerns (best if can pass through digestive tract)
- Techniques include structural studies, combinatoral chemistry and various kinds of predictive modeling.

Toxicology

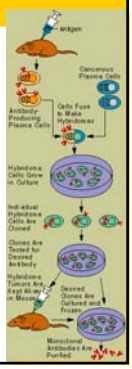
- Many compounds that would be good drugs have some kind of associated toxicity
  - Generally discovered in animals (although some computational and *in vitro* approaches now used)
- Determine the mechanism of toxicity, e.g.
  - Cross reactivity?
  - Toxic breakdown product?
- Reoptimize to avoid toxic effect.

Clinical Trials

- A drug that is effective and nontoxic in animals (preclinical studies) gets human tests
- Phase I: safety testing
  - Usually in healthy volunteers
  - Gradually increasing doses provide pharmacokinetic data
- Phase II: preliminary studies
  - First tests of effectiveness, usually in small populations
- Phase III: complete assessment
  - Gather enough data to demonstrate safety and effectiveness for the general population

Antibodies

- Antibodies can specifically recognize many molecules (and be tagged with fluorescent reporters)
- *Monocolonal antibodies are created by hybridomas:*
  - *Mouse is exposed to an antigen of interest, and plasma cells are harvested from spleen*
  - *These cells are fused with immortalized (cancerous) plasma cells*
  - *Transformed immortalized cells are tested for activity, and then produce large supplies*



Artificial Molecular Evolution

- *Selex: uses enzymatic RNA, since information and activity on the same molecule*
  - *Generate large numbers of random RNAs; select ones that have activity (e.g. binding affinity), amplify & repeat*
  - *Commercially, aptamers for protein chips*
- *Directed Evolution*
  - *Large scale mutagenesis, and artificial selection*
  - *Many specific successes, e.g. 10^5 increase in activity, changed substrates, etc.*