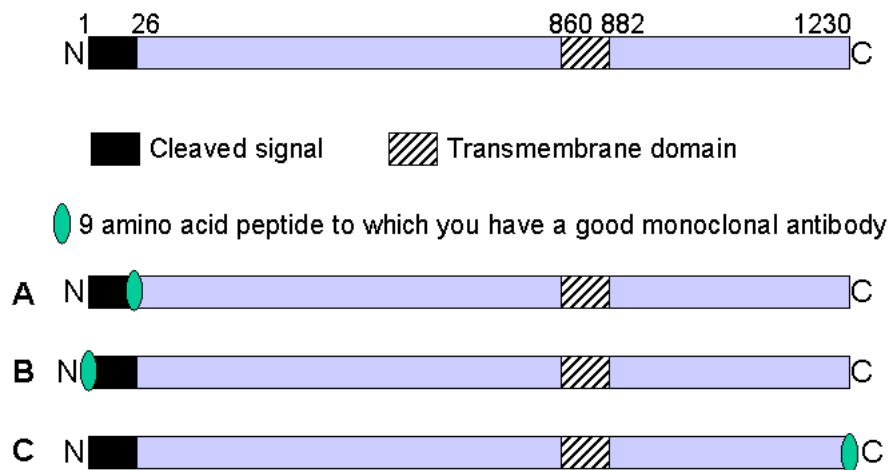


1. You just got a cDNA clone that encodes what looks like a new receptor tyrosine kinase (RTKX). You express it in a mammalian cell line and then make a homogenate so that you can try to purify and characterize this new receptor. You find a bottle of 20 mM sodium phosphate, pH 7.4, in the refrigerator and decide to use it to make your initial homogenate. You do a slow spin to remove nuclei and then a 1 h spin at 100,000 x g.

- Where would you expect to find RTKX and why?
- How might you be able to detect RTKX? Think of as many ways as you can.
- You resuspend the pellet in the same buffer with 2 M KCl and re-centrifuge it. Where should RTKX be now?
- You wash the remaining pellet in Na₂CO₃, pH 11 and spin again – where would you expect RTKX to be?
- You take out some sodium dodecyl sulfate, a strong, ionic detergent, and resuspend the pellet in buffer with SDS. After centrifugation, where would you expect RTKX to be? Which of the methods you thought of above could still be used to detect RTKX?

2. You just started on your rotation project, which you kind of took over from the previous student. You were handed a diagram showing you some features predicted for Blob, the protein you are supposed to be studying. Although your PI tried several times to raise antisera to Blob, the rabbits weren't cooperative. The previous student worked hard and generated expression vectors encoding the proteins diagrammed below.



You transfect your favorite fibroblast cell line with each construct and then harvest the cells for Western blot analysis.

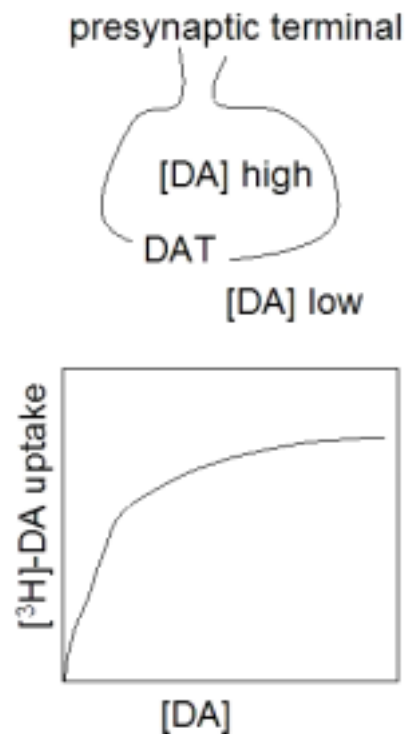
- You find a band of the right size for constructs A and C, but see almost nothing for construct B. What might have happened?
- You carry out a subcellular fractionation, carefully separating plasma membranes from endoplasmic reticulum. While construct A is largely localized to the plasma membrane fraction, construct C is recovered in the ER fraction.

What might be going on and how might you decide where Blob is supposed to be?

- You carry out a metabolic labeling experiment, incubating your transfected cells with radiolabeled Met ($[^{35}\text{S}]\text{-Met}$) and isolating your epitope-tagged Blob protein using your monoclonal antibody. After the pulse incubation you see a band of 140 kDa for both construct A and construct C. After a 1 hour chase, you see a band of 155 kDa for construct A and almost nothing for construct C. What might be going on? How could you check?

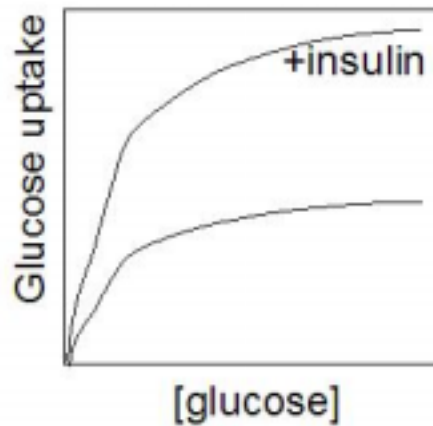
3. Cocaine blocks the sodium-dependent reuptake of dopamine (DA: a neurotransmitter) that would normally remove DA from the synaptic cleft after it is released by exocytosis. You are trying to study a mouse with a mutation that is thought to affect the function of the dopamine transporter.

- Do you think the dopamine transporter (DAT) is likely to be a pore or a carrier? How might you distinguish these possibilities?
- Your mutant mice are very sensitive to cocaine, going “crazy” on the first injection, and you want to assess DAT function. Thus you have prepared a synaptosome preparation (pinched off purified nerve endings) and purchased $[^3\text{H}]\text{DA}$ to use. Briefly describe how you would try to study dopamine transport using this system. Pay special attention to the choice of buffers and proper controls.



4. When insulin is given to skeletal muscle, glucose transport is seen to change as diagramed.

- What would happen if insulin were added to muscle along with a protein synthesis inhibitor?
- Do you expect the K_m for glucose transport to change, or not, following application of insulin, and why or why not?
- Your muscle specimen was incubated with a NaK-ATPase inhibitor for varying periods of time. What might you expect to see after:
 - a 3 minute incubation?
 - an an hour in drug?



5. In 1952, Hodgkin and Huxley published 5 landmark back-to-back papers in *J Physiol* which defined many of the properties we take for granted in how the action potential works. Aside from predicting that 4 similar units (now we'd say protein domains or subunits) had to work together to open one channel, and that the Na^+ and K^+ channels would be separate, they found that the Q_{10} for the action potential was about 1.3. The Q_{10} is a measure of the temperature sensitivity of a process, and is typically 2 or 3 or bigger for enzymatic steps. Such a low value suggested to them that the ions moved through a pore.

- thinking about valinomycin and gramicidin, how might you explain their 50-year-old data?
- how does their idea fit with current data about the structure of ion channels?