25 points for each main question

**Question 1: Cytokine receptor**

1) Binding assays with labeled cytokine X should be used to screen cell lines. There should be a discussion of binding assays and Scatchard analysis as a method to determine total receptor number and affinity.

2) 1500 x $10^7$ x ($5x10^4$g/$6x10^{23}$ molecules/g)=$1.25x10^{-9}$g= 1.25 ng. This is much too little for biochemical purification/peptide sequencing.

3) The Fc fusion can act analogously to an antibody.

There should be a description of expression cloning, including:
-construction of a cDNA from the high-expressing cell line
-generation of cDNA pools
-transfection of pools into COS cells
Three possible selection methods are: FACS to select for cells transfected with the correct clone, binding of pools to labeled Fc fusion protein, "panning" (The Fc fusion is deposited onto a plate, and transfected pools are placed onto the plate to select for cells that stick)
-retrieval of positive pools and further sib selection till a single clone is identified.

4) The receptor has to be encoded by a single gene (i.e., cDNA), and it must be capable of being functionally produced in COS cells.

5) Typical cytokine receptors signal through the JAK/STAT pathway. A C-terminal truncation could affect (a) affect JAK binding or (b) remove tyrosine phosphorylation sites that are docking sites for STAT.

**Question 2: Apoptosis**

1) The ectopic masses in the forebrain are likely due to lack of developmentally programmed cell death in the forebrain. The masses can be shown to of neuronal origin by staining with neuronal markers. To test the idea of defective apoptosis, the occurrence of apoptosis in normal versus mutant embryos can be evaluated by TUNEL staining.

2) The Apaf1 mutant cells would be resistant to etoposide but remain sensitive to the anti-Fas antibody. Apaf1 operates in the intrinsic pathway; Fas-mediated cell killing occurs via the extrinsic pathway.

3) No difference is expected. Cytochrome *c* release occurs independently of Apaf1 and therefore would occur normally in Apaf1 mutant cells. However, the Apaf1 mutant cells would be resistant to UV-induced cell death.

4) Cytochrome *c* has an additional function as an electron carrier in the respiratory chain. The resulting defect in oxidative phosphorylation causes mid-gestation lethality.

5) From the CED-9 gain-of-function mutation (as well as loss of CED4 or CED3), the survival of the 131 cells normally destined for programmed cell death appears to be compatible with normal development. This is in contrast to the mammalian situation. In the CED-9 loss-of-function mutation, however, the ectopic cell deaths cause embryonic lethality, because many of these cells are essential for viability.

**Question 3: Oncogenesis**

1) [2-hit hypothesis of Knudson.] The BRCA1 mutations are loss of function alleles. All cells would inherit one inactive allele, but at low frequencies the other allele can become inactivated (loss of heterozygosity). Such cells occur at a low frequency, but high enough to give rise to bilateral disease. BRCA1 is thought to play a role in DNA repair.

In sporadic breast cancer with BRCA1 mutations, both alleles of BRCA1 must be mutated. This is a much rarer event, and so bilateral disease is not expected.

2) Many cancer cells show genomic instability (increased rates of mutations due to loss of repair mechanisms), causing the presence of tumor cells resistant to a targeted therapy.

3) RSV is an acute virus because it contains an activated v-src oncogene and activates proliferation in all infected cells, leading to polyclonal tumors.

Some other retroviruses do not carry an activated oncogene, but can lead to cancer after a long latency. In this case, oncogenesis is due to promoter/enhancer insertion, but this is a much less efficient process. It causes tumorigenesis in a rare cell, leading to monoclonal tumors.

4) Phosphorylation of the tyrosine in the C-terminal tail creates a binding site for the SH2 domain. This intramolecular interaction constrains the protein and creates an inactive conformation of the Src kinase domain.

5) Binding of a phosphorylated ligand to the SH2 domain of Src would prevent the intramolecular interaction above, thereby allowing an active conformation of the kinase domain.

**Question 4: Membrane protein**

1)
Immunocytochemistry:

pros: This is the better method for examining *endogenous* localization.
cons: The cells need to be fixed and permeabilized and this can lead to artifacts. The protein may be expressed at low levels and may be difficult to detect.

GFP fusion
cons: It is not examining the *endogenous* protein.

It usually involves overexpression, which can cause artifacts.
The fusion construct can also create artifacts; it may not localize to the endogenous location.

2) Collect antibodies against various organelle markers and perform co-localization studies with immunofluorescence.

3) A polyclonal antibody is a collection of antibodies recognizing multiple epitopes on the protein. A monoclonal antibody comes from a single B cell clone and consists of a single antibody species recognizing a single epitope.

Monoclonal antibody

pros: potentially more specific reagent

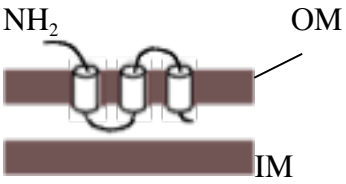
con: lower signal because only one epitope is recognized

Polyclonal antibody

Pros: higher signal because multiple epitopes can be recognized

Cons: potentially dirtier reagent

4) The N-terminus is outside the mitochondria, and the C-terminus is inside. This places the protein in the outer membrane of mitochondria. There has to be 3 transmembrane segments.




5) No, the mitochondrial membrane potential is important only for proteins going through the inner membrane.