Cell and Molecular Neuroscience, Example Exam 2 from Fall 2015

1. (a few sentences each) Compare and contrast the catecholamines and steroid nuclear receptor hormones

    a. synthetic pathways

    b. storage and release

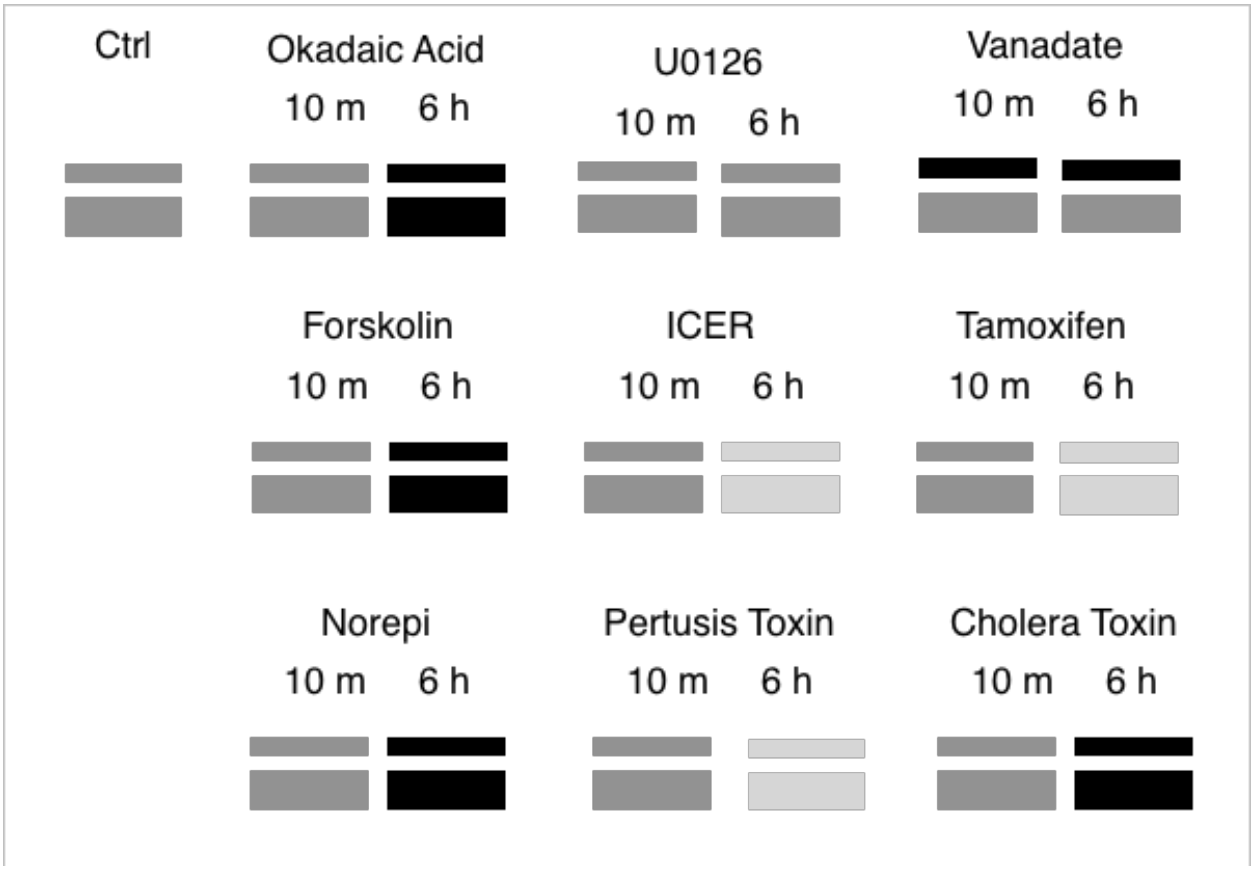
    c. types of receptor

    d. intracellular effects

2. The figure below shows a series of Western Blots for a specific protein extracted from treated cells. The protein is visualized with a specific antibody that yields 2 bands: a major band and a slightly larger molecular weight band that contains a subset of the total protein. For each blot, the cells were treated for either 10 min or 6 h with the indicated compound. The compounds decreased or increased the intensity of one or both bands as indicted.

Outline the regulation of the protein's gene expression and phosphorylation (i.e. arrows connecting receptors, enzymes, transcription factors.)

(See solution at end of example exam).



3. These questions relate to the two figures (Figure 1 and Figure 2) below on the analysis of the tryptophan hydroxylase (TPH) promoter. (Answer each subquestion with a few sentences.)

a. In Figure 1, what are the critical promoter regions for TPH expression, based on how the TPH-reporter expression drops with truncation of the TPH promoter?

b. What is a possible explanation for why expression is generally lower in PC12 cells than in Pinealocytes, especially with the longer promoter regions?

c. In Figure 2, what can you conclude about TPH gene regulation, and specific transcription factors or pathways, based on the cAMP sensitivity of TPH gene expression?

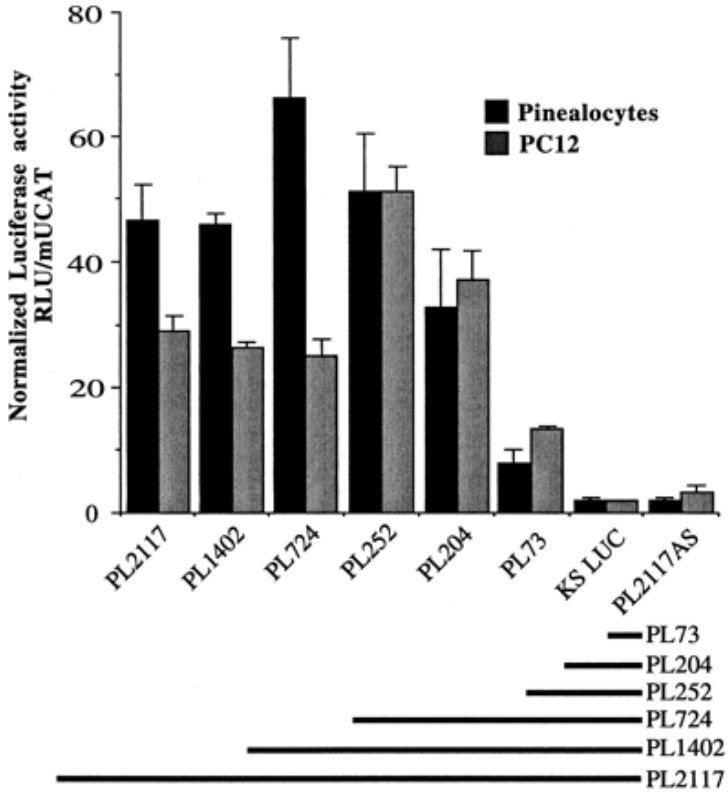
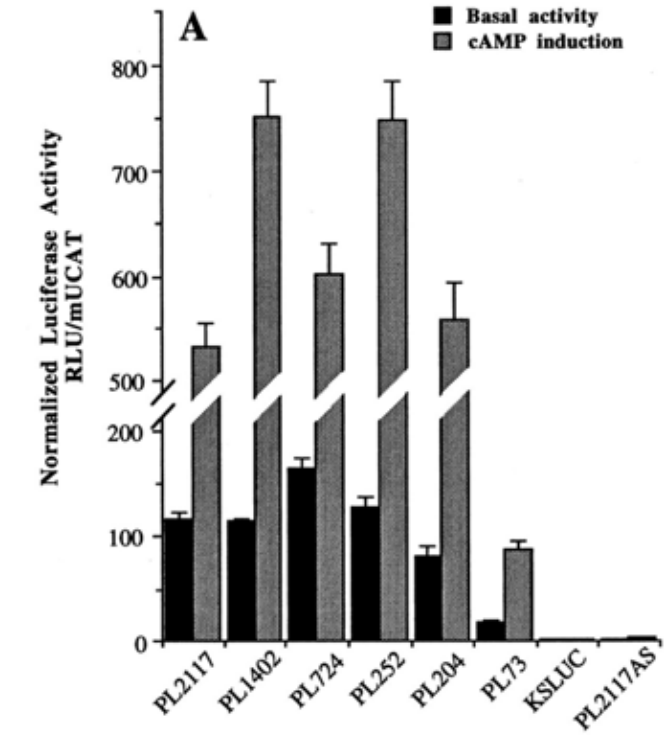
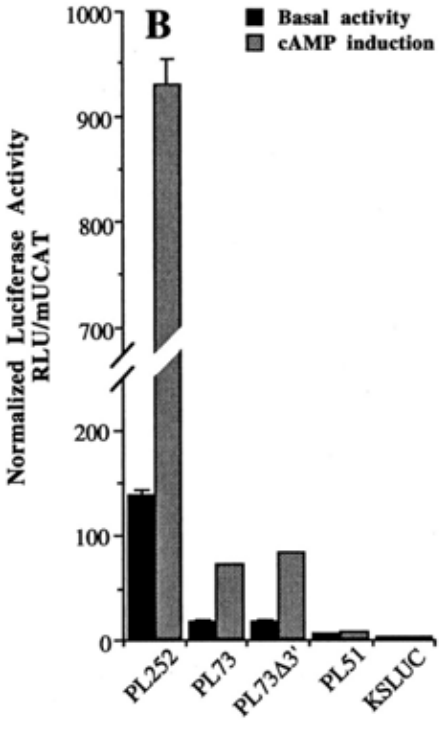


Figure 1. Deletional analysis of cis-active DNA elements in the human TPH gene 5′-flanking region. Top, a series of constructions containing various lengths of the 5′-region of the hTPH promoter plus 29 bp of exon 1 were fused to the structural gene for firefly luciferase. The hTPH promoter-luciferase constructs were introduced into primary cultures of rat pinealocytes and PC12 cells for transient expression assays. Luciferase activity is expressed as relative light units (RLU) normalized to CAT activity in the same cell extract (RSV-CAT) expressed in milliunits. The deletion constructs of the hTPH promoter are indicated on the abscissa. PL corresponds to the hTPH promoter fragment of 2,117 bp inserted into the promoterless luciferase vector (KSLuc) in the reverse orientation. Data are mean ± S.E. (bars) values from at least three independent experiments done in triplicate. Bottom, schematic representations of the fragments of the hTPH promoter.



Basal Activity	116.4 ±5.8	114.6 ±2.0	165.2 ±9.6	127.9 ±9.3	81.9 ±9.2	17.5 ±1.9	2.0 ±0.2	1.9 ±0.2
cAMP fold increase	4.6*	6.5*	3.6*	5.8*	6.8*	5.1*	1.4	1.5



	137.4 ±3.7	16.2 ±1.2	16.3 ±1.9	4.4 ±0.2	2.0 ±0.2
	6.7*	4.4*	5.0*	1.4	1.5

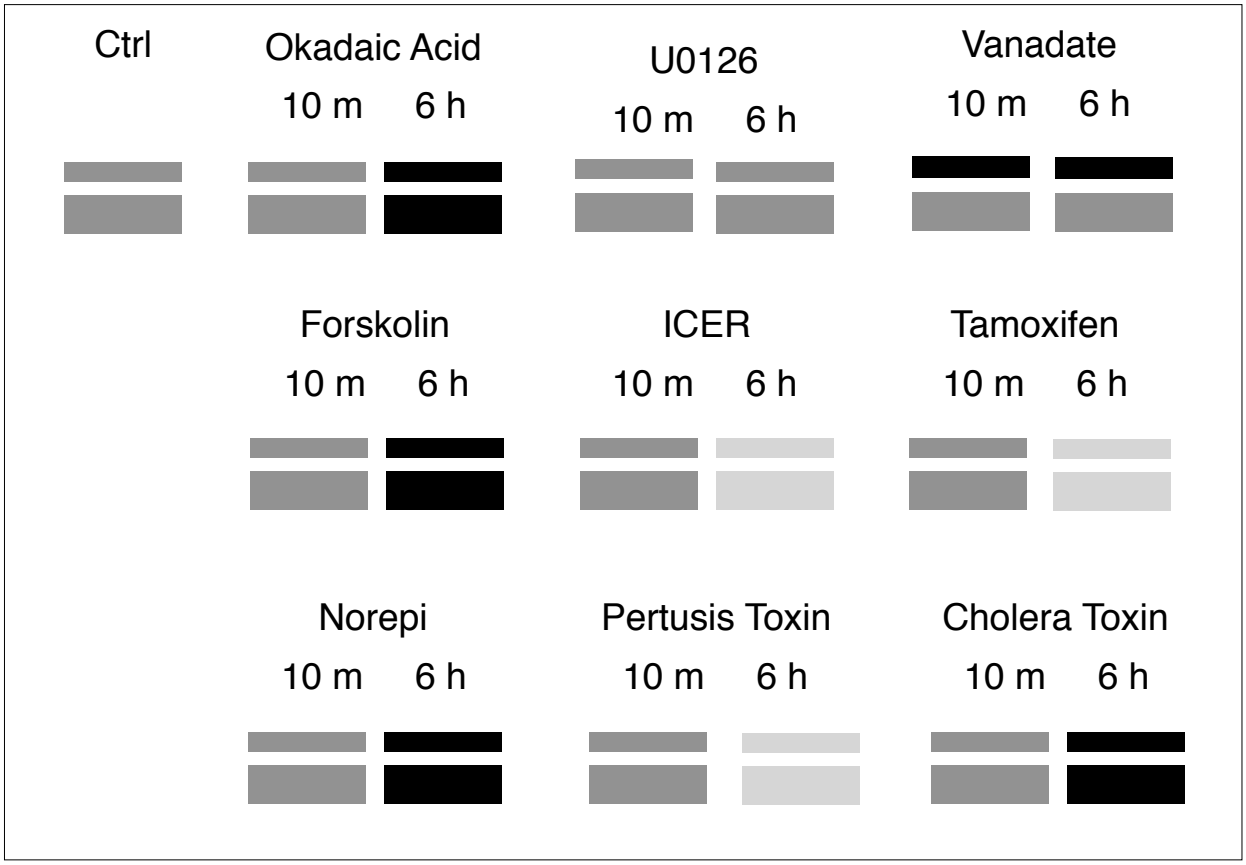
Figure 2. Luciferase activity of human TPH promoter-luciferase fusion genes in pinealocyte cultures. A and B, pinealocytes were transfected with the hTPH promoter-luciferase plasmids defined in Figure 1 exposed or not exposed to 1 mM 8-Br-cAMP (a membrane permeable analog of cAMP, which activates adenylate cyclase). Luciferase activity is expressed as relative light units (RLU) normalized to CAT activity in the same cell extract (RSV-CAT) expressed in milliunits. The abscissa indicates the names of the deletion constructs of the hTPH promoter. Data are mean ± S.E. (bars) values from at least three independent experiments done in triplicate. Bottom, the two tables give the values of the basal level of the reporter genes expression (RLU/mUCAT) and the -fold induction of the reporter genes activity after 8-Br-cAMP treatment (, $p < 10$; t test).*

4. (about 250-500 words) A technique is rarely perfect. Choose a specific technique for detecting nucleic acids, and concisely 1) describe the attributes of the nucleic acids (size, sequence, charge, etc.) which the technique can determine and 2) describe the limitations and/or ambiguities of the technique (i.e. what can go wrong, or how the result of the technigue might not be the definitive identification of the target nucleic acid).

5. (about 250-500 words) Same as Question 4, but apply to a protein technique.

Question 2: The Figure belwo shows a series of Western Blots for a specific protein extracted from treated cells. The protein is visualized with a specific antibody that yields 2 bands: a major band and a slightly larger molecular weight band that contains a subset of the total protein. For each blot, the cells were treated for either 10 min or 6 h with the indicated compound. The compounds decreased or increased the intensity of one or both bands as indicted.

Outline the regulation of the protein's gene expression and phosphorylation (i.e. arrows connecting receptors, enzymes, transcription factors.)



Solution:

1. make a table of the western blot results

Drug	Target	10 m	6 h	Conclusion?
Okadaic Acid	Ser/Thr PPs	none	increase	
U0126	MAPK	none	none	
Vanadate	pTyr PPs	increase pX	increase pX	
Forskolin	cAMP	none	increase X and pX	
ICER	CRE	none	decrease X and pX	
Tamoxifen	Estrogen Receptor	none	decrease X and pX	
Norepi	Adrenergic receptors	none	increase X and pX	
Pertussis	block G proteins	none	decrease X and pX	
Cholera Toxin	activate G proteins	none	increase X and pX	

Solution: (cont)

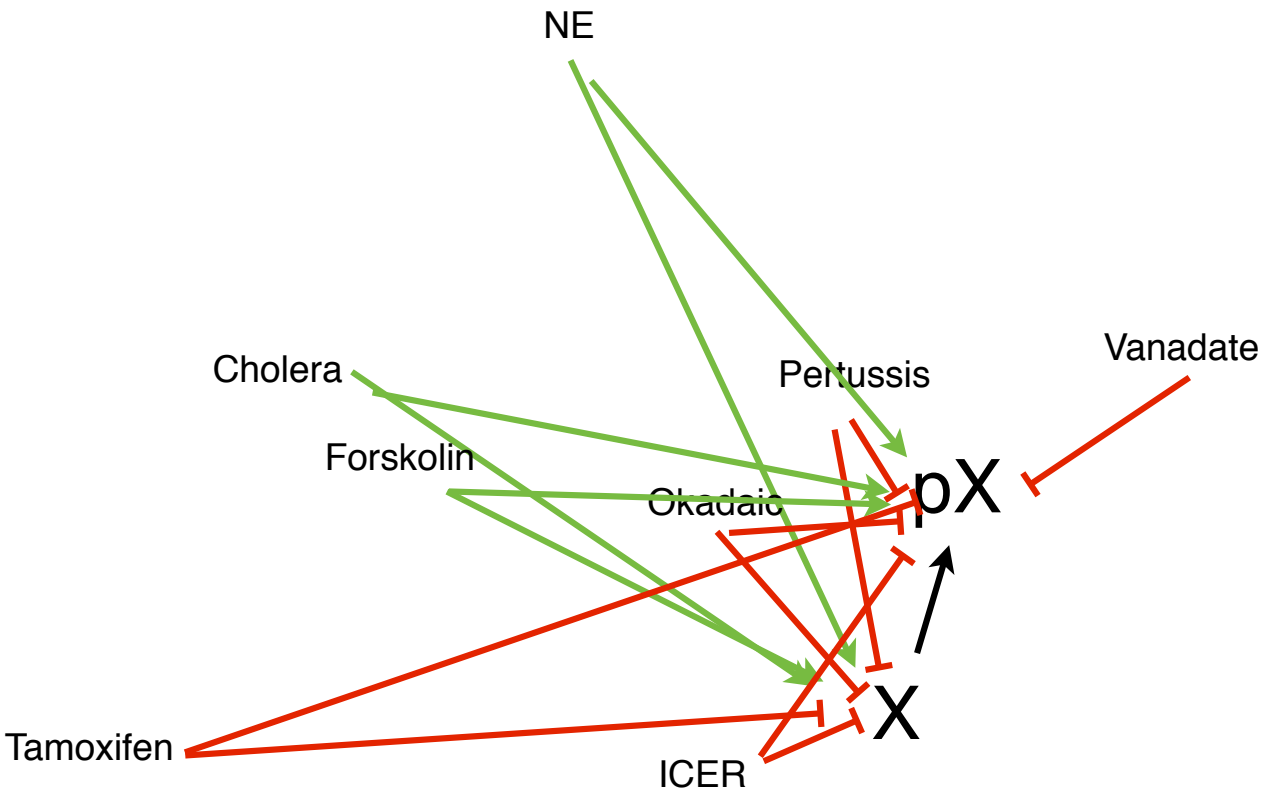
2. add the conclusion that can be drawn from each western blot (i.e. what the effects of the different treatments tell you about the regualtion of X).

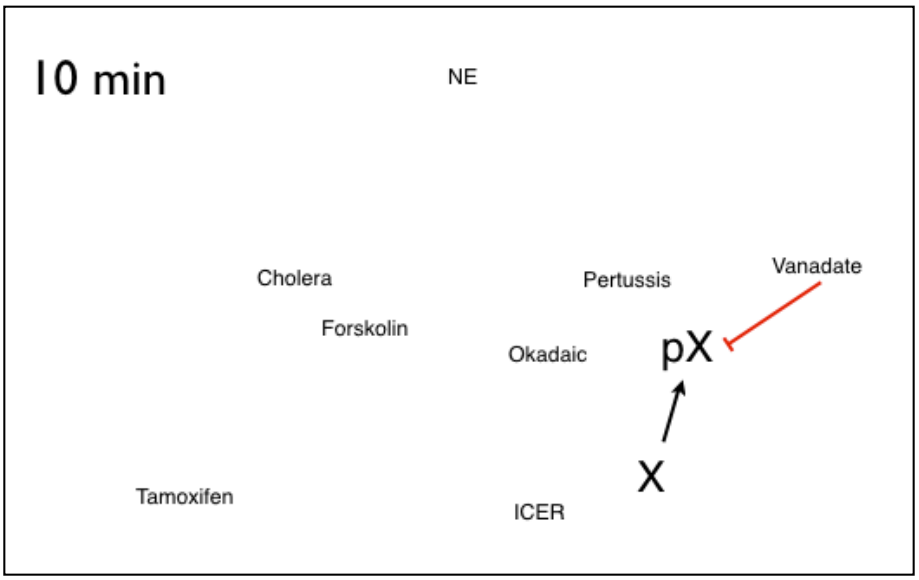
Drug	Target	10 m	6 h	Conclusion
Okadaic Acid	Ser/Thr PPs	none	increase	PPs constrain gene X
U0126	MAPK	none	none	MAPK not involved
Vanadate	pTyr PPs	increase pX	increase pX	pTyr PPs constrain phosphorylation but not gene X expression
Forskolin	cAMP	none	increase X and pX	cAMP stimulates gene X expression but not phosphorylation
ICER	CRE	none	decrease X and pX	CREB stimulates gene X expression (but not phosphorylation)
Tamoxifen	Estrogen Receptor	none	decrease X and pX	estrogen stimulates gene X expression (but not phosphorylation)
Norepi	Adrenergic receptors	none	increase X and pX	Norepi stimulates gene X expression but not phosphorylation (consistent with B-adrenergic receptors -> cAMP)
Pertussis	block G proteins	none	decrease X and pX	G-proteins stimulate gene X expression but not phosphorylation
Cholera Toxin	activate G proteins	none	increase X and pX	G-proteins stimulate gene X expression but not phosphorylation

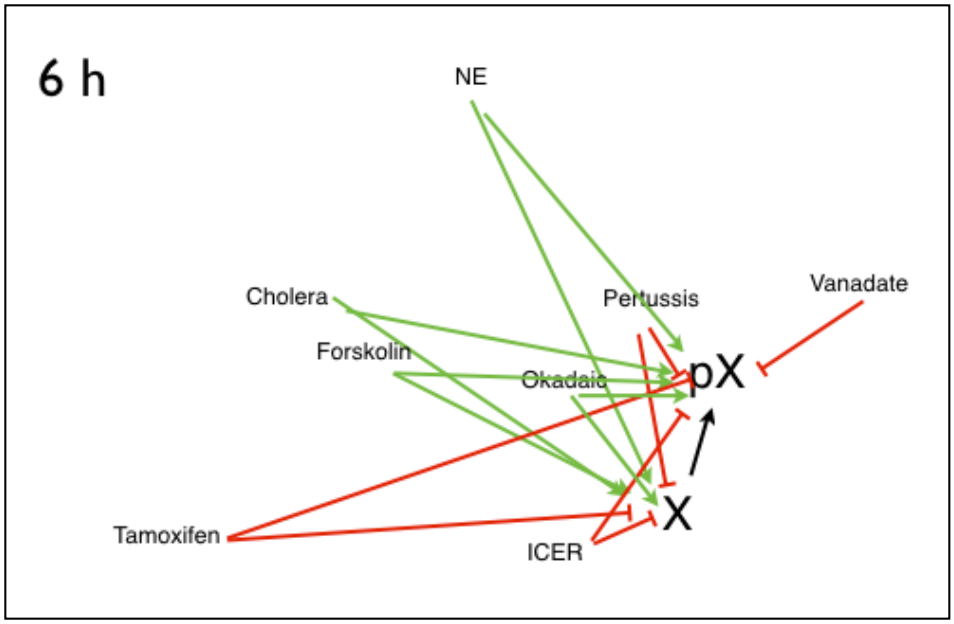
Solution: (cont)

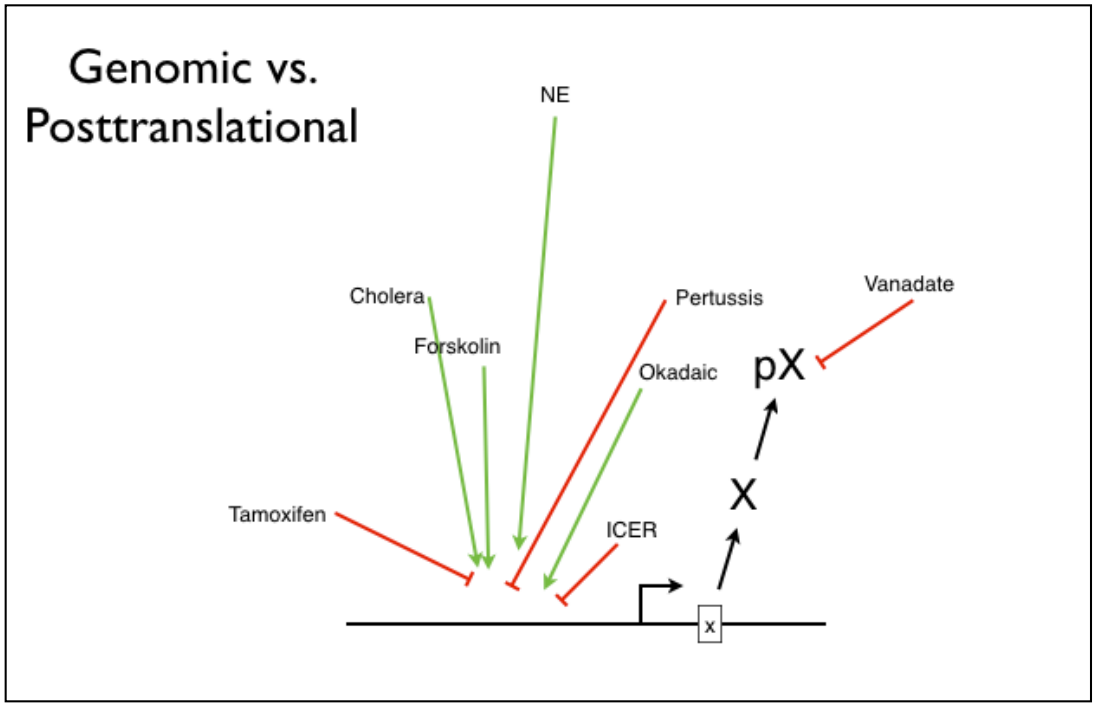
3. Try making a pathway, by looking at overall effects, then breaking down in to different timing of effects (10 mn vs. 6h) and those effects which are likely mediated regualting gene expression of X (so effects on promoter and transcription) vs. those post translational effects on X itself.

Overall




10 min
NE
Cholera
Pertussis
Vanadate
Forskolin
Okadaic
pX
X
Tamoxifen
ICER


6 h
NE
Cholera
Pertussis
Vanadate
Forskolin
Okadaic
pX
X
Tamoxifen
ICER


Genomic vs.
Posttranslational
NE
Cholera
Pertussis
Vanadate
Forskolin
Okadaic
pX
X
Tamoxifen
ICER
x

Solution: (cont)

4. Put it all together (it helps to model it on some of the other signaling pathways we reviewed in class…)

Model