Question 1

E. coli prosigma factor Crl protein interacts directly with stress response sigma factor RpoS (σ^{38}) to increase affinity between RpoS and the core polymerase during stress conditions. The *crl* gene (shown in Figure 1) is under the regulation by two different promoters, the canonical RpoD (σ^{70}) promoter and the RpoN (σ^{54}), the nitrogen-stress σ factor.

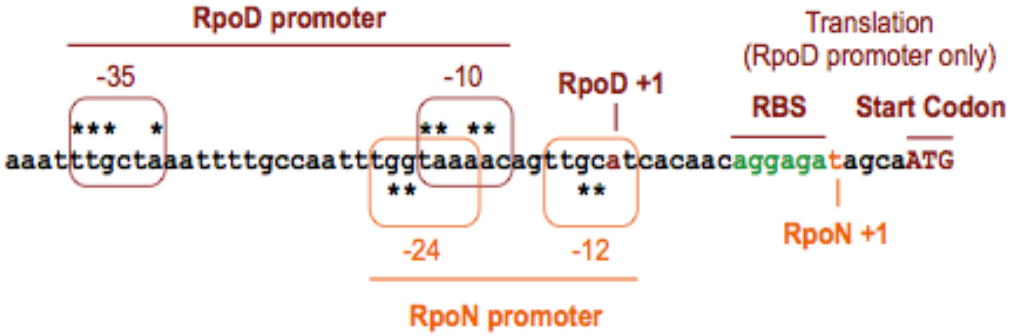


Figure 1: The *crl* promoter sequence with both RpoD (σ^{70}) and RpoN promoters highlighted. The Transcriptional Start Sites (TSSs) are indicated with a +1, and the ribosome binding site (RBS or Shine Dalgarno) is indicated in green; asterisks mark the consensus sequence for each of the promoters

A) In response to stress, RpoS expression is tightly regulated at the transcriptional, translational and overall stability levels (both RNA and protein). It is thought that Crl must also be highly regulated in response to stress but this has not been shown experimentally. Describe experimental approaches to test how expression of Crl is regulated. You should design specific approaches that allow you to test whether Crl expression is regulated through the same mechanism(s) as RpoS. Please include required controls. **(7 points)**

B) You have discovered that although Crl protein levels are significantly decreased during stress, mRNA levels are increased. Based upon the information provided, design a model to explain these results. **(2 points)**

C) The *dps* gene contains an RpoS promoter. Below (Figure 2) is the quantification of RNA levels of dps during different growth conditions (indicated on Y axis). Interpret the data provided below. **(1 point)**

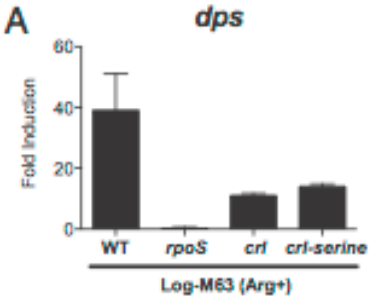


Figure 2: X axis indicates strains- WT (wild type), *rpoS* deficient strains and *crl* deficient strains. Log-M63 (Arg+) is denoted as nitrogen-stress medium. The fold-induction in response to stress is indicated on the Y-axis.

Question 2

Assume you PCR-amplify a 1.5kb full-length human cDNA encoding a liver enzyme, which is encoded by the gene *GR8*; you subclone it into a high copy yeast expression vector with a strong constitutive promoter and transform it into *S. cerevisiae*. Next, you assay lysates of the resulting yeast transformants for GR8 enzyme activity, but instead of finding high levels of GR8 enzymatic activity in transformants you find levels that are barely detectable above the level found in untransformed yeast. You know you got the plasmid into the yeast because your transformants are all prototrophic for leucine, indicating that the *LEU2* gene carried on the plasmid backbone has complemented the *leu2* genomic deletion in the host yeast.

What went wrong?

A) As a first step toward troubleshooting this situation you decide to make a list of reasonable possible "levels" at which the problem might have occurred. Here is one to get you started: There might be a PCR-induced mutation in your *GR8* cDNA sequence, killing the activity of the encoded enzyme. Please list FOUR conceptually DIFFERENT levels at which the problem might have occurred. **(2 points)**

i.

ii.

iii.

iv.

B) Assume you fully sequence the plasmid insert and the *GR8* cDNA sequence looks perfect with no mutations. Explain what experiment(s) you would do next and what results you would expect to obtain that would let you rule in or rule out two of the potential problems you listed in **(A)** above. **(4 points)**

C) Pick one of the four levels you listed in part **(A)** and assume the results of your experiments in part **(B)** indicated that was the problem. What could you do to overcome this problem and finally obtain high levels of GR8 activity from yeast transformants? Explain. **(4 points)**

Question 3

A) You have been tasked with identifying novel binding partners for your protein of interest, X, and have opted to try co-immunoprecipitation (co-IP) to do so. You find on-line a great looking commercial antibody to X that they show on the company website as yielding a strong, single band in immunoblots of total cell homogenate from HeLa cells. So you buy the antibody and perform a standard co-IP protocol from bovine brain because you have plenty in the freezer. But not only do you get no partners, you get no X in your IP. Provide a prioritized list of **your top three** potential explanations for why this result occurred. Then describe a plan to get around this road block, with an explanation of how your plan will remedy the situation for your top explanation. Include appropriate positive and negative controls in your description. **(4 points)**

B) You are now attempting to purify X from HeLa cell lysates. Full length human X is 1,000 residues in length and is thus predicted to be _____ Da (write your estimate here). **(1 point)**

C) You have identified a novel protein, Y, which is present in plaques in Alzheimer's disease brains at autopsy. You purchase a commercial antibody to Y that identifies a single band migrating at 45 kDa in immunoblots from control brains and a band of the same electrophoretic mobility is seen to increase upon over-expression of Y in HeLa cells. However, in brains from Alzheimer's disease patients the immunoblot reveals a second band, migrating ~2 kDa higher than the other (i.e., at 47 kDa). You hypothesize that Y is hyperphosphorylated in Alzheimer's disease.

(i) Describe one approach other than the use of mass spectrometry (LC-MS/MS) to confirm that Y is phosphorylated. **(1 point)**

(ii) Again, without the use of mass spectrometry, describe how you might determine if there is more than one site of phosphorylation. **(1 point)**

(iii) Based upon homology to a close paralog that is known to be phosphorylated at a conserved site, Serine 45, you speculate that your protein Y is also phosphorylated there. How would you test that? (note: you still do NOT have access to LC-MS/MS) **(1 point)**

(iv) Finally, you now are convinced Y is a phosphoprotein and are tasked with identifying every site of phosphorylation. Fortunately, you have access to an antibody to Y that works very efficiently for IP and you now have access to a state of the art LC-MS/MS facility. Describe your approach and whether you think it will necessarily identify every site of phosphorylation. And if not, why not? **(2 points)**

Question 4

Herpes simplex virus (HSV) infects human cells and then enters the nucleus of the host cells. A genetically engineered HSV strain expressing green fluorescent protein tags was used to infect human epithelial cells, and virus particles were observed in live cell imaging (Fig. 1). Researchers found that these virus particles move inside the nucleus (Fig. 1).

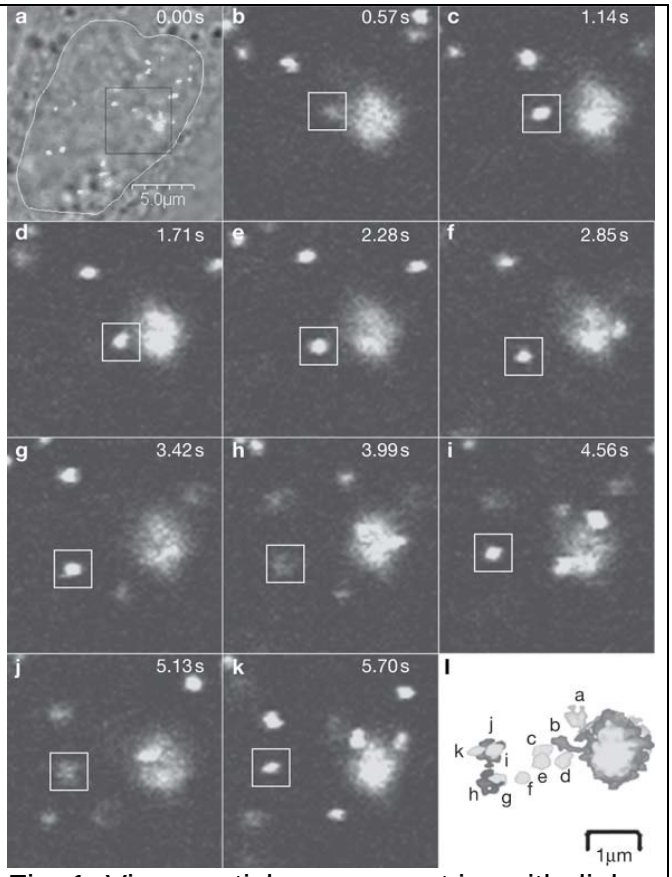


Fig. 1. Virus particle movement in epithelial cells. Panel **a** shows a transmitted light channel, with the nucleus outlined in white. From the boxed area in panel **a**, the GFP channel is expanded in **b–k**, in which an individual virus particle is indicated by squares. The particle images are superimposed in **l**, which has the background subtracted. Scale bar, 1 μm. The figure is from Forest et al. *Nature Cell Biology* **7**, 429 - 431 (2005).

A) You became interested in the mechanism of virus movement and hypothesized that actin is involved in the movement. However, your skeptical advisor disagreed and said that actin plays major roles in the cytoplasm. To convince your advisor, describe **TWO** experiments to demonstrate that actin is present in the nuclei in the cells used in this study. Assume that these cells are from a commonly available cell line. Include proper controls, provide a rationale for your selection, and explain one limitation in each experimental approach. **(4 points).**

B) You found that Latrunculin A (a small compound that sequesters actin monomers) efficiently blocks the virus movement.

(i) Based on this observation, describe your hypothesis on the mechanism of the HSV movement **(2 points).**

(ii) Then, describe **TWO** experiments to test your hypothesis. Include proper controls, provide a rationale for your selection, and explain one limitation in each experimental approach. **(4 points)**.

Question 5

You have become interested in studying the role of initiator caspase-10 in cell death. This is a difficult molecule to study because it does not exist in mice so you will be limited to using human cell lines for your study. Caspase-10's closest relative in the caspase family is caspase-8, with which it shares all functional domains.

A) Describe the features of an initiator caspase that distinguish it from an effector caspase. Feel free to diagram these differences. Include both domain structure differences **(1 point)** and how each type of caspase is activated **(1 point).**

Before testing the role of caspase-10 in cell death, you first perform a control experiment to test the effect of treating cell lines with a peptide inhibitor of caspase 10 (Q-AEV-OPH) under normal growth conditions. The graph below shows the surprising results you get from this control experiment. (Figure 1).

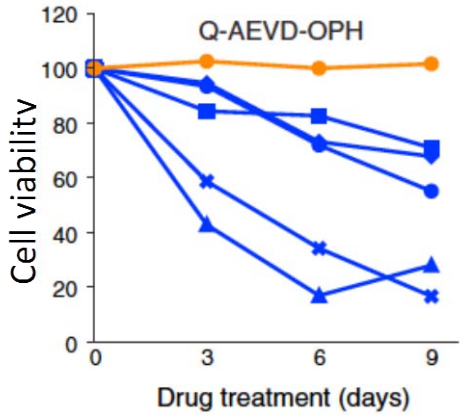


Figure 1. Cells were treated with the caspase-10 inhibitor Q-AEVD-OPH for the indicated time (days) and viability was determined by propidium iodide exclusion and quantitated by flow cytometry. Each line on the graph represents a different cell line. The cells whose results are indicated by blue lines are all derived from patients suffering from a common disease (myeloma), while the orange line represents results from a cell derived from a patient with a different disease.

Answer the following questions based on these data.

B) What would you predict the role of caspase-10 to be in the blue cell lines? **(2 points)**

C) What experiment could you perform to test whether your answer in **B** is correct? Be sure to include details of what your experimental conditions and controls are and how you would measure cell death. **(3 points)**

Continue on the next page

Question 5 Continued from the previous page

D) You repeat the experiment shown above with a pan-caspase inhibitor (Q-VD-OPH) and get the results shown in Figure 2 below. Given these findings, what is the most likely explanation of how these cells are dying? **(2 points)**

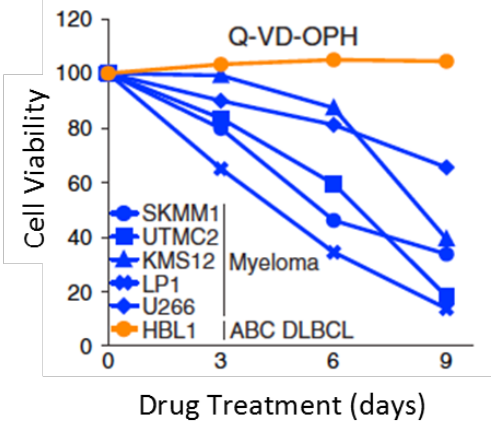
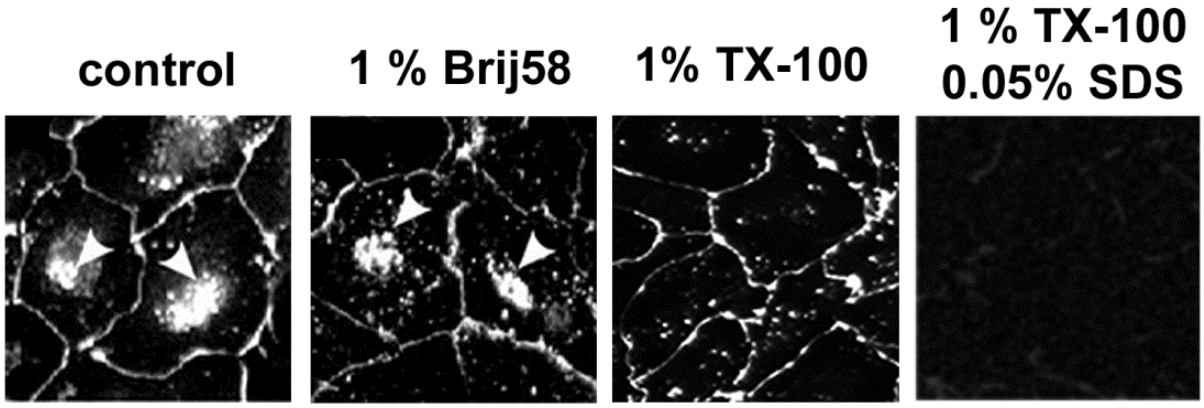


Figure 2. Cells were treated with the pan-caspase inhibitor Q-VD-OPH for the indicated time and viability was determined by propidium iodide exclusion. Data are representative of 3 experiments. Blue lines show data from myeloma lines while the orange line shows data from a line derived from a different disease.

E) Given your answer to **D**, what do you think the most likely substrate for caspase-10 is in these cells? **(1 point)**

Question 6

You are interested in a transmembrane junction protein, Klng-2. By immunofluorescence microscopy, control cells show two major pools for this protein, at cell-cell contact sites and in the perinuclear region of the cell (arrow head)



Pretreating the cells with a detergent solution containing 1% Brij58 before fixation and staining had little, if any effect on the staining pattern for Klng-2, where junctions and the perinuclear localized pool were present. However, pretreatment with 1% Triton X-100 (TX-100) prior to fixation caused the perinuclear pool to disappear, while the junctional pool of Klng-2remained. Using a solution of 1 % TX-100 + 0.05% sodium dodecyl sulfate (SDS) prior to fixation caused the entire Klng-2 signal to disappear.

A) Provide an interpretation of the results presented in the figure above considering the nature of the detergent solutions used? **(2 points)**

B) Provide a hypothesis for how Klng-2 at cell-cell junctions is retained following TX-100 treatment. How would you test this hypothesis? Suggest the functional significance for your hypothesis as it relates to formation of junctions containing Klng-2? **(4 points)**

C) Provide a hypothesis for the identity of the perinuclear pool of Klng-2, including a role for this intracellular compartment in regulating the junctional pool of Klng-2. How would you test this hypothesis? **(4 points)**

Question 7

A recent genome-wide association study on a large human population identified mutations in the CHD7 gene, a putative chromatin remodeling enzyme, as being strongly associated with congenital heart defects. You would like to understand the role of this protein in heart development. Given that the fruit fly *Drosophila* has a highly conserved ortholog of the human CHD7 gene and a very simple heart that can be dissected out for a variety of analyses, you have decided to begin your studies in this model organism.

Design experiments to address the following issues, and include all relevant controls:

A) Describe one approach to determine whether *Drosophila* CHD7 plays a role in development of the fly heart. **(1.5 points)**

B) You find that mutations in CHD7 affect the specification of both cell types in the fly heart, the contractile cells and the pericardial cells, leading to incomplete differentiation of both cell types and a partially functional heart. Given your observations, you would like to purify CHD7 from the heart in order to identify other proteins that form a complex with it. Describe how you would isolate the complex and identify the component proteins. **(2.5 points)**

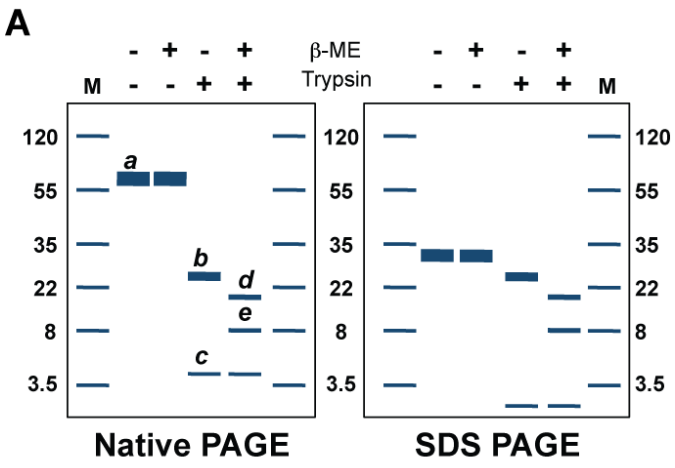
C) You find that CHD7 forms a complex with 14 other proteins including a histone deacetylase called HDAC1. You find two different forms of HDAC1 (HDAC1A and HDAC1B, which are encoded by different genes) in your purified material. Given that HDAC1A and 1B are unlikely to be present in the same complex at the same time, this suggests to you that two distinct forms of the CHD7 complex are present in the heart. You hypothesize that one form of the CHD7 complex is present in the contractile cells while a different form is present in the pericardial cells. Describe how you would determine whether each cell type contains a different form of the CHD7 complex with respect to the HDAC1 subunit. **(3 points)**

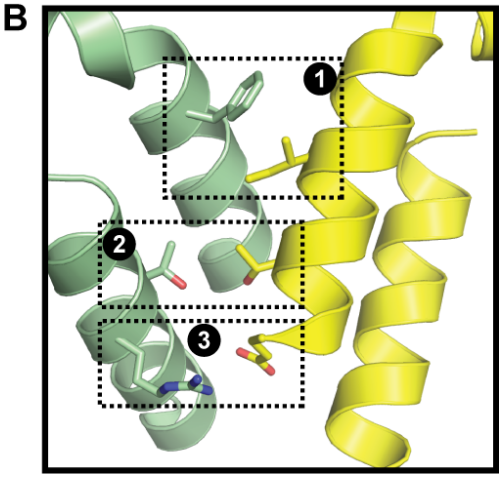
D) You suspect that the CHD7 complex is required for proper expression of cell type-specific genes in the contractile cells during heart development. Describe how you would determine which genes CHD7 acts on in this cell type and whether it has a positive or negative effect on the expression of its target genes. **(3 points)**

Question 8

You have cloned, expressed and purified a protein that is proposed to be a novel ~30 kDa protein kinase. It is thought that a key step in conversion of this kinase from its latent to active state is an initial self-phosphorylation reaction, after which the protein exists in an exclusively monomeric form (as determined by gel filtration chromatography).

To begin characterizing the latent enzyme structure you perform a limited proteolytic digest with trypsin under native conditions and compare this to untreated protein by both native and SDS-denaturing polyacrylamide gel electrophoresis (PAGE). You also examine each of your samples with and without the reducing agent β-mercaptoethanol (β-ME) in the sample loading solution. You stain the gels with Coomassie Blue dye and obtain the results shown in **Figure A** [M = molecular weight markers with the sizes indicated. *Note: you may assume for both gels that your protein fragment masses are accurately represented by the molecular weight markers shown*]. Ultimately, you are able to crystallize and determine the X-ray crystal structure of this kinase. Part of this structure is shown in **Figure B**.





A) From the data shown in **Figure A**, what do you deduce about the protein quaternary structure in solution and the nature of disulfide bond(s) in the protein? Justify your answer. **(2 points)**

B) Sketch **TWO** different protein domain structures that are consistent with the observed tryptic digests. On each, indicate the locations of the trypsin sensitive sites, the approximate size of the structural domains, and disulfide bonds present in the protein. **(4 points)**

C) Describe the three boxed interactions within the molecular interface shown in **Figure B**, including the identities of the amino acids involved and the type of interaction for EACH case **(2 points).** Explaining your reasoning, suggest a possible reason for the observation that active kinase exists as a monomer **(2 points).**

Question 9

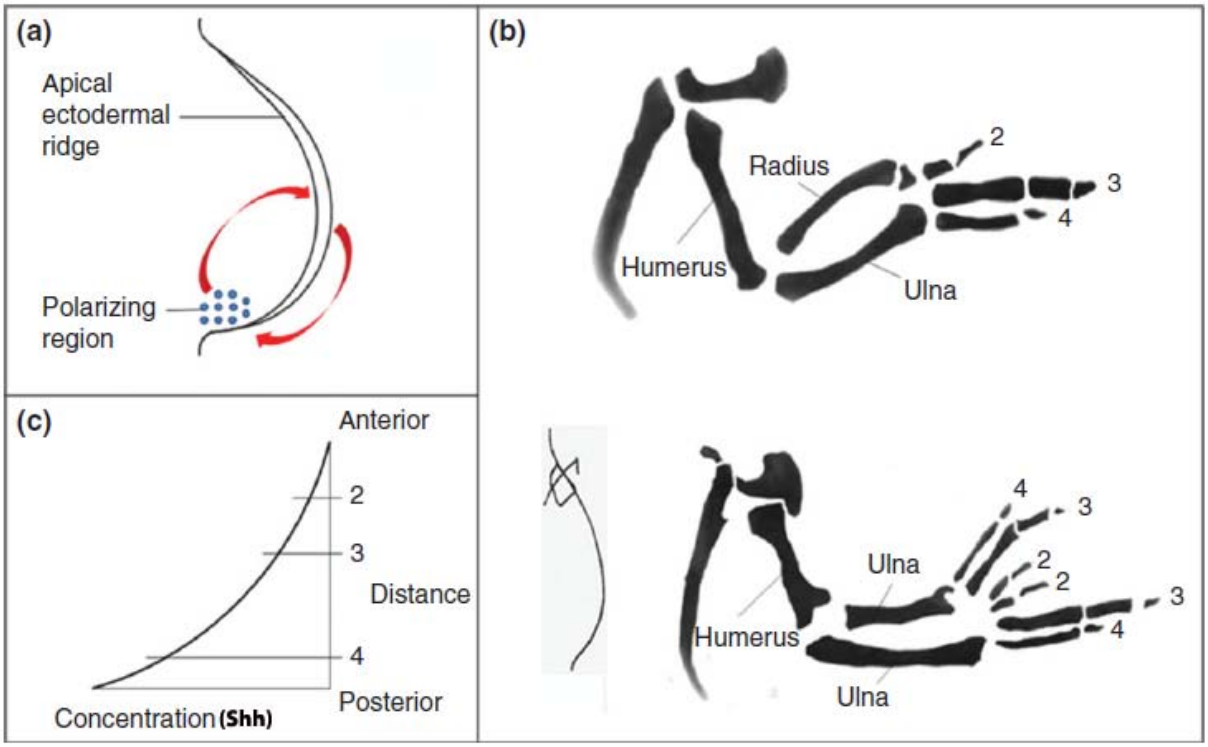


FIGURE: (a) Diagram of the early chick wing bud showing the polarizing region (blue dots) at the posterior margin and the apical ectodermal ridge rimming. Red arrows indicate reciprocal interactions that sustain signaling by both the polarizing region and the apical ectodermal ridge. (b) Images of whole mounts of skeletons of the wings of a 10-day chick embryo stained with alcian green (for skeleton); control wing (upper skeleton) and the wing that received a polarizing region graft (lower skeleton). Sketch showing a polarizing region grafted to anterior margin of host wing bud, taken from a research notebook. Note each of the three digits (2, 3, 4) is morphologically distinct and there is a mirror-image symmetrical pattern of digits (4, 3, 2, 2, 3, 4) when a polarizing region is grafted to the host (b, lower skeleton). (c) Morphogen model for specifying the digit (2, 3, or 4) pattern in a chick wing.

For your Development mini-module, we asked you to propose a morphogen-based mechanism for the patterning of the digits in a developing limb bud. The figure above shows the actual mechanism that was discovered, in chick originally, where the polarizing region at the posterior end (blue dots above) secretes *sonic hedgehog* (*shh*). In this context, *shh* acts both (1) as a morphogen to specify digit identity, and (2) as a mitogen to stimulate growth of the limb bud. This latter function explains why transplantation of the polarizing region into the anterior of a limb bud (lower part of panel b) leads to an increase in the number of digits, along with a mirror image pattern.

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Question 9 Continued from the previous page

The text below is taken from an abstract of a paper published by Zhu et al. Please remember that this is a mouse model, where there are 5 digits (with 1 at the anterior and 5 at the posterior), not 3 as shown above for chick.

Abstract
Sonic hedgehog (Shh), which regulates proliferation in many contexts, functions as a limb morphogen to specify a distinct pattern of digits. How Shh's effects on cell number relate to its role in specifying digit identity is unclear. Deleting the mouse Shh gene at different times using a conditional Cre line, we find that Shh functions to control limb development in two phases: a very transient, early patterning phase regulating digit identity, and an extended growth-promoting phase during which the digit precursor mesenchyme expands and becomes recruited into condensing digit primordia. Our analysis reveals an unexpected alternating anterior-posterior sequence of normal mammalian digit formation. The progressive loss of digits upon successively earlier Shh removal mirrors this alternating sequence and highlights Shh's role in cell expansion to produce the normal digit complement.

A) Briefly describe the expected outcome of adding a polarizing region to the anterior of a limb bud if *shh* only acted as a morphogen and was NOT required for cell proliferation. **(2 points)**

B) Please describe the experiments that these authors should have carried out to temporally delete the mouse Shh gene. Please assume that all of the reagents that they may have needed were available. In your answer, make sure to briefly describe the key elements of the conditional *shh* knock-out construct and how temporal control using a "conditional Cre line", as mentioned in the abstract, is achieved for knocking out *shh* at different time points in limb development. **(8 points)**

Question 10

The neuron-specific protein that you are studying, neuron-specific protein 1 (NSP1), is readily detectable when you perform a western blot on lysate prepared from an N2A neuronal cell line but is not detectable in lysate prepared from a fibroblast cell line. Analysis of RNA from the same cell lines indicates that the mRNA transcript encoding NSP1 is also undetectable in the fibroblast cell line but is readily detected in the N2A cell line.

A) Design an experimental approach to determine whether the absence of NSP1 expression in the fibroblast cell line is due to transcriptional or post-transcriptional regulation. **(2 points)**

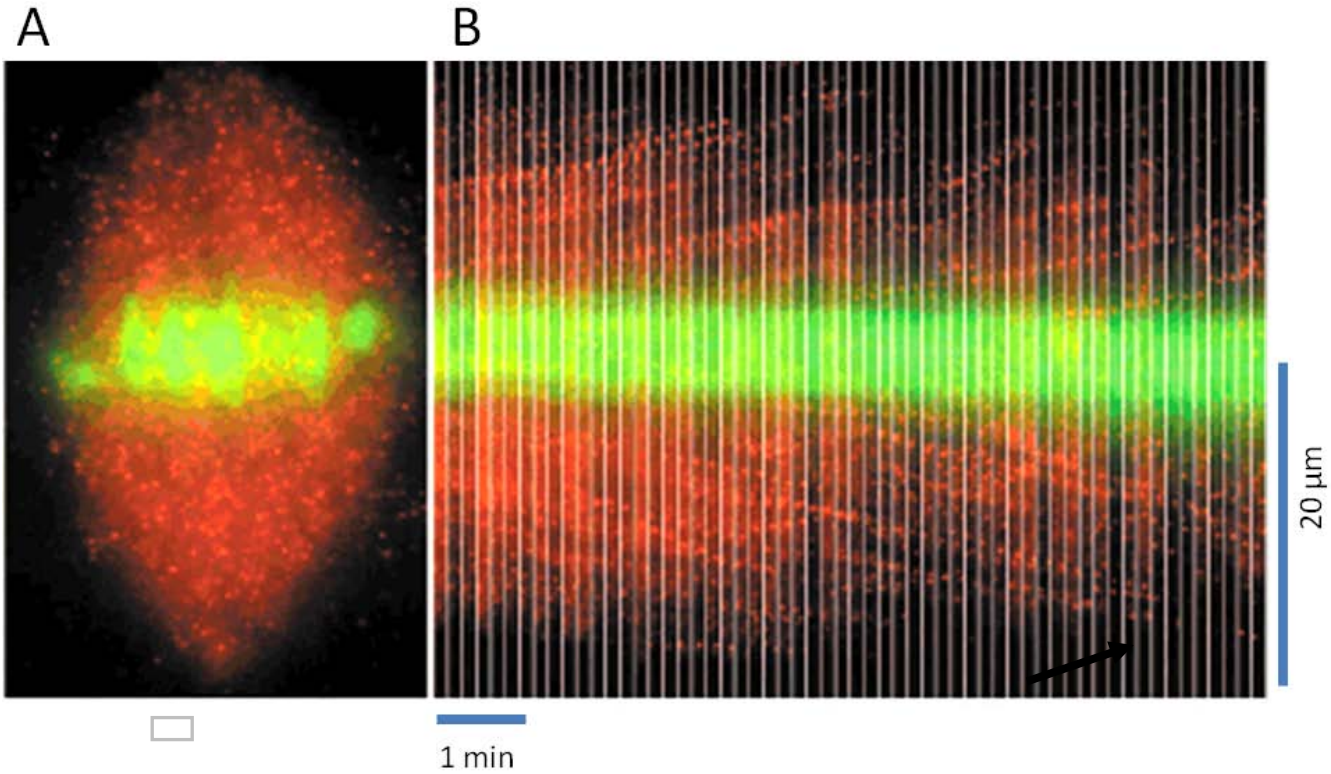
B) From the experiment in **(A)**, you find that *NSP1* transcription is, in fact, decreased in the fibroblast cell line relative to the N2A cells. The transcription factor, TrnX, is the only known trans-activator for *NSP1* and a single well-characterized (10-base pair) TrnX binding site is present in the promoter of the *NSP1* gene. You show by western blot analysis that TrnX protein is expressed at equal levels in both the neuronal and fibroblast cell lines. You hypothesize that TrnX should be able to bind to this sequence. Design an experiment to test your hypothesis that the TrnX protein can bind directly to the 10-base pair TrnX binding site found in the *NSP1* gene promoter. Be sure to include appropriate controls to demonstrate the specificity of this direct, biochemical interaction. **(3 points)**

C) Your experiment shows that TrnX can recognize the specific TrnX binding sequence found in the *NSP1* promoter. Now you need to test to see whether TrnX is bound to this site in the fibroblasts and in the neuronal cell line. Describe an experimental approach to assess TrnX binding to the promoter region in the different cell lines. Be sure to mention appropriate controls. **(2 points)**

D) Your experiments from **(B and C)** indicate that while TrnX can bind directly to the TrnX sequence found in the *NSP1* promoter, you only detect this binding in the neuronal cell line and not in the fibroblast cell line. You sequence the *TrnX* gene in both cell lines and find no polymorphisms or sequence changes that could explain your results. There are also no changes in the TrnX binding site or the whole *NSP1* gene when you compare the two cells lines. Propose a model to explain your results (which are correct) and describe an experimental approach to test your model. Be sure to include appropriate controls to explain the differences that you observe between the two cell lines. **(3 points)**

Question 11

Visualization of the dynamics of individual microtubules in a metaphase spindle studied by "fluorescence speckle microscopy" in live cells



(From Mitchison and Salmon, 2001 Nature Cell Biology)

Panel A: Fluorescence micrograph of a metaphase mitotic spindle in a living cell. Chromosomes are stained in green and speckled microtubules are red. **Panel B:** The movement of individual speckles within the boxed region (A, black arrow) can be followed by time lapse video microscopy and images pasted side by side (Kymograph-like). The results indicate that individual speckles move continuously toward the spindle poles (represented by diagonal lines parallel to the red arrows). The data shown was restricted to metaphase, and during this period the spindle did not change in length and the chromosomes did not move relative to the speckles. Although not illustrated here, the cell subsequently completed mitosis and cell division normally.

A) Based on the kymograph in Panel B, please calculate the approximate rate of movement of MT speckles towards the pole. **(1 point)**

B) Describe the principle and advance of "fluorescence speckled microscopy" in live cells. Illustrate as necessary, and assume you have already prepared purified rhodamine-tubulin. **(2 points)**

Continue on the next page

Question 11 Continued from the previous page

C) What is the orientation of a kinetochore microtubule relative to the spindle structure? Illustrate as necessary. **(2 points)**

D) Postulate a mechanism for the observed motion of microtubule speckles. Please design a simple experiment to test if GTP hydrolysis is involved in this process. **(2 points)**

E) Although not included in the figure, the cell proceeded through mitosis and cell division. During anaphaseA, chromosomes move at 10 μm/min, yet the microtubule speckles continue to move at the rate you have calculated. Based on this information, where is the primary site of tubulin subunit loss in kinetochore microtubules during anaphaseA, and what is the basis for your answer? **(2 points)**

F) Please describe an alternative experimental approach that can observe the same movement of tubulin towards the spindle pole. **(1 point)**

Question 12

Table 1 | Mutations identified in known genes for intellectual disability or related disorders

Family	Gene	Mutation	Diagnosis, clinical features
8500306	*AHI1*	R329X	Joubert's syndrome 3
M332	*AHI1*	R495H	Joubert's syndrome 3
M254	*AP4E1*	V454fs	Microcephaly, paraplegia
M004	*AP4M1*	E193K	Microcephaly, paraplegia
M324	*BBS7*	533del2aa	Bardet–Biedl's syndrome
M107	*CA8*	R237Q	Ataxia, cerebellar hypoplasia
M175	*COL18A1*	L1587fs	Knobloch's syndrome (eye and brain development)
G026	*FAM126A*	Splice site*	Hypomyelination-cataract
M198	*FOLR1*	Splice site*	Folate receptor deficiency
M165	*HEXA*	C58Y	Psychomotor delay, mild Tay–Sachs' disease
8600276†	*L2HGDH*	R335X	Hydroxyglutaric aciduria
M142	*MED13L*	R1416H	Non-syndromic ID, no cardiac involvement
8600486	*NAGLU*	R565Q	Sanfilippo's syndrome, MPS IIIB
8500234	*PDHX*	R15H	Pyruvate dehydrogenase defect
M331	*PEX6*	L534P	Peroxisome biogenesis disorder
8307998	*PMM2*	Y106F	Glycosylation disorder CDG Ia
8600273	*PRKCG*	V177fs	Spinocerebellar ataxia 14
M146	*PRKCG*	D480Y	Spinocerebellar ataxia 14
8600162	*PRKRA*	S235T	Non-syndromic ID
8600042	*SLC2A1*	V237M	Non-syndromic ID
8700017	*SRD5A3*	Y169C	Kahrizi's syndrome, CDG
M069†	*SRD5A3*	A68fs	Kahrizi's syndrome, CDG
G008	*SURF1*	W227R	Leigh's syndrome, very mild form
8600041	*TH*	R202H	infantile parkinsonism, Segawa's syndrome
M017N	*VRK1*	R133C	Pontocerebellar hypoplasia
M196	*WDR62*	G705G	Microcephaly, cerebellar atrophy

Common diseases are often complex because they are genetically heterogeneous, with many different genetic defects giving rise to clinically indistinguishable phenotypes. This has been amply documented for early-onset cognitive impairment, or intellectual disability, one of the most complex disorders known. The more frequent forms of intellectual disability are autosomal yet the genes involved are mostly unknown. Next-generation sequencing in 136 consanguineous families with autosomal-recessive intellectual disability reveals novel mutations in single disease-causing variants in 50 novel candidate genes (Table 1).

A) You want to prove that one of the mutations is causally involved in the disease it is associated with. Pick one mutation/disease and describe an experimental strategy designed to test your idea **(4 points)**. Be specific with respect to your experimental system and your interpretation of the outcome.

B) The conservative change of Y106F mutation in the gene *PMM2* causes a severe syndrome characterized by epilepsy, intellectual disability and macrocephaly. Due to the discrete nature of this mutation, what mechanism would you hypothesize could be impaired by the loss of one –OH group in *PMM2* Y106F? Provide a rationale for your answer **(2 points)**.

Continue on the next page

Question 12 Continued from the previous page

C) Genome-wide analysis of people with cognitive impairment identified three single nucleotide polymorphisms in the non-coding region 5-10 kb upstream of the *PMM2* initiating methionine. How could any one of these polymorphisms affect the expression of *PMM2*? Propose **TWO** hypotheses. Then, select **ONE** of them and propose an experiment to test this hypothesis **(4 points)**.