Q1.b)

Cyclin B is degraded by the ubiquitin pathway, which is the key step governing exist from mitosis and progression into the next cell cycle [1]. More detailed, Cdk1/cyclin B has a direct and major role in the phosphorylation and activation of anaphase promoting complex (APC), and then APC, which has cyclin-ubiquitin E3 ligase activity, provides a negative feedback loop, by which Cdk1/cyclin B triggers its own inactivation at the end of mitosis by degradation of cyclin B. Actually, cyclins are named because of their cycled levels during cell cycle. **Cyclin E** synthesis controls late G1 progression and degradation cyclin E is essential for the cell entering S phase [2]. Cyclin B synthesis is required for the cell entering mitosis and degradation of cyclin B is the key step that controls exit from mitosis. Cyclin A presents in S and M phase, and removal of cyclin A is carried out by ubiquitin-mediated proteolysis [3]. **Cyclin A** in S phase is important for initiation of DNA replication and cyclin A in M phase contributes to the control of cyclin B stability. **Cyclin D** plays essential roles in the response to mitogens, transmits their signal to the Rb/E2F pathway and progresses cell cycle through G1 phase.

CDK "inhibitors" **$p21^{Cip1}$** and **$p27^{Kip1}$** are negative regulators of cyclin dependent kinases that have functions at cell cycle checkpoints, but also are essential activators of cyclin D dependent kinases, which progress the cell through G1 phase. These CKIs, especially $p27^{Kip1}$, have been found to be degraded through the ubiquitin/proteasome pathway [4]. The degradation event of $p27^{Kip1}$ is necessary for cell cycle inhibition by affecting cyclin D dependent kinases.

p53 is a homotetrameric transcription factor that can induce cell cycle arrest. Generally, mdm2, which has E3 ligase activity, proteasomal degrades p53 by constant ubiquitination to keep a low level p53 activity which is important for normal cell cycle progression. When the cell is under stress, ARF, which is alternatively translated from *INK4a* locus, interacts with mdm2 and neutralizes mdm2's inhibition of p53, which leads to cell cycle inhibition.

Q1.c)

Rb is a nuclear phosphoprotein that arrests cells during the G1 phase of cell cycle by repressing transcription of genes required for the G1 to S phase transition [5]. This function is through the target of Rb, E2F family of transcription factors.

Rb can directly bind the transactivation domain of E2F, and block the ability of E2F to activate transcription. [6] The E2F transactivation of cell cycle genes is important for cell progression.

Rb can bind to the promoters of cell cycle progression genes as a complex with E2F and actively repress transcription. This repression correlates with histone deacetylation at the promoters and chromatin remodeling complexes formation.

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[1] *Science*, 1991, **349**:132-138.

[2] *Progress in Cell Cycle Research*, 2003, **5**:441-446.

[3] *Cell Mol Life Sci*, 2002, **59**:1317-1326.

[4] *Science*, 1995, **269**:682-685.

[5] *Nature Cell Biology*, 2000, **2**:E65-E67.

[6] *Mol Cell Biol*, 1993, **13**:6501-6508.

Q2.a)

Intrinsic stresses from Rb deficiency and E2F deregulation can activate the intrinsic apoptotic pathway, and p53 is a critical initiator of this pathway. p53 can initiate apoptosis by transcriptionally activating pro-apoptotic Bcl-2 family members (Bax *etc*), and repressing anti-apoptotic Bcl-2 protein (Bcl-2, Bcl-X_L) and IAPs. There is a report in the latest issue of *Nature Cell Biology* shows that interaction of p53 with Bak (a member of Bcl-2 family protein) causes oligomerization of Bak and release of cytochrome c from mitochondria [7]. However, p53 can transactivate other genes that may contribute to apoptosis including *PTEN, Apaf-1 etc*, and can transcriptionally activate both CD95 and TRAIL receptor 2 thereby sensitizing cells to death-receptor-mediated apoptosis. p53 may also have transcription-independent activities that progress cell death. [8] In a word, p53 functions as a master regulator of the apoptosis program. Rb deficiency and E2F deregulation increase the stress on p53 by activating ARF, and progress cells more easily to apoptosis by a p53-dependent pathway.

Actually, unrestrained E2F activity can result in the accumulation of caspase proenzymes through a direct transcriptional mechanism [9]. Loss of Rb and increased E2F expression coordinately increase caspases (casp-8, casp-9, casp-3 and casp-7) expression. Although not sufficient for apoptosis, high levels of signaling caspases may increase the probability and the proceeding efficiency of apoptosis.

Therefore, Rb deficiency results in increased apoptosis in cells by p53-denpedent and p53 independent pathways. Both mechanisms are correlated with deregulation of E2F.

Q2.b)

Neural development is a complex process, involving cell proliferation, differentiation, and apoptosis. The nervous system is initially generated with an excess number of neurons, half of which are eliminated by apoptosis during a restricted embryonic period. Mature neurons are in a state of terminal differentiation and incapable of undergoing cell division. The cell cycle apparatus is incomplete in mature neurons. [10] As we know that the orderly progression of cell cycle is driven by sequential activation of CDKs to pass checkpoints. While in postmitotic neurons lacking the required components for cell cycle regulation, the dynamic balance between cyclins, CDKs and CKIs, which helps cell cycle repression and DNA damage repair, doesn't exist, and causes the neurons just like facing the gulf.

All the cell cycle repression falls on Rb in the neurons, as G1/S checkpoint is often the prime target for cell cycle regulation. This consequence is consistent with the phenomena that the central and peripheral nervous systems are both tissues that normally express high levels of Rb [10], and the levels of Rb in the neurons decrease dramatically as the apoptotic program unfolds [11].

In Rb^{Null} mice, E2F and selective genes are deregulated. Usually, deregulation of cell cycle can either directly trigger apoptosis or increase sensitivity to apoptotic inducers, especially in the neurons, where no other cell cycle inhibitor is "available". So it is wise for Rb^{Null} mice to eliminate damaged cells simply by apoptosis.

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[7] *Nature Cell Biology*, 2004, **6**:443-450.

[8] *Cell*, 2002, **108**:153-164.

[9] *Nature Cell Biology*, 2003, **5**:28-33.

[10] *Cell Tissue Res*, 2001, **305**:217-228.

[11] *J Neurosci*, 1999, **19**:8747-8756.

Q3.a)

Maternal RNA, which is pre-cleaved and capped with ^{7m}G at 5' end, usually has a very short 3' poly(A) tail. The maternal RNA translation is repressed by a CPE-mediated mechanism, which requires the 5' cap. This form of maternal RNA is the translationally inactive form stored in the oocyte cytoplasm.

Progesterone binds the receptor on the oocyte surface, and leads to a transient but essential decrease in cAMP concentration, which is followed by activation of Eg2 (Aurora family of protein kinase). Active Eg2 phosphorylates CPEB and causes CPEB to recruit CPSF into an active cytoplasmic polyadenylation complex. Poly(A) polymerase is recruited to the complex, which leads to the cytoplasmic polyadenylation and translational activation of specific mRNA. [12]

Translational regulation is important for oocyte maturation. During oocyte maturation, different maternal RNA must be precisely spatio-temporally regulated, which is achieved by 5'/3' UTR consensus sequences mediated RNA translocation and cytoplasmic polyadenylation. During maternal RNA translocation, specific RNA is well regulated by repression and activation at certain site at an appropriate stage.

After fertilization, the cell enters embryogenesis. Comparing to oocyte maturation, the cell needs to progress in cell cycle. The *cis* element directed deadenylation leads to specific translation repression, which is very important for the cell to go to differentiation.

Q3.b)

As there is no CPE in the *cyclin Bx* mRNA, *cyclin Bx* is regulated by cytoplasmic polyadenylation independent pathway. My preferred hypothesis is about how microRNA regulates *cyclin Bx* mRNA translation.

HYPOTHESIS: a gene named *anti-Bx* encodes a microRNA, binds the 3' UTR of *cyclin Bx*, and represses *cyclin Bx* translation during early stage of meiosis. After certain time, another protein is expressed, and inhibits the expression of anti-Bx to release cyclin Bx mRNA and activate its translation.

EXPERIMENTAL APPROACHES:

I. Competition experiment to approve that *cyclin Bx* regulation is through its 3' UTR. Make different truncations based on the 3' UTR sequence of *cyclin Bx*, and microinject them respectively into cells going to meiosis. If my hypothesis is right, when you microinject full length *cyclin Bx* 3' UTR expression vector into the cell, which is also the target of microRNA *anti-Bx*, the cyclin Bx protein expression will be moved up comparing with control, which can be detected by immunoblotting. Through this experiment, you can also identify the exact sequence that the microRNA binds.

II-a. EP element insertion experiment to clone *anti-Bx* gene. According to Julius B. 2003 *Cell* paper, I decide to use transposable elements randomly insert into the genome, screen and find the *anti-Bx* locus. When EP element is inserted at the *anti-Bx* locus, the cyclin Bx protein expression will be moved up.

II-b. Bioinformatics analysis. You got the exact sequence that microRNA binds through Experiment I, you can predict the microRNA sequence, blast the database and find some candidates. Use six ORF analysis to narrow down the pool. This method can be used to valid the results from Experiment II-a.

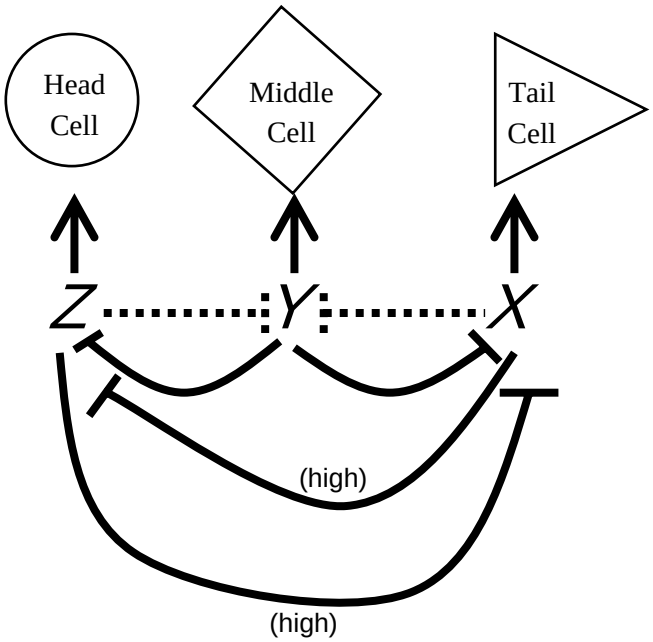
[12] *PNAS*, 2001, **98**:7069-7071.

III. Rescue and frame-shift experiments. After you identify the *anti-Bx* gene, it is better to make a knockout or use the EP element inserted construct, and try to rescue phenotype by expression exogenous *anti-Bx*. It is also valuable to do frame-shift of *anti-Bx* gene to confirm it still can produce functional microRNA after mutation.

IV. *In suit* hybridization and immunostaining. Now it is the time to correlate the cyclin Bx protein expression with the level of *anti-Bx* microRNA. Use RNA probe to label the microRNA and use cyclin Bx antibody to label the protein. If my hypothesis that specific microRNA represses the cyclin Bx protein translation is right, you will find the increase of cyclin Bx expression correlates the decrease of *anti-Bx* microRNA.

It is very important to set appropriate controls during the experiments. If I can get all positive results from these experiments, I will be very convincing about my hypothesis. The next step is trying to identify the protein that regulates *anti-Bx* microRNA. I think using artificial reporter combined with yeast 2/3/4 hybridization may reveal this mystery.

Q5.a)



According to the mutants we have, I draw the genetic scheme shown on the right. My model is that maternal effects generate axial polarity and result in the different mRNA gradients of *X/Z* genes. High concentration of *X/Z* genes produce high concentration of X/Z protein and inhibit *Z/X* genes translation respectively. In the middle of the single cell, *X/Z* genes generate equal amount of X/Z protein, which synergic affect *Y* gene translation (pointed out by the dashed lines). Y protein inhibits X/Z and leads to the middle cell development. As *XZ* double mutant will produce all middle cells, I predict that high concentration of either X or Z will inhibit Y function (pointed out by the dashed lines).

It is better to have both the mRNA and protein distribution of X/Y/Z to build a model. If my model is right, you can find out that Z protein localizes to the head area, Y protein localizes to the middle area and Z protein localizes to the tail area during the wild type single cell stage.

Q5.b)

	Hypotheses 1 (X/Y/Z Nuclear Localization**)**	**Hypotheses 2 (**Z extracellular signaling**)**
Possible amino acid sequence	Nuclear localization signal, which can be grouped into mono-partite and bipartite motifs [13]; DNA binding element, zinc finger *etc*.	Secret signal; Extracellular transport element.
Apply antibody to distinguish two possibilities	I. Centrifuge cell extract, collect the nuclear part, and use antibodies to check the existence of X/Y/Z; II. Immunostaining X/Y/Z, use fluorescent microscopy to check whether they co-localize with nuclear (DAPI stains DNA, Hoechst stains chromatin, antibody against nuclear pore complex *etc*); III. Predict the DNA sequences that X/Y/Z could bind, make radio labeled DNA probe and do antibody super-shift experiments; IV. Use chromatin immunoprecipitation to determine whether X/Y/Z binds a specific DNA sequence. Alternatively, you may try metabolic radiolabel DNA in vivo to test whether X/Y/Z binds DNA.	I. Transient transfect cells to over-express Z protein and check whether there are secreted Z in the culture media; II. Make mutants in the *Z* secret signal sequences to see whether Z accumulates in the cytoplasm by immunostaining or check phenotype change of the organism; III. Add Z specific antibody into the culture media to immunodeplete the secreted Z protein, which inhibits Z's function and will affect the normal cell development.

[13] http://maple.bioc.columbia.edu/predictNLS/

Q5.c)

The definition of MORPHOGEN is any of various chemicals in embryonic tissue that influence the movement and organization of cells during morphogenesis by forming a concentration gradient. [14] Morphogen has graded effects.

I would like to do the following experiments.

I. GAL4-USA system. Suppose that GAL4-UAS system works well in this new organism. I would like to use genomic enhancer to express Z protein ectopically. If Z is the master morphogen, the sites where *Z* ectopically expressed will differentiate into tail cell autonomously. Alternatively, check whether overexpression Z protein will disturb the original Z gradient and influence morphogenesis.

II. Magnetic beads. Coat magnetic beads with Z protein, control the relative location of the bead on the surface of the single cell by magnet, and inspect the development to see whether there will be a tail cell differentiated at the bead-attached site autonomously.

III. Microinjection. Microinject Z protein to different locations in the single cell and check whether it leads the tail cell differentiation or not.

IV. CALI. Make a transgenic organism that only express Z protein tagged with EGFP. Thought the Z protein is still functional, you can use laser irradiation to inactivate the Z protein by some photochemical mechanism and see whether localized destroy of Z protein affects localized cell differentiation.

V. RNAi or immunodepletion. Use siRNA technique to globally inhibit Z protein expression and check whether it can abolish the organism's morphogenesis or not. Actually, add antibody against Z protein into the media may globally repress Z function as well, if Z protein is an extracellular morphogen.

It is very important to set appropriate controls during the experiments. If I can get all positive results from these experiments, I will be very convincing about the hypothesis.

[14] http://education.yahoo.com/reference/dictionary/entries/73/m0427350.html