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RESPONDING TO THE CELLULAR ENVIRONMENT

REVIEW THE CONCEPTS

1. Release of insulin from pancreatic β cells is regulated by elevated blood glucose level (above 5mM), as after a meal. The entry of glucose at the concentration above 5mM into pancreatic islet β cells leads to a change in membrane potential and the release of insulin-containing vesicles. One function of this released insulin is the inhibition of secretion of glucagon from pancreatic α cells; on the other hand, secretion of glucagon is enhanced by signals from the central nervous system when blood glucose should increase.
Insulin decreases blood glucose levels by triggering the uptake of glucose by fat and muscle cells. Glucagon increases blood glucose level by binding to its receptor, primarily on liver cells. This binding induces a rise in cAMP, leading to activation of protein kinase A, which inhibits glycogen synthesis and promotes glycogenolysis, yielding glucose 1-phosphate. Liver cells convert glucose 1-phosphate into glucose, which is released into the blood, thus raising blood glucose back toward its normal fasting level.
2. GLUT4 is an insulin-responsive glucose transporter, found only in fat and muscle cells. (These cells also express the GLUT1 transporter that does not respond to insulin.) In unstimulated cells, GLUT4 is found in vesicles (GSVs) that are tethered to the Golgi matrix by TUG proteins. The binding of insulin to its receptor activates the cleavage of TUG, releasing the GSVs to the plasma membrane, to function in glucose import into cells.
3. Type 1 diabetes mellitus, the form common in children and young adults, results from an autoimmune process that destroys the insulin-producing β cells in the pancreas. Type 2 diabetes mellitus or non-insulin-dependent diabetes, the form with adult onset, results from two factors: a decrease in the ability of muscle, fat, and liver cells to respond to insulin, and then a loss of functional insulin-producing β cells as the body tries to compensate for an elevated glucose level by overproducing insulin.

4. mTORC1 activates the following pathways:
 - enhancing the rate of messenger RNA translation and protein synthesis – and therefore increasing the rate of protein synthesis which produces the components needed for cell growth;
 - enhancing the synthesis of ribosomal RNAs and tRNAs:rRNAs and tRNAs are needed for the step above, to enhanced protein synthesis;
 - stimulating glycolysis – which provides the supply of ATP needed by growing and dividing cells; and
 - inhibiting autophagy - since cells use autophagy to consume their own components for energy and raw material, this process is turned off when nutrients are plentiful and cells are growing and dividing.Overall, mTORC1 upregulates pathways that produce proteins and energy needed for cell division, and turns off a pathway that is active during starvation, when cells cannibalize themselves to generate those components.
5. The levels of only three amino acids - leucine, arginine, and the methionine metabolite S-adenosylmethionine (SAM) - regulate mTORC1 kinase activity. To be active, the mTOR complex needs to be anchored to the lysosomal membrane. This takes place by binding to the RagA and RagC GTP-binding proteins. Only RagA GTP can recruit mTOR to the lysosomal membrane. RagA is regulated by GATOR1, a GAP that converts active RagA GTP to inactive RagA GDP. GATOR1, in turn, responds to the levels of amino acids as follows: when amino acid levels are low, GATOR1 activity is high and further increased by SAMTOR, a SAM-binding protein. When amino acid levels are high, SAM binds to SAMTOR and blocks its activation of GATOR1. GATOR1 activity is further inhibited by GATOR2, a protein that is normally held in check by CASTOR and Sestrin, proteins whose GATOR2-binding is inhibited by Arg and Leu, respectively. With GATOR1 activity blocked, the active RagA GTP can recruit mTOR to the lysosomal membrane, where it can be activate by further signals.
6. Rheb is a small GTP-binding protein. In the cell, Rheb is tethered to the cytosolic surface of the lysosomal membrane. When in its GTP-bound state, Rheb can bind to and activate the mTOR complex that is localized to the lysosome surface by RagA GTP. GEF proteins drive the dissociation of GDP from Rheb GDP, which allows formation of Rheb GTP and the activation of mTORC1. Rheb regulation takes place by a GAP composed of subunits TSC1 and TSC2, which converts the active Rheb GTP to inactive Rheb GDP. The GDP-bound Rheb does not bind mTOR or activate mTORC1 kinase activity. In the absence of this regulation, high level of Rheb GTP can result in high, unregulated activity of mTORC1 – this is found in some cancers and correlate with a poor prognosis.
7. In cells that have adequate levels of cholesterol, SREB is found in the ER membrane, in complex with SCAP and insig-1 (or 2). The interaction between these three proteins retains SREB in the ER membrane and prevents the expression of genes containing sterol regulatory elements. SREBP is comprised of three domains: an N-terminal domain that contains a DNA-binding motif that, when released into the nucleus, functions as a transcription factor; a central membrane-anchoring domain; and a C-terminal regulatory domain. The C-terminal domain of SREB is bound to the C-terminal domain of SCAP. SCAP also contains a sterol-sensing central domain, and an N-terminal domain that is bound to insig-1(or 2). When cholesterol binds to the sterol-sensing domain in SCAP, SCAP assumes a conformation such that allows it to bind to insig-1(or 2). This interaction induces a conformational change on the SCAP-cholesterol that blocks its binding to the Sec24 subunit of the COPII vesicle coat complex This prevents incorporation of the SCAP-SREBP complex into COPII ER-to-Golgi transport vesicles, where the N-terminal DNA binding motif would be cleaved and released. As a consequence the SCAP-SREBP complex is retained in the ER and the expression of genes that contain sterol regulatory elements are kept in check.

8. At low cholesterol levels, SCAP releases its bound cholesterol. Since insig-1(2) cannot bind to the cholesterol-free SCAP, the SCAP-SREBP complex is free to move from the ER to the Golgi complex via COPII vesicles. SREBP is then cleaved sequentially at two sites by two proteases that are resident in the Golgi membrane. The resulting nSREBP can translocate to the nucleus and activates transcription of genes containing sterol regulatory elements (SREs) in their promoters, resulting in the transactivation of specific genes. SREBP-1a and SREBP-1c preferentially activate transcription of genes required for fatty acid and triglyceride synthesis. SREBP-2 primarily activates transcription of genes required for cholesterol synthesis and uptake (e.g. LDL receptor and HMG-CoA reductase).
9. nSREBP (nuclear SREBP) is generated by a two-step cleavage of the SREBP protein in the Golgi membrane, resulting in the N-terminal bHLH-containing domain of SREBP being released into the cytosol. This nSREBP protein is then translocated into the nucleus, where it activates transcription of genes containing sterol regulatory elements (SREs) in their promoters.
10. Hif-1 α activates the synthesis of vascular endothelial growth factor (VEGF). This is a secreted hormone that stimulates the formation of capillaries. These tiny blood vessels increase oxygen delivery to the surrounding tissues. Hif-1 α also activates multiple genes encoding glycolytic enzymes, and specifically - genes that encode several proteins that catalyze regulated and rate-limiting steps in the glycolytic pathway. Activation of these genes promotes ATP synthesis in an oxygen-independent manner.
11. There are two checkpoints for Hif-1 α inactivation. At oxygen levels just above 0.5 percent, the asparaginyl hydroxylase FIH becomes activated and transfers an OH group to a specific asparagine residue in the C-terminal transactivation domain of the Hif-1 α protein; this blocks the interaction between the HRE-bound Hif-1 α and the transcriptional co-activators CBP and p300, which blocks transcription of hypoxia-responsive genes.
The second checkpoint is activated at slightly higher (above 4 percent or ambient) oxygen concentrations. The PHD2 prolyl hydroxylase becomes activated and adds a hydroxyl group to two proline residues in the Hif-1 α protein. Each of these residues then forms a part of a separate binding site for the VHL subunit of an E3 ubiquitin ligase, which ubiquitylates Hif-1 α and targets it for proteasomal degradation.
12. All ERF proteins have a cysteine residue at their N-terminus. At ambient oxygen levels, this residue undergoes a series of enzyme-catalyzed reactions, which results in the targeting of the ERF for proteasomal degradation. First, in an oxygen-sensing step, two oxygen atoms are transferred to the N-terminal cysteine residue of ERF, forming cysteine sulfinic acid. This cysteine sulfinic acid, in turn, serves as a binding site for an enzyme that catalyzes the transfer of the arginine residue from an arginyl tRNA to the amino group of the cysteine sulfinic acid. Proteins with N-terminal arginine residues are unstable, and ERF is immediately subjected to ubiquitination and degradation in the proteasome.

13. To optimize structural and enzymatic function, proteins have evolved to be conformationally flexible at the normal growth temperature of the organisms in which they occur. Because of this, many proteins are only marginally stable at the normal growth temperature and a small increase in temperature can cause protein unfolding.
14. HSPs (notably HSP70) bind to misfolded regions of polypeptides, unfold that region, and then release it to allow proper refolding. The protein-binding domain of an HSP70 recognizes and binds a sequence of a specific seven mainly hydrophobic amino acids that normally only occur only in the hydrophobic core of properly folded proteins or in transmembrane helices. Exposed hydrophobic stretches like this indicate a misfolded protein. HSP70 is initially weakly bound to an unfolded region of the client protein. An ATP → ADP hydrolysis results in a conformational change of the HSP70 protein-binding domain and an unfolding of the bound region of the client protein into a conformation that can spontaneously refold when it is released. The client protein is then released. If it does not successfully fold into the proper conformation, it is rebounded by HSP70 and the cycle repeats until proper conformation is achieved.
15. In unstressed cells, most HSF1 is located in the cytosol in complex with HSC70s, which are in excess over HSF1. This association of HSP70 with HSF1 inhibits HSF1 import into the nucleus by blocking its interaction with importins. In vertebrates, HSP70 also dissociates a large fraction of the HSF1 trimers into inactive HSF1 monomers bound by HSP70. Only following heat shock, when HSP70 is bound to misfolded peptides and pools of free HSP70 are depleted and not available to bind to HSF1 can HSF1 translocate to the nucleus and activate genes with promoter proximal response elements.
16. The key genes/proteins of the circadian clock in *D. melanogaster* are: two transcriptional repressors, PERIOD (PER) and TIMELESS (TIM), encoded by the *per* and *tim* genes; two transcriptional activators, CLOCK (CLK) and CYCLE (CYC), encoded by the *clock* (*clk*) and *cycle* (*cyc*) genes; the DOUBLETIME (DBT) kinase; an E3 ubiquitin ligase; and CRY, a cryptochrome, encoded by the *cry* gene.
The central components of the clock in *Drosophila* cells make up a negative feedback loop, where the expression of some of the *per* and *tim* genes is inhibited by their own protein products. The levels of PER and TIM are lowest around in the first part of the day. Around noon, the expression of the *per* and *tim* genes is induced by the CLK and CYC heterodimer, which binds the *per* and *tim* promoters and drives the expression of PER and TIM in the cytoplasm during the daylight hours. Around dusk, PER and TIM are at their highest levels and are imported into the nucleus, where they bind to CLK/CYC heterodimer and block its ability to stimulate transcription of the *per* and *tim* mRNAs throughout the night, when PER and TIM levels start to fall. At dawn, the CRY-photosensitive protein is stimulated by light and binds to TIM, releasing it from both PER and CLK/CYC. PER and TIM are then shuttled to the cytoplasm for DBT phosphorylation and targeting for degradation by ubiquitin ligases, and CLK/CYC can once again bind to and transactivate *per* and *tim* promoters, restarting the cycle.
17. The circadian clock in the cyanobacteria consists of three proteins: KaiA, KaiB, and KaiC. KaiC possesses intrinsic kinase and phosphatase activity. At dawn, KaiC exists in a dephosphorylated state. Over the next six hours, it binds KaiA, which activates its kinase activity, and KaiC autophosphorylates itself at two residues. Over the following six hours, one of the residues in KaiC becomes dephosphorylated which leads to the binding of the KaiB protein. Then, over the next six hours, KaiA is released. KaiB binding to KaiC inhibits its kinase activity, leading, within the final six hours of the 24-hour cycle, to dephosphorylation of KaiC and release of KaiB.

18. A zeitgeber is an external cue that can entrain the organism's endogenous, self-running clock to regulate the organism's circadian rhythm. The zeitgeber can either entrain the current circadian rhythm or reset it. Some examples of zeitgebers include light, medication, temperature, social interactions, exercise, eating and drinking, and weather.
19. The Hippo protein kinase pathway controls the sizes and morphologies of several organs during embryonic development. It is also required for regeneration of damaged tissues and organs in many after injury.
20. The type of substrate on which a cell is plated in laboratory experiments affects the abundance of actin stress fibers in the cell. Cells that are plated on a stiff substratum contain extensive actin stress fibers (that can be experimentally visualized by staining with the fluorescently labeled phalloidin). In these cells, the Hippo pathway is off. By contrast, no actin stress fibers are observed in cells plated on a soft substratum, and in these cells Hippo signaling is active (as indicated by the presence of cytoplasmic YAP). Similar phenomena are observed in cells that are allowed to spread out on a fibronectin-coated slide or have to adhere to only a small area of the slide – spreading out induced stress fibers and turns off the Hippo pathway. This suggests that information about the ECM is communicated via actin stress fibers.
In cells grown on stiff substratum or allowed to spread out, YAP and TAZ were nuclear. In cells on a soft substrate or forming focal adhesion, YAP and TAZ are cytoplasmic.
21. The Hippo pathway is active only in the inner cells of the morula - the cells that are surrounded on all sides by other cells and connected to them by adherens junctions. In these cells angiomotin (Amot) is associated with adherens junctions, and the Nf2 protein, on all cell surfaces. In this adherens-junction-associated protein complex, a specific Amot serine residue becomes phosphorylated by the Hippo pathway protein kinases LATS1 and 2. The phosphorylation of Amot stimulates the phosphorylation of YAP/TAZ by LATS1/2, and causes the retention of YAP/TAZ in the cytosol. No differentiation program is initiated in these cells.
The Hippo pathway is inactive in the outer cells of the morula – the cells that have a free surface not attached to other cells. Under this free segment of the plasma membrane are long filaments of F-actin. These filaments sequester the Amot protein, which can no longer activate the Hippo pathway. As a result, YAP/TAZ are imported into the nucleus where, together with a TEAD, they activate transcription of genes that initiate the trophectoderm differentiation program.