BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 11)

Your Name : _____ Grade : _____

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EXERCISE 1 "GENETIC NETWORKS IN BACTERIAL SPORULATION" :

1. Bacterial spores are metabolically inactive. However, the cytoplasm of bacterial spores is densely packed with ribosomes, which are more abundant in spores than in actively growing cells. Explain why.

Ribosomes are required for synthesis of all the cell's proteins, including ribosomal proteins. When the last ribosome is degraded (or inactivated) the cell is effectively dead (incapable of resuming growth). Despite being metabolically inactive, spores can remain viable (capable of germinating and resuming vegetative growth) for millions of years. During this "long wait" there is, despite the spores defenses, slow accumulation of damage (oxidative, chemical, light, etc.), including damage that may inactivate ribosomes. Thus, the larger the number of ribosomes packed into the spore the greater the chance that at least one ribosome will survive and allow the cell to reactivate when environmental conditions become favorable for growth again.

2. The *spoIIA* operon encoding *spoIIAA* (anti-anti-σ), *spoIIAB* (anti-σ), and *spoIIAC* (σF) is located next to the chromosomal replication *terminus*. Explain how this chromosomal location contributes to activation of σF in the forespore but not in the mother cell.

At the beginning of sporulation there are two full-length chromosomes bundled into an "axial filament" that stretches along the long axis of the cell from pole to pole. When the cell wall separating the forespore and mother cell compartments is created, the first chromosome is located entirely in the mother cell, while the second chromosome is divided between the forespore and mother cell. Specifically, the origin-proximal 30% of the second chromosome is located in the forespore while the terminus-proximal 70% of the second chromosome is located in the mother cell. Since the spoIIA operon is located next to the terminus, this means that there are 2 spoIIA operons in the mother cell and 0 spoIIA operons in the forespore. Consequently, de novo transcription and translation of the spoIIA genes continues in the mother cell but does not occur in the forespore. Because the SpoIIAB (anti-σ) protein is unstable, it is rapidly degraded in the forespore, resulting in release and activation of the SpoIIAC (σF) protein, which is stable. Free σF can then bind to RNA polymerase and activate transcription from σF-specific promoters. Meanwhile, synthesis of the SpoIIAB (anti-σ) protein continues in the mother cell; therefore, the SpoIIAC (σF) protein continues to be bound to the SpoIIAB (anti-σ) protein in the mother cell and is consequently unable to bind to RNA polymerase, which prevents transcription from σF-specific promoters in the mother cell. After about 15-20 minutes, the remaining terminus-proximal 70% of the second chromosome is pumped into the forespore by the SpoIIIE protein to ensure that the mature spore receives a full chromosome, but by then σF is no longer needed because it has already finished activating the downstream σ factors in the σ-factor cascade.

3. Sporulation involves a "sigma (σ) factor cascade". This motif is common in differentiation programs in bacteria. Explain: what are sigma factors, what is their primary biochemical and biological function, and what is a "sigma factor cascade"?

Sigma factors are proteins that bind to the core RNA polymerase and direct them to bind with high specificity to promoter regions in the chromosome. In the absence of a sigma factor, RNA polymerase has only weak and non-specific DNA binding activity. After binding to a promoter, the promoter DNA is isomerized from a "closed complex" to an "open complex" and transcription initiates. Then the sigma factor is released from the RNA polymerase and elongation factors bind instead. In most bacterial species, a cell can

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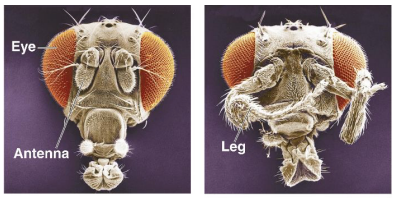
produce many distinct types of sigma factors. Each distinct type of sigma factor recognizes a distinct type of promoter to direct the transcription of a distinct set of genes. In a sigma factor cascade, sigma factors are transcribed in a temporal sequence, one after the other, in which sigma factor A activates sigma factor B, which activates sigma factor C, which activates sigma factor D…and so on (hypothetical example). Thus, the genes controlled by sigma factor A are transcribed before the genes controlled by sigma factor B, which are transcribed before the genes controlled by sigma factor C, which are transcribed before the genes controlled by sigma factor D…and so on. The time-dependent expression of different sets of genes is essential in developmental processes like sporulation, which require that different functions be activated at different points in time. Otherwise, you might end up with legs growing out of your forehead, for example… ;-)



Wild type Mutant

By analogy: when you build a house, first you dig the cellar, then you make the foundation, then you make the walls, and only then do you make the roof. You do not make all parts at the same time, or start with the roof…

4. If you mutate (inactivate) the gene encoding anti-anti-σF, what effect do you predict this would have on σF activation in the prespore? Explain.

I predict that inactivation of the spoIIAA gene encoding anti-anti-σF would delay (or maybe even prevent) the activation of σF in the prespore. The job of the anti-anti-σF is to bind to anti-σF and thereby displace it from σF; free σF can then bind to RNA polymerase to direct it to the promoters of σF-dependent genes. There are other mechanisms that restrict σF activation to the prespore (for example, the transient genetic asymmetry mentioned in problem #2 above), so it is possible that σF might still occur in the prespore, even in the absence of anti-anti-σF, but this activation would presumably be delayed.

5. Positive feedback loops are common genetic network motifs in differentiation programs like sporulation. Do positive feedback loops generate graded responses ("all the same") or threshold responses ("all-or-nothing") to a signal such as nutrient starvation? Explain.

Positive feedback loops tend to generate "all-or-nothing" (threshold) responses. In a positive feedback loop, when a transcription factor is activated by some external signal it begins to activate transcription of its target genes, which include the gene encoding the transcription factor itself. Thus, the more the transcription factor is activated, the more the transcription factor is produced, which leads to higher levels of the activated transcription factor, which leads to still-higher expression of the transcription factor…and so on, in a self-amplifying circuit. A signal that is strong enough to activate the transcription factor above the threshold required for it to bind to and activate its own promoter will become self-amplifying, resulting in a "fully-on" response. Conversely, a signal that is too weak to activate the transcription factor above the threshold required for it to bind to and activate its own promoter will fail to activate the feedback loop and the level of activated transcription factor may gradually decay, resulting in a "fully-off" response.

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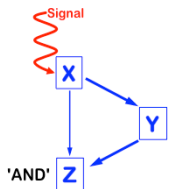
EXERCISE 2 "GENETIC NETWORKS IN BACTERIAL SPORULATION" :

1. Draw a diagram of the "coherent feed-forward loop" genetic network motif and identify each component.



X is a primary transcription factor, which is activated by a signal. In the sporulation system that we discussed in class, this could be σF.

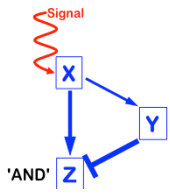
Y is a secondary transcription factor, which is activated by X (in its signal-activated form). In the sporulation system that we discussed in class, this could be one of the transcription factor genes activated by σF.

Z is the target gene, which is activated by X and Y together (as the sum of their inputs). The system is called "coherent" because the inputs on the target gene (Z) are the same in sign: X is a positive input and Y is a positive input.

2. Does the "coherent feed-forward loop" increase or decrease the time for a genetic network's output to reach the steady-state expression level following a stimulus? Explain.

Paradoxically, the "coherent feed-forward loop" can be used to increase the time for a transcription network's output to reach steady state following a stimulus, compared to a "simple system" with no feed-forward or feed-back loops. The key to understanding this is to recall that the level of the activated target gene's product is tuned by evolution to achieve a specific steady-state value that is neither too low nor too high for optimal fitness. In the "simple system" the rate of target (Z) transcription is constant so approach to the steady-state value is hyperbolic. In the "coherent feed-forward loop" system the rate of Z transcription is lower at the beginning (when only X is active) and higher at the end (when both X and Y are active). Consequently, approach to the steady-state value is sigmoidal. This kinetic delay (fast weak activation followed by slow strong activation) is created by the time required for activated X to bind to the promoter of the Y gene and transcribe the Y gene, and for the mRNA encoding Y to be translated into protein, which can then diffuse to the Z gene and activate it.

3. Draw a diagram of the "incoherent feed-forward loop" genetic network motif and identify each component.



X is a primary transcription factor, which is activated by a signal. In the sporulation system that we discussed in class, this could be σF.

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Y is a secondary transcription factor, which is activated by X (in its signal-activated form). In the sporulation system that we discussed in class, this could be one of the transcription factor genes activated by σF.

Z is the target gene, which is activated by X but repressed by Y (as the sum of their inputs). The system is called "incoherent" because the inputs on the target gene (Z) are opposite in sign: X is a positive input but Y is a negative input.

4. Does the "incoherent feed-forward loop" increase or decrease the time for a genetic network's output to reach the steady-state expression level following a stimulus? Explain.

Paradoxically, the "incoherent feed-forward loop" can be used to decrease the time for a transcription network's output to reach steady state following a stimulus, compared to a "simple system" with no feed-forward or feed-back loops. The key to understanding this is to recall that the level of the activated target gene's product is tuned by evolution to achieve a specific steady-state value that is neither too low nor too high for optimal fitness. In the "simple system" the rate of target (Z) transcription is constant so approach to the steady-state value is hyperbolic. In the "incoherent feed-forward loop" system the rate of Z transcription is higher at the beginning (when only X is active) and lower at the end (when both X and Y are active). Consequently, approach to the steady-state value is initially fast, leading to a transient overshoot of the steady-state level of Z that gradually decays back down to the correct steady-state value. This kinetic delay (fast activation followed by slow repression) is created by the time required for activated X to bind to the promoter of the Y gene and transcribe the Y gene, and for the mRNA encoding Y to be translated into protein, which can then diffuse to the Z gene and repress it.

5. The "incoherent feed-forward loop" can also function as a "pulse generator". Explain how this works.

In the "incoherent feed-forward loop" activation of Z by X is fast and repression of Z by Y is slow. This kinetic delay (fast activation followed by slow repression) is created by the time required for activated X to bind to the promoter of the Y gene and transcribe the Y gene, and for the RNA encoding Y to be translated into protein, which can then diffuse to the Z gene and repress it (see my answer to question #4 above). The relative strengths of these interactions, X-to-Z and Y-to-Z, can be tuned by evolution. If the repressing effect of Y on Z completely dominates over the activating effect of X on Z, then initially (when there is not yet any Y in the system) X will activate Z but later (after X activates the Y gene and Y protein accumulates) Y will completely repress Z. The resulting pattern is a pulse: first off (before the signal arrives to activate X), then on (activated X alone) then off again (both activated X and Y are present but Y completely dominates). Note that the pulse (off-on-off) occurs even if the signal persists! Which is a pretty nifty piece of microbial engineering, in my opinion…