Transcription

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Transcription

▼ What are the key structures of bacterial RNA polymerase

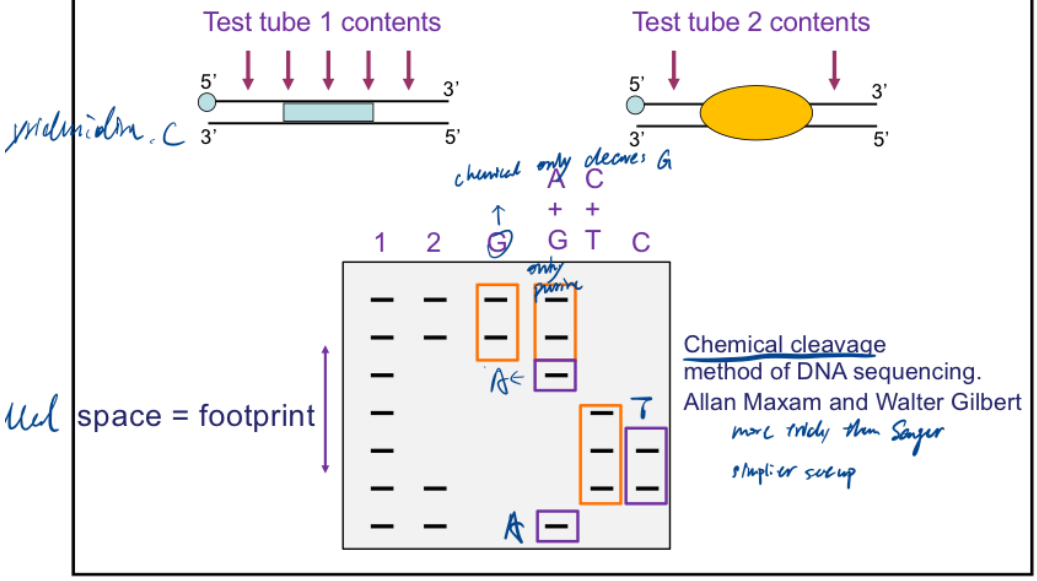
- Core enzyme:
  - two identical alpha subunits binding at the most upstream sequences: promoter recognition
  - Beta subunit — bind to the coding strand — **Catalytic domain**
  - Beta’ subunit — bind to the template strand
- Sigma subunit: recognises and binds to specific DNA sequence
  - binds to the promoter region: **-35 — -10 region**
    - -35: recognition site
      - The -35 region typically contains a conserved DNA sequence with the consensus sequence 5'-TTGACA-3'. This sequence is **recognized by specific amino acid residues within the sigma factor**
    - -10: **orients** RNA polymerase holoenzyme correctly
- Together forms the RNA polymerase holoenzyme!
- Other factors associate once sigma subunit is bound

▼ Experimental evidence to identify the base sequence that has the protein bound

DNase 1 footprint

- DNase 1
  - catalyzes the hydrolysis of phosphodiester bonds within double-stranded DNA molecules, resulting in the **cleavage of DNA into smaller fragments**
- Chemical cleavage: method that only cleaves the DNA at specific base
- Radioactive labelled DNA
- Set up:
  - Test tube 1: -ve control : DNA + DNase 1
  - Test tube 2: + ve control: DNA + DNase 1 + RNA polymerase
  - Test tube 3, 4, 5: add DNA + chemical cleavage

Identifying the footprint
i.e. to which base sequence has the protein bound



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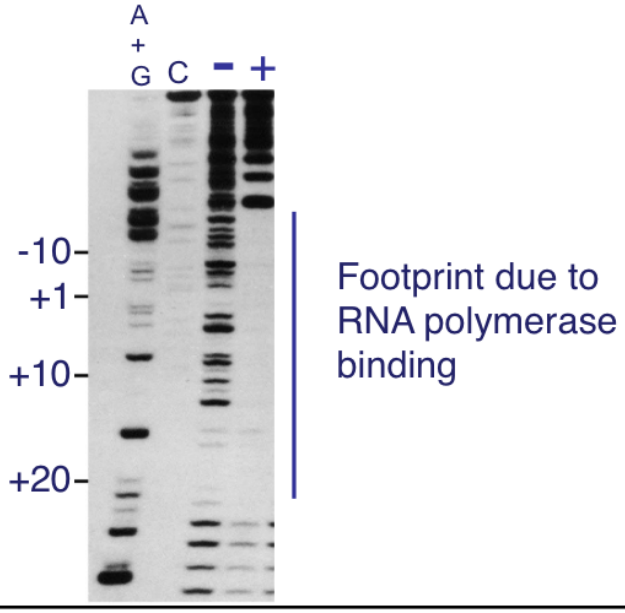
DNase 1 footprint experiment identifies region to which RNA polymerase binds



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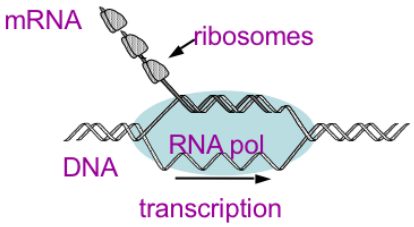
Phases of transcription

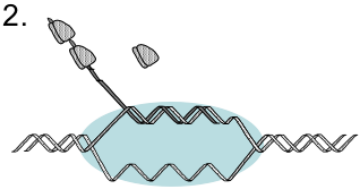
- Initiation
- Elongation
- ▼ Termination
  - ▼ What are the requirements:
    - RNA synthesis is ceased
    - newly synthesised RNA is released
    - RNA pol is dissociated from the DNA template
  - ▼ Rho-independent
    - requires **intrinsic terminator:** specific DNA sequence in the template strand
    1. G-C rich palindromic repeats and poly U sequence are transcribed
    2. Formation of G-C hairpin structure, followed by the poly Us stretch
       a. The hairpin structure is formed by a palindromic sequence
       b. poly U: weak hydrogen interactions (2 per base pair) between A and U
    3. Hairpin structure destabilises RNA-DNA hybrid
    4. The destabilised hybrid generates resistance for RNA pol, which then ceases the transcription elongation
    5. The halt then dissociates RNA pol, and releases RNA transcript
  - ▼ Rho-dependent
    - Requires Rho factor (ρ), which is an **ATP-dependent helicase**
    - Recall that in bacteria, translation occurs simultaneously with transcription

Rho-dependent termination of transcription

1.



2.

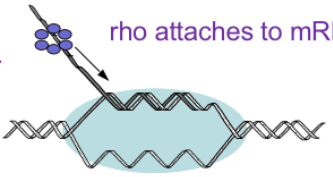


ribosomes reach stop codon

dissociate

3.

rho factor



rho attaches to mRNA at a rho utilization site (rut)

rho is a hexamer of 6 identical subunits

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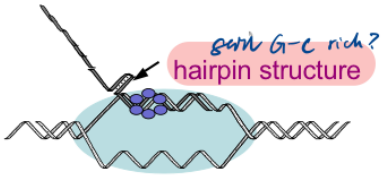
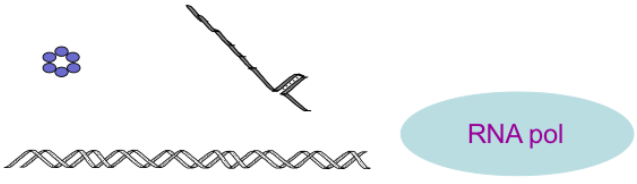
Rho-dependent termination of transcription

4. rho stalls near a hairpin loop



Note: the hairpin is NOT followed by a string of U residues

5. rho has helicase activity –requires ATP
H-bonds between DNA and RNA broken



Transcription complex dissociates

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One stop codon: UGA

Operon

▼ Promoter strength

- The efficiency that RNA polymerase **leaves the promoter region and start synthesising** the full length of transcript from 5′ to 3′ direction
- depends on the **optimal consensus sequence**
- However, the bacteria usually have a weak promoter for regulation

▼ Polycistronic mRNA

- allow for translation initiation at two or more sites along a single mRNA transcript
- so for bacteria:
  - one promoter and operon to transcribe one mRNA, which encodes several genes
    - contains multiple Shine-Dalgarno and AUG sequence, allow for multiple ribosome attachment

▼ **Inducible & Repressible**

▼ Inducible

- In inducible transcription, gene expression is normally turned off or expressed at **low levels under basal conditions**.
- However, in response to specific signals or inducers, transcription of the gene is activated or "induced."
- Inducers can be molecules such as hormones, nutrients, toxins, or environmental factors that trigger the activation of transcription factors or regulatory proteins.
- Example: lac operon — in the presence of lactose, the transcription of genes is turned on to catabolise lactose

▼ Repressible

        - gene expression is normally turned on or expressed at high levels under basal conditions
        - in response to specific signals or repressors, transcription of the gene is repressed or "turned off”
        - Example: trp operon — in the presence of tryptophan in the environment, tryptophan biosynthesis is repressed
- operon is OFF when repressor is bound
- Blue-white screening: beta-galactosidase can dimerise X-gal and then blue-pigment

Lac operon

▼ Operons

- promoter
- operator
- lac z: beta-galatosidase
- lac y: lactose permease to transport lactose into the cell
- lac a : helps remove toxic metabolites, which are then transported out of the cell by lacY protein

▼ Upstream of operon: Promoter and lac i — encoding for the repressor

- repressor: binds to the operator, to prevent RNA pol from binding
- The lac i is constitutively expressed

▼ Negative regulation

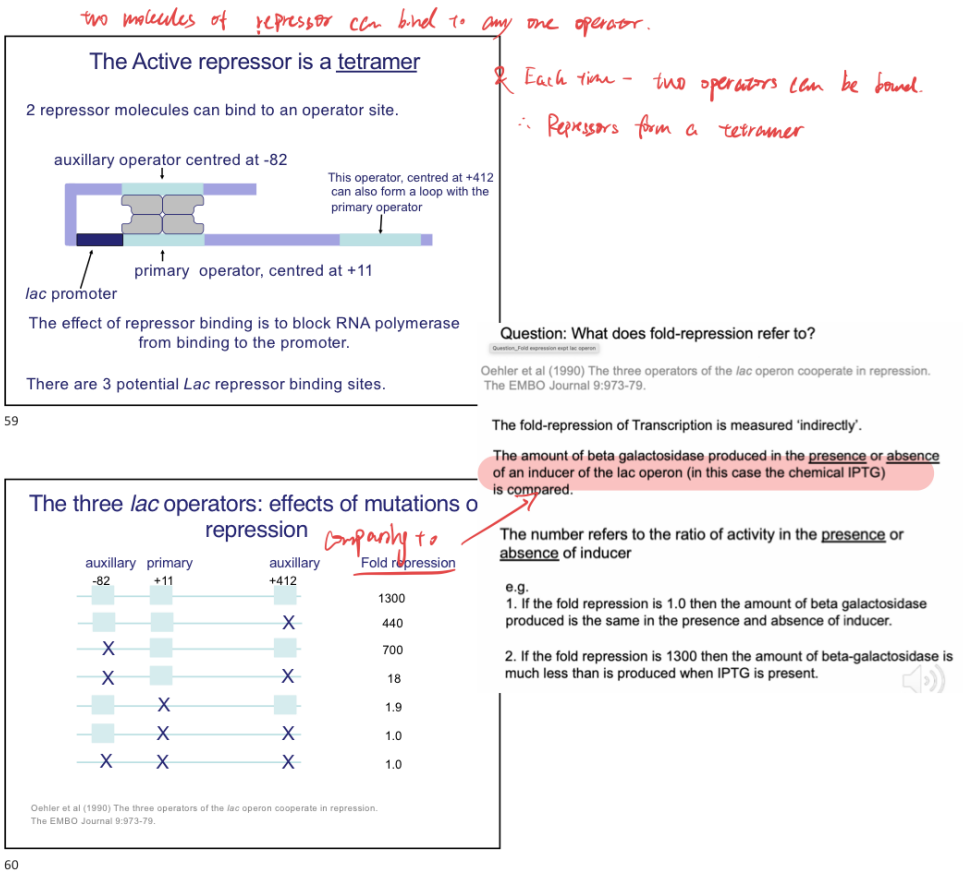
- When lactose is present:
    - The true **inducer** is allolactose
    - Requires basal level of beta-galactosidase activity to isomerise the lactose

    - Also, permease is required to transport the inducer

This indicates that:

- Binding of repressor is never infinitely strong
- Drop off approx. once every cell cycle
- To ensure always have low basal level of lac transcription

- Operator and Repressor
    - Operator sequence is a near perfect palindromic repeat — each operator can bind to two molecules of repressors
    - Lac operon contains three operator regions
        - Primary operator: directly downstream promoter (+11)
        - auxillary operator: -82
        - The active is actually a tetramer — can BEND the DNA
        - 3 rd operator: +142, also forms a loop with primary operator



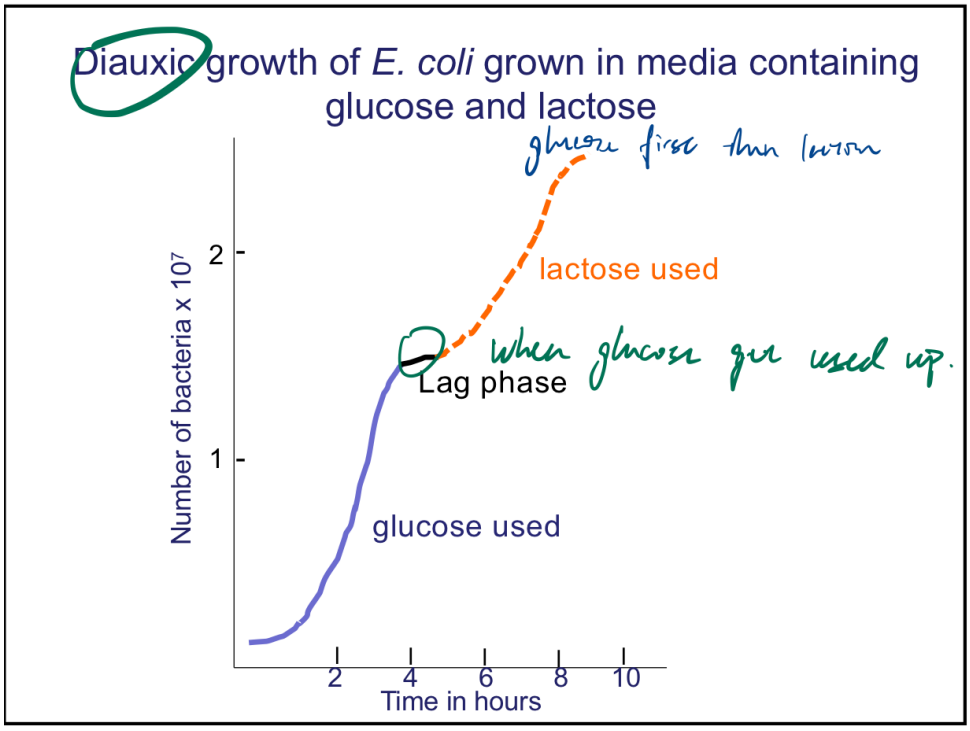
▼ Experimental evidence

To assess the strength of the operator, investigate the effects of single mutated operators, and dual mutated operators on fold-repression

- Fold-repression
  - The number refers to the ratio of activity in the presence to absence of inducer:
    - 1300: the amount of beta-galatosidase in the presence of allolactose is 1300 fold to the amount when the inducer (IPTG) is absence
- Result: fold-expression from largest to smallest
  - 3 operator non-mutated > auxillary operator mutated > 3rd operator mutated > primary operator mutated = all three mutated

▼ Positive regulation

- Because when glucose is present, bacteria can directly utilise is as the nutrient source & glucose metabolism does not require expression of new proteins
- Therefore, when glucose is present, transcription of lac operon is inhibited
- So, when the media contain lactose and glucose, there is a diauxic growth:
  - glucose used up first — lag phase to initiate lac operon transcription — lactose used up



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- When glucose is present:
  - enter the bacteria via the transporter complex called phosphotransferase system
  - In this system, IIAGlc phosphorylates the cytosolic glucose to ensure that the extracellular glucose concentration remains low, as

phosphorylated glucose cannot easily leave the cell

- IIAGlc can also phosphorylate the adenylyl cyclase, but the affinity to it is not as high as to the glucose transporter complex
- Therefore, in the presence of glucose, adenylyl cyclase is not activated — cannot catalyse ATP into cAMP
- [cAMP] low — few bind to CAP (catabolite activator protein)

- When glucose is absent:
  - IIAGlc can activate adenylyl cyclase — convert ATP into cAMP
  - cAMP binds to CAP
  - CAP can now bind to the DNA region upstream of RNA pol binding site
    - interact with the 2 alpha subunit
    - increases the affinity of RNA pol to the “weak” promoter
      - the -10 region does not contain the consensus sequence, allow signal-mediated regulation to adjust the affinity and efficiency, sensitive to environmental nutrient change
    - By bending DNA > 90º around the symmetry — conformational change of DNA
- glucose is present and lactose is absent:
  - cAMP levels are low so cAMP-CAP is not formed
  - lac repressor will be bound to the operator
  - RNA polymerase will not be able to bind and the operon is not transcribed.
- Glucose and lactose are present:
  - lac repressor not bind to the operator but
  - cAMP levels will remain low so cAMP-CAP will not bind to assist RNA polymerase initiation.
  - small amount of lac mRNA
- Glucose is absent and lactose is present:

- lac repressor is not bound and the
- levels of cAMP will rise enabling the formation of cAMPCAP and hence initiation of transcription is possible.
- The genes are now transcribed and lactose can be metabolized by large amount

Experiment: repressor acts as trans; operator acts as cis

Discovery of the regulation of the *lac* operon

1. Isolated mutants that made β-galactosidase even when lactose was absent called 'constitutive' mutants.
2. Two classes of mutants could be distinguished using the *cis-trans* test.
3. Mutants in which the *lac* repressor gene was inactive.
4. Mutants in which the operator site was defective.

understood: repressor

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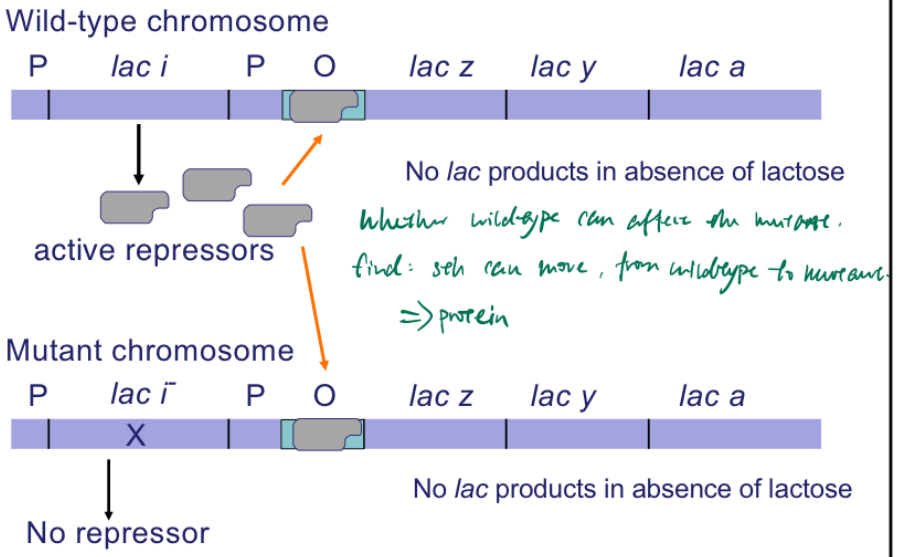
E.coli cells, two operons

Merodiploid cells – One wild-type and one mutant gene



whether wild-type can affect the mutant.
find: sth can move, from wildtype to mutant
⇒ protein

Conclusion: Repressor acts in *trans* sth crossed

but operons

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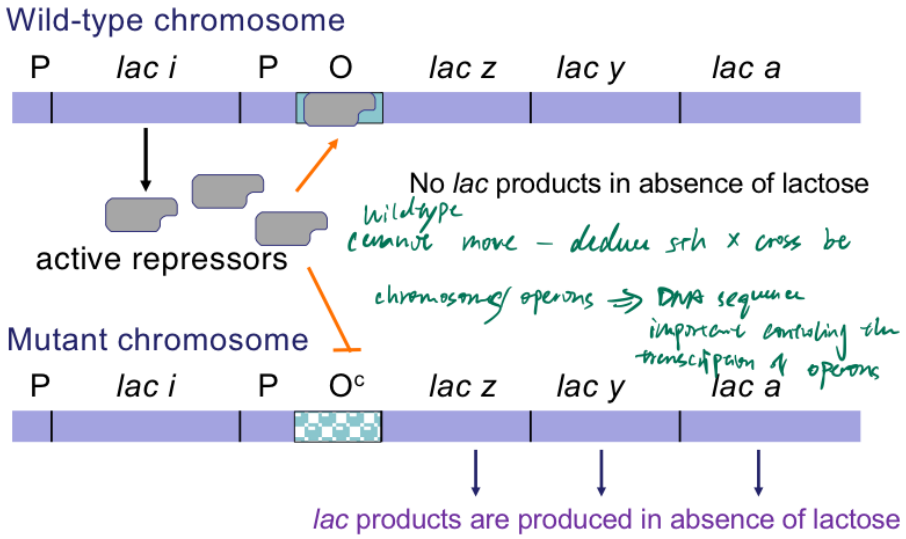
Merodiploid cells – One wild-type and one mutant operon



Conclusion: Operator acts in *cis*

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Conclusion

Laci encodes a protein that acts in *trans* i.e. it can cross the cell to exert its effect.

Operator acts in *cis* i.e. it exerts its effect on its own chromosome (no information flows between the wild-type and mutant chromosome).

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Trp operon

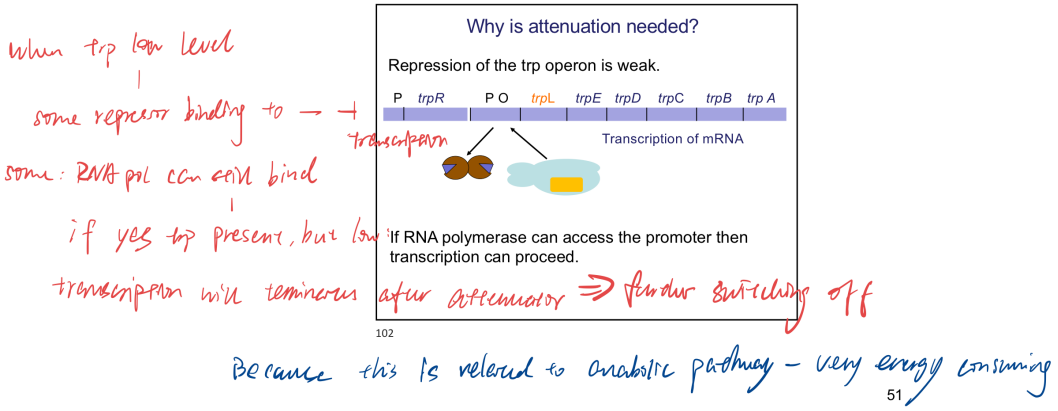
▼ Components

- trp: **co-repressor**
- *trpR* — aporepressor
- trp + aporepressor — active repressor
  - active repressor binds to the operator — block RNA pol from binding to the promoter
- trp operon is under -ve control of the active repressor
- ***trpL* — leader sequence**
- ***trpa* — attenuator sequence**
- trp EDCBA — enzymes for tryptophan synthesis
  - conversion of chorismic acid

▼ Attenuation

- Prevents complete transcription when [trp] is high
- incomplete transcription — only *trpL* is transcribed
- *trpL* encodes for 14 amino acid + *trpa* attenuator
- Attenuation depends on:
  - Within leader-attenuator **mRNA** there are 4 complementary mRNA, **G-C rich sequences**
    - 1 pair to 2
    - 2 pair to 3
    - 3 pair to 4 — attenuation occurs in this case, acts as the transcription terminator
    - after 4, followed by polyU tail, similar to rho-independent termination

- **extent to which a ribosome has translated the leader mRNA**
  - **leader mRNA encodes 2 trp (UGG) at GC-rich region 1**
  - therefore, to proceed the co-translation, cytosol requires adequate tryptophan to bind to the tRNA
- if trp level is low:
  - the ribosome stalls at GC-rich region 1 — region 2 pairs with region 3, forming a temporary stem loop, which **will not leads to the dissociation of RNA pol**
  - This will not lead to the termination of cleavage of mRNA
- if trp level is high:
  - the ribosomes will not stall at region 1, and can dissociate from the mRNA when reach the stop codon
  - therefore, 1 pairs with 2, 3 pairs with 4, forming the similar structure as Rho-independent stem loop
  - RNA pol stops and dissociates from mRNA



▼ Repression + Attenuation — greater control

- absence of trp:
  - repression: the aporepressor will not bind to the operator — transcription occurs

        - attenuation: ribosomes stall at the UGG codons at GC-rich region 1 — no transcription termination
    - Presence of trp:
        - if [trp] is low: although there are some active repressor blocking the operator, some RNA pol can still start transcription from promoter, but:
        - attenuation: leads to transcription termination, the tryptophan synthesis is still switched off
    - Because anabolism is very energy-consuming, it requires tight regulation, and should prevent from synthesising excess nutrients. Attenuation is used to regulate many operons involved in amino acid synthesis
        - repressed level of transcription is only 70-fold lower than when the repressor is not bound
        - attenuation contributes to the control over the operon another 10 fold
        - in total: 700 fold

Pho operon

▼ Regulon

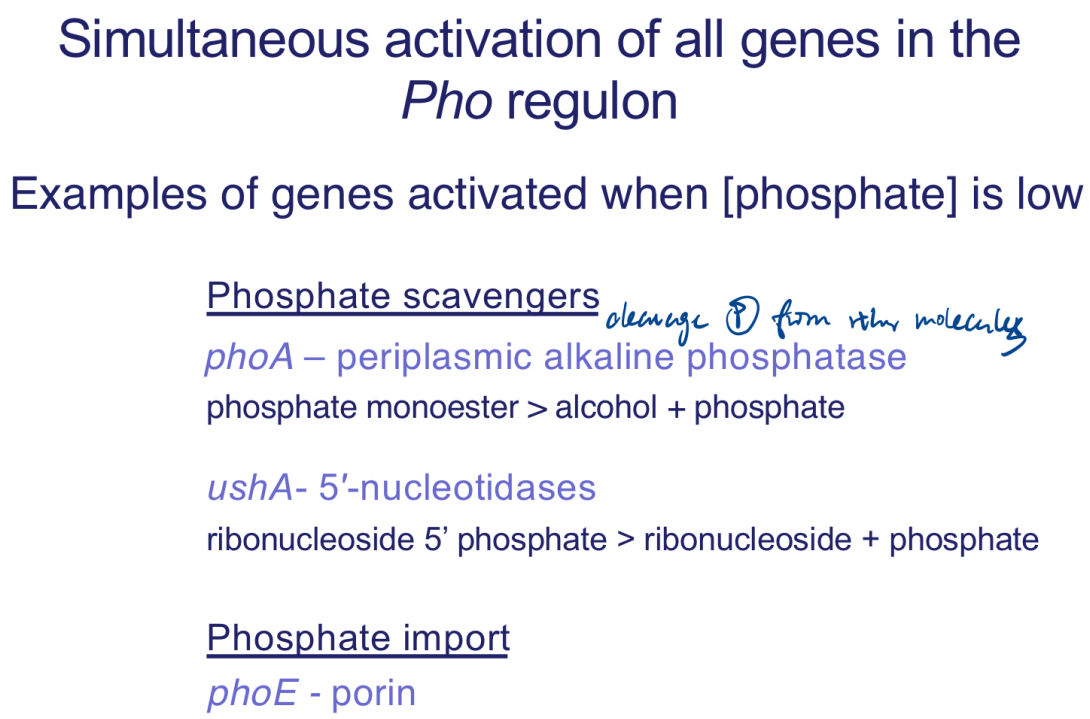
- collection of genes regulated as a unit
- transcribed at the same time
- Function:
    - sense the drop of [phosphate] in Gram -ve cells (e.g. E.coli)
    - to increase the cellular phosphate level, for survival
    - P usually exists as inorganic form in bacteria (PO4 3-)
    - important element: DNA, RNA, ATP

- Components
  - sense [Phosphate] concentration
    - PhoR — Histidine kinase
  - activator for gene transcription
    - PhoB — DNA binding protein
  - ~30 genes, whose transcription increases the [phosphate], promote cell survival

▼ How is the [Phosphate] sensed in the cell

- PhoR — only reside in periplasmic membrane & does not have a periplasmic phosphate binding domain
- Require other protein complex **(Pst - phosphate-specific transporter)** to transport P to regulate PhoR
- Phosphate enters the periplasm via porin, and then is transferred to the periplasmic membrane via PstS complex, which together mimic the channel for phosphate
  - PstA & Pst C at periplasmic membrane
  - two PstB at cytoplasm
  - PhoU — phosphate-specific transport system accessory protein
- When phosphate level is high:
  - The “channels” open → Phosphate is transported into the cytoplasm →PhoU cannot activate PhoR
- When phosphate level is low:
  - No phosphate is transported into the cytoplasm
  - PhoU now adapts a different confirmation than that when phosphate is present
  - So now the PhoR can autophosphorylates a histidine by ATP hydrolysis
  - p-PhoR now can phosphorylate regulator protein PhoB at aspartate
- How does PhoB activate transcription

    - The phosphorylated PhoB dimerise → bind to the Pho box (upstream of -35) → recruit RNA pol
- Activate the transcription level of Pho regulon — upregulate [phosphate]



Lux operon

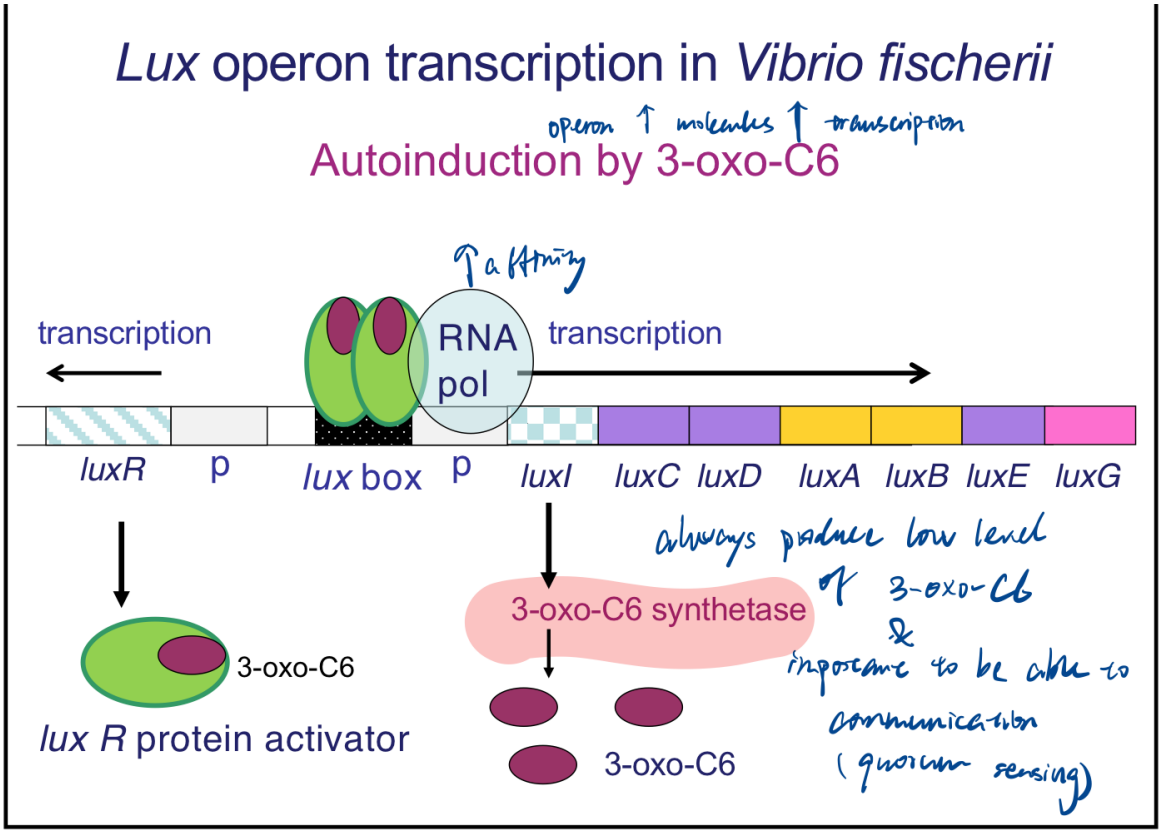
- Exploited by Hawaiian squid Euprymna scolopes to form a natural symbiosis with the bacterium Vibrio fischeri
- Form a mutually beneficial symbiotic relationship
    - **Squid light organ** provides the bacteria with a nutrient rich environment
    - Bacteria population luminesce to provides the squid with camouflage mechanism (mimicking moonlight) to hunt its prey

▼ lux operon allows luminescence & for quorum sensing, which is a communication way between bacteria

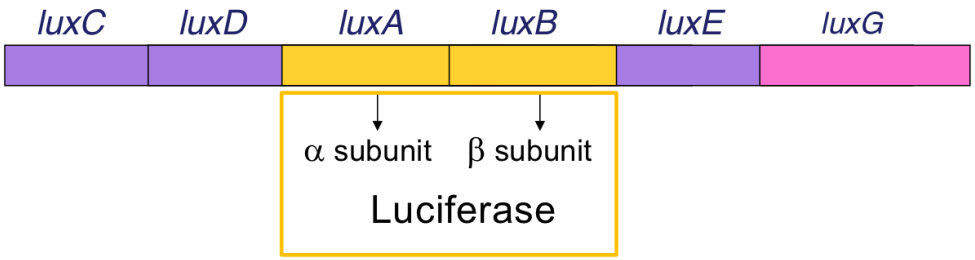
- If there is a high density of bacteria (quorum) in the light organ of the squid — leads to luminescence
- In small population of the bacteria, no luminescence

▼ Components of lux operon

- luxR: activator protein
- *lux* box: upstream of promoter, similar to the Pho box, and CAP binding site
- lux I: encodes for 3-oxo-C6 synthase — synthesise the autoinducer: 3-oxo-C6
- lux A, lux B — luciferase (alpha and beta subunit)
- lux C, D, E — fatty acid reductase complex, produce the aldehyde substrate
- lux G — flavin reductase — reduces FMNH2 to FMN



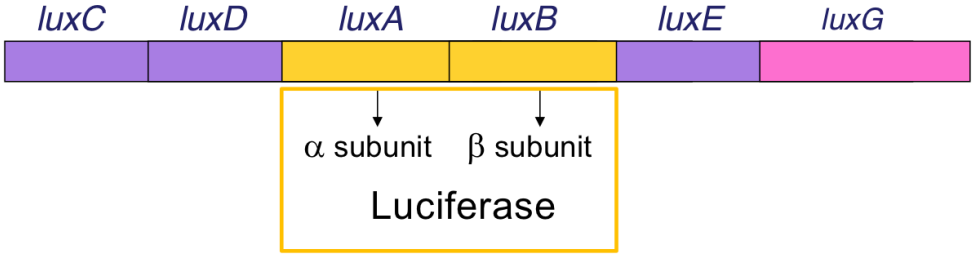
Light production



luxC, luxD, luxE encode a fatty acid reductase complex.
Produce the substrate a long chain aldehyde, RCHO.

luxG encodes flavin reductase and reduces FMN to $FMNH_2$.

Light production



luxC, luxD, luxE encode a fatty acid reductase complex.
Produce the substrate a long chain aldehyde, RCHO.

luxG encodes flavin reductase and reduces FMN to $FMNH_2$.

▼ Regulation of transcription

- Under low probability, in basal condition RNA pol binds to the promoter and starts transcription of lux I
- Therefore, always low activity of 3-oxo-C6 synthetase — always low level of 3-oxo-C6
- 3-oxo-C6 can diffuse rapidly across the cell membrane
- Therefore, when there is a quorum of bacteria in the light organ, in each bacterium, the level of 3-oxo-C6 increases
- More inducer binds to the lux R activator protein — can binds on lux box — interact with RNA pol — increases its affinity to the promoter
- transcription level increases
- more luminescent protein — greater luminescence

▼ The symbiotic relationship regulation

- During the night, the light organ offers nutrients for bacteria — quorum sensing — population expands —
- The light-producing bacteria can activate the genes that regulate crypt cell swelling — able to accommodate more bacteria
    - bacteria with mutant lux do not stimulate the swelling of crypt cells of the light organ

- During the day:
    - squid buries itself in the sand
    - ejects 95% bacteria
    - when bacteria leave the squid, they divide
    - they replenish the light organ during the night