

Regulation of Gene Expression in *E. coli*

Constitutive, Inducible and Repressible Expression · Positive and Negative Control · the Lactose and Tryptophan Operons

Bacteria constantly adjust which genes they transcribe in response to the nutrients and stresses in their environment, rather than making every protein at a fixed rate all the time. In *E. coli*, this regulation happens mainly at the level of transcription initiation, and is most easily understood through two classic, extensively studied gene clusters (operons): the **lac operon**, which is switched ON in response to a nutrient (lactose), and the **trp operon**, which is switched OFF in response to a product (tryptophan). Together they illustrate essentially all the logical strategies bacteria use to control gene expression.

1. Patterns of Gene Expression

Not all bacterial genes are regulated the same way. Based on how their expression responds to environmental signals, genes can be grouped into three broad patterns.

1.1 Constitutive expression

Constitutive genes are transcribed at a roughly constant rate under essentially all growth conditions, because their products are needed continuously. These are typically "housekeeping" genes encoding core machinery - for example, genes for glycolytic enzymes, RNA polymerase subunits, or ribosomal proteins. Their promoters are efficiently recognised by RNA polymerase without requiring activator proteins, and they are largely unaffected by regulatory repressor or activator proteins.

1.2 Inducible expression

Inducible genes are expressed at a very low (or negligible) basal level and are switched ON (induced) when a specific small molecule - the **inducer** - becomes available, typically a nutrient the gene products are needed to metabolise. The classic example is the lac operon: its enzymes are made at high levels only when lactose (via its isomer allolactose) is present, sparing the cell the cost of making lactose-digesting enzymes when there is no lactose to digest.

1.3 Repressible expression

Repressible genes are expressed at a high basal level by default and are switched OFF (repressed) when a specific small molecule - the **corepressor** - accumulates, typically the end product of a biosynthetic pathway the gene products build. The classic example is the trp operon: its biosynthetic enzymes are made continuously until tryptophan becomes abundant, at which point tryptophan itself acts as a corepressor and shuts the operon down, since the cell no longer needs to manufacture what it can now obtain ready-made.

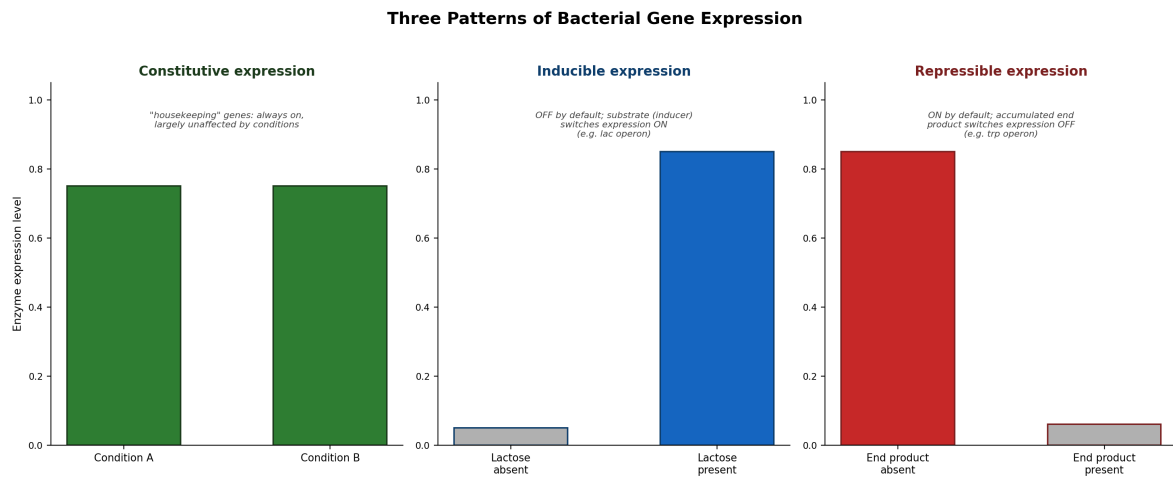


Fig. 1 - The three patterns of bacterial gene expression: constitutive (always on), inducible (off, then switched on by a substrate/inducer), and repressible (on, then switched off by an accumulated end product).

Key points

- Constitutive = always on; inducible = off by default, turned ON by a signal; repressible = on by default, turned OFF by a signal.
- Inducible systems typically control *catabolic* (breakdown) pathways; repressible systems typically control *anabolic* (biosynthetic) pathways - this matches the underlying logic of "make the enzyme only when there is substrate to break down" versus "stop making the enzyme once there is enough product".

2. Positive and Negative Control of Gene Expression

Separately from whether a gene is inducible or repressible, bacterial operons can also be classified by the *type* of regulatory protein that controls them - a repressor or an activator - and by the default state of the operon in the protein's absence.

2.1 Negative control

In **negative control**, a regulatory protein called a **repressor** binds to the operator sequence and physically blocks RNA polymerase from transcribing the operon. The gene is ON by default (if the repressor is absent or inactivated) and is switched OFF when an active repressor binds. Whether the repressor is active depends on a small effector molecule: in the lac operon, the inducer (allolactose) binds the repressor **LacI** and inactivates it (removing the "off switch"), while in the trp operon, the corepressor (tryptophan) binds the repressor **TrpR** and activates it (engaging the "off switch").

2.2 Positive control

In **positive control**, a regulatory protein called an **activator** must bind DNA near the promoter to help RNA polymerase bind efficiently and initiate transcription. The gene is OFF (or only weakly transcribed) by default and is switched ON (or strongly enhanced) when an active activator is present. The clearest example in the lac operon is **CAP (catabolite activator protein, also called CRP)**, which - when bound to cyclic AMP (cAMP), whose levels rise when glucose is scarce - binds just upstream of the lac promoter and substantially increases RNA polymerase binding there.

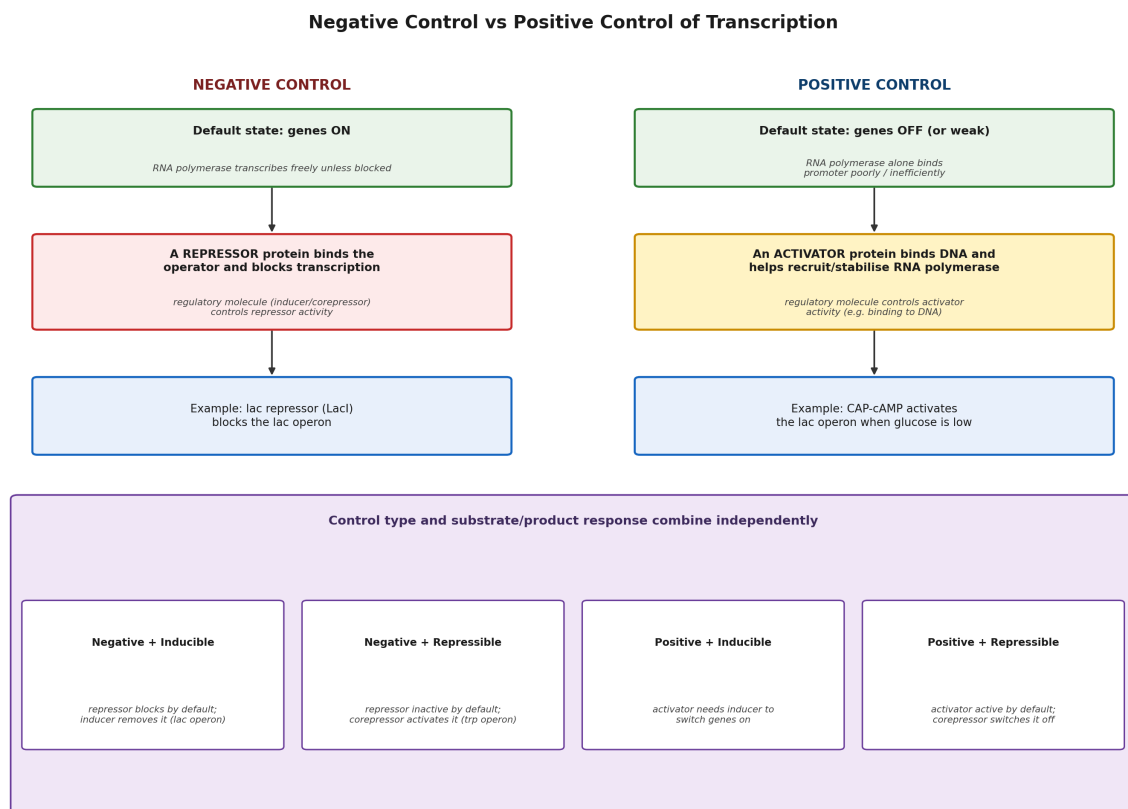


Fig. 2 - Negative control (repressor blocks transcription) versus positive control (activator assists transcription), and how the two axes - control type, and inducible/repressible response - combine independently in real operons.

2.3 The two axes are independent

"Positive vs negative" (which kind of protein, and what the default state is) and "inducible vs repressible" (which direction a signal moves the system) are two separate classification axes, and a real operon is described using both. The lac operon is simultaneously under negative control (via LacI) and positive control (via CAP), and its LacI-based regulation is inducible; the trp operon is under negative control only (no activator is required), and its TrpR-based regulation is repressible.

Worth noting

- A common exam trap: "negative" and "positive" describe the *type of protein and default state*, not whether the outcome is "bad" or "good" for the cell - both mechanisms are equally purposeful regulatory strategies.
- The lac operon is the standard teaching example of a system combining negative control (LacI repressor) with positive control (CAP activator) at the same promoter.

3. The Lactose (lac) Operon

The lac operon is an inducible operon that allows *E. coli* to metabolise lactose only when it is needed. It consists of a promoter (P) and operator (O) controlling three structural genes transcribed as a single polycistronic mRNA, plus a separate, constitutively expressed regulatory gene.

3.1 Genetic organisation

- **lacZ** encodes β -galactosidase, which hydrolyses lactose into glucose and galactose (and converts a small fraction of lactose into the true inducer, allolactose).
- **lacY** encodes lactose permease, a membrane transporter that imports lactose into the cell.
- **lacA** encodes thiogalactoside transacetylase, whose precise physiological role is less central to lactose catabolism but is thought to help detoxify non-metabolisable thiogalactosides.
- **lacI**, transcribed from its own separate, constitutively active promoter, encodes the **Lac repressor**, which in the absence of inducer binds the operator (O) and blocks RNA polymerase.
- The **CAP site**, just upstream of the promoter, is where the activator CAP-cAMP binds to enhance transcription when glucose is scarce.

3.2 The operon under three physiological conditions

The operon's output is set jointly by two independent signals - the presence/absence of lactose (controls LacI) and the presence/absence of glucose (controls CAP-cAMP, via cAMP levels) - giving three physiologically important states.

The lac Operon Under Three Physiological Conditions

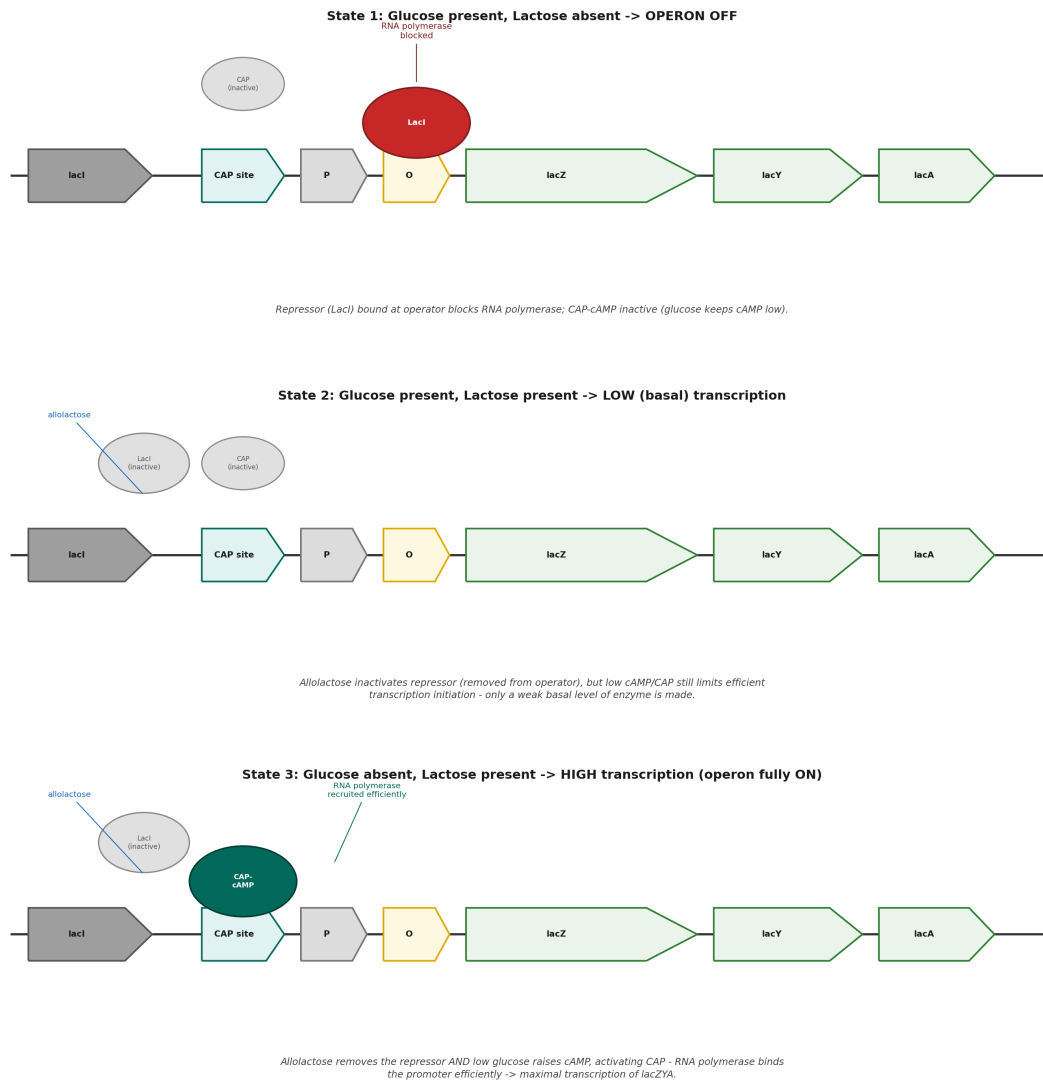


Fig. 3 - The lac operon gene map and its regulatory state under three combinations of glucose and lactose availability.

State	Glucose / Lactose	LacI	CAP-cAMP	Transcription
1	Present / Absent	Active - bound at O	Inactive	OFF (repressor blocks polymerase)
2	Present / Present	Inactive (allolactose bound)	Inactive (glucose keeps cAMP low)	LOW / basal (repressor off, but CAP not helping)
3	Absent / Present	Inactive (allolactose bound)	Active (low glucose raises cAMP)	HIGH (repressor off AND CAP actively recruiting polymerase)

3.3 Catabolite repression and diauxic growth

When *E. coli* is grown in a medium containing both glucose and lactose, it preferentially consumes glucose first - because glucose keeps intracellular cAMP low, keeping CAP inactive and lac transcription at only a low basal level even though lactose (and allolactose) is present. This glucose-mediated suppression of the lac (and other sugar-catabolism) operons is called **catabolite repression**. Once glucose is exhausted, cAMP rises, CAP-cAMP activates the lac promoter, and lac

expression rises sharply - producing the classic biphasic ("diauxic") bacterial growth curve, with a brief lag phase between the two growth phases while the cell resets its enzyme complement.

Key points

- The lac operon is regulated by two independent controls acting on the same promoter: negative control by LacI (responds to lactose/allolactose) and positive control by CAP-cAMP (responds to glucose, via cAMP).
- Maximal ("fully ON") transcription requires BOTH conditions at once: lactose present (removes the repressor) AND glucose absent (activates CAP) - explaining the sharp induction only in state 3.
- The true inducer is allolactose (an isomer of lactose made by basal levels of β -galactosidase acting on the small amount of lactose entering via basal permease), not lactose itself.

4. The Tryptophan (trp) Operon

The trp operon is a repressible operon encoding the five enzymes needed to synthesise the amino acid tryptophan from chorismate. Unlike the lac operon, it is controlled purely negatively (no activator is involved), but it is regulated at two independent levels - a coarse on/off repressor switch, and a finer-scale mechanism called attenuation.

4.1 Genetic organisation

- **trpE, trpD, trpC, trpB, trpA** are five structural genes, transcribed as a single polycistronic mRNA, encoding the enzymes of the tryptophan biosynthetic pathway.
- **trpL**, a short leader sequence at the start of the transcript (upstream of trpE), encodes a 15-residue leader peptide and contains the regulatory sequences responsible for attenuation.
- **trpR**, located elsewhere on the chromosome, encodes the **Trp repressor**, which is synthesised constitutively but is functionally inactive on its own.

4.2 Level 1: repressor/corepressor control

The Trp repressor protein cannot bind the operator by itself. It becomes an active DNA-binding repressor only when it binds two molecules of free tryptophan, which acts as a **corepressor**. When tryptophan is scarce, the repressor stays inactive and RNA polymerase can initiate transcription freely; when tryptophan is abundant, the Trp-repressor complex binds the operator and blocks initiation. This sets a baseline (on/off) response, analogous in logic to lac repression but with the opposite outcome of ligand binding (here, binding the effector activates the repressor, whereas in lac it inactivates the repressor).

4.3 Level 2: attenuation

Even when transcription does initiate, a second, more sensitive control mechanism called **attenuation** can prematurely terminate the transcript within the trpL leader region before RNA polymerase ever reaches the structural genes. This relies on a feature unique to bacteria: because there is no nuclear envelope, ribosomes begin translating an mRNA while RNA polymerase is still transcribing it downstream, so the two processes are physically coupled. The trpL leader mRNA folds into one of two alternative secondary structures depending on how far a ribosome gets while translating the leader peptide, whose sequence contains two consecutive tryptophan codons.

- When tryptophan (and therefore charged tRNA-Trp) is **scarce**, the ribosome stalls at the two Trp codons in region 1, leaving region 2 free to base-pair with region 3 and form an **antiterminator** structure. This prevents the alternative 3:4 terminator hairpin from forming, so RNA polymerase reads through into the structural genes.
- When tryptophan is **abundant**, charged tRNA-Trp is plentiful, so the ribosome translates straight through regions 1 and 2 without stalling, physically blocking region 2 from pairing with region 3. Region 3 is then free to pair with region 4, forming a **3:4 terminator hairpin** followed by a run of U residues, which causes RNA polymerase to terminate transcription within the leader - well before it reaches trpE.

The trp Operon: Two Layers of Negative-Repressible Control

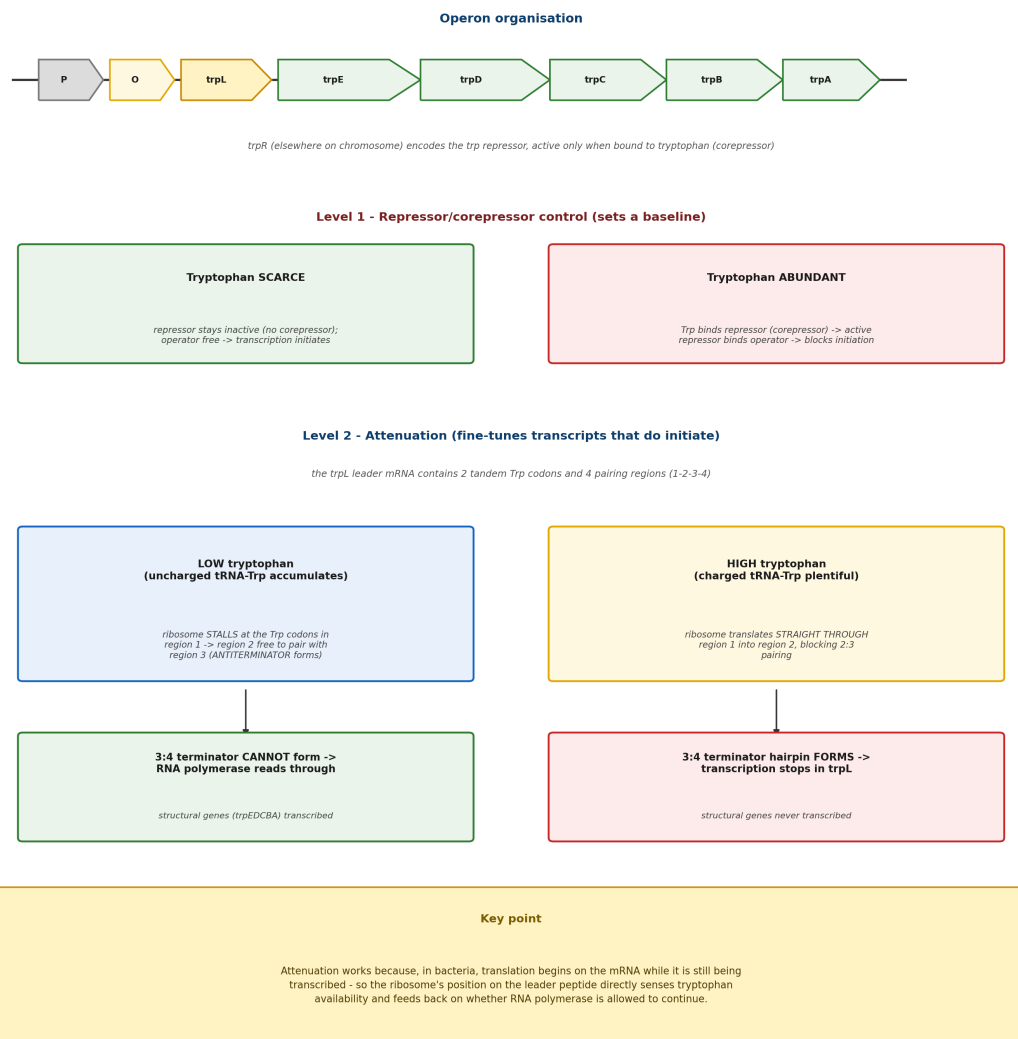


Fig. 4 - The trp operon's two layers of negative-repressible control: repressor/corepressor binding at the operator (Level 1), and translation-coupled attenuation within the trpL leader (Level 2).

Key points

- The trp operon is under negative control only, and is repressible: high tryptophan represses expression via an active Trp-repressor complex at the operator.
- Attenuation is a second, finer layer of negative-repressible regulation layered on top of the repressor switch, and works only because bacterial transcription and translation are physically coupled - a mechanism not available to eukaryotes, whose nuclear envelope separates the two processes.
- Both levels respond to the same signal (tryptophan availability) but sense it in different ways: the repressor senses free tryptophan directly, while attenuation senses the charging state of tRNA-Trp via ribosome stalling during translation of the leader peptide.

5. Quick Revision Summary

Concept	Essence
Constitutive expression	Roughly constant transcription regardless of conditions (housekeeping genes)
Inducible expression	OFF by default; switched ON by an inducer (usually a substrate)
Repressible expression	ON by default; switched OFF by a corepressor (usually an end product)
Negative control	A repressor blocks transcription; gene is ON unless repressor is active
Positive control	An activator assists transcription; gene is OFF/weak unless activator is active
lac operon genes	lacZ (β -galactosidase), lacY (permease), lacA (transacetylase); regulated by lacI
Lac repressor (LacI)	Binds operator by default; inactivated by allolactose (inducer) -> negative control
CAP-cAMP	Activates lac promoter when glucose is scarce (cAMP high) -> positive control
Catabolite repression	Glucose keeps cAMP/CAP low, suppressing lac even if lactose present
Diauxic growth	Two-phase growth curve: glucose consumed first, then lactose, after a lag
trp operon genes	trpEDCBA (biosynthetic enzymes) + trpL (leader); trpR regulatory gene is separate
Trp repressor (TrpR)	Inactive alone; activated by binding free tryptophan (corepressor) -> negative control
Attenuation	Translation-coupled premature termination in trpL, tuned by ribosome stalling at Trp codons

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