

***nif* genes functions and regulation of expression**

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nif genes

- The two main classes of nitrogen-fixing genes, the *nif* genes and *fix* genes, are present in rhizobia.
- The *nif* genes encode nitrogenase and show structural and functional resemblance with the nitrogen-fixing genes present in *Klebsiella pneumoniae* and other microbial groups.
- Although most of the *nif* genes are found on plasmids of rhizobia, it was also reported to be found on chromosomes of *Bradyrhizobium*.
- *Klebsiella pneumoniae* is the best studied nitrogen-fixing species because of its similarity to *E. coli*.
- *Klebsiella pneumoniae* is a free-living facultative anaerobic nitrogen-fixing bacterium. It contains a total of 20 *nif* genes located on the chromosome in a 24-Kb region. *nifH*, *nifD*, and *nifK* encode the nitrogenase subunits, while *nifE*, *nifN*, *nifU*, *nifS*, *nifV*, *nifW*, *nifX*, *nifB*, and *nifQ* encode proteins involved the assembly and incorporation of [iron](#) and [molybdenum](#) atoms into the nitrogenase subunits. *nifF* and *nifJ* encode proteins related to electron transfer taking place in the reduction process and *nifA* and *nifL* are regulatory proteins in charge of regulating the expression of the other *nif* genes .

- The nonsymbiotic *K. pneumoniae* carries at least 20 *nif* genes which are organized in about eight operons.

<i>Nif</i> Genes	Role in Nitrogen Fixation
<i>nifH</i>	Dinitrogenase reductase
<i>nifD</i>	α -Subunit of dinitrogenase
<i>nifK</i>	B-subunits of dinitrogenase. B clusters are present at B subunit-interface
<i>nifY</i>	In <i>Klebsiella pneumoniae</i> , aids in the insertion of FeMo-co into apodinitrogenase
<i>nifE</i>	Forms a ₂ B ₂ tetramer with <i>nifN</i> . Required for FeMo-co synthesis
<i>nifN</i>	Required for FeMo-co synthesis
<i>nifX</i>	Involved in FeMo-co synthesis
<i>nifU</i>	Involved in mobilization of Fe–S cluster synthesis and repair
<i>nifS</i>	Involved in mobilization of S for Fe–S cluster synthesis and repair
<i>nifV</i>	Homocitrate synthesis involved in FeMo-co synthesis
<i>nifW</i>	Involved in stability of dinitrogenase. Proposed to protect dinitrogenase from O ₂ inactivation
<i>nifM</i>	Required for the maturation of <i>nifH</i>
<i>nifF</i>	Flavodoxin, physiologic electron donor to <i>nifH</i>
<i>nifL</i>	Negative regulatory element
<i>nifA</i>	Positive regulatory element
<i>nifB</i>	Required FeMo-co synthesis. Metabolic product. NifB-co is the specific Fe and S donor to FeMo-co
<i>fdxN</i>	Ferredoxin serves as electron donor to nitrogenase
<i>nifQ</i>	Involved in FeMo-co synthesis. Proposed to function in early MoO ₄ ²⁻ processing
<i>nifJ</i>	Pyruvate flavodoxin (ferredoxin) oxidoreductase involved in electron transport to nitrogenase

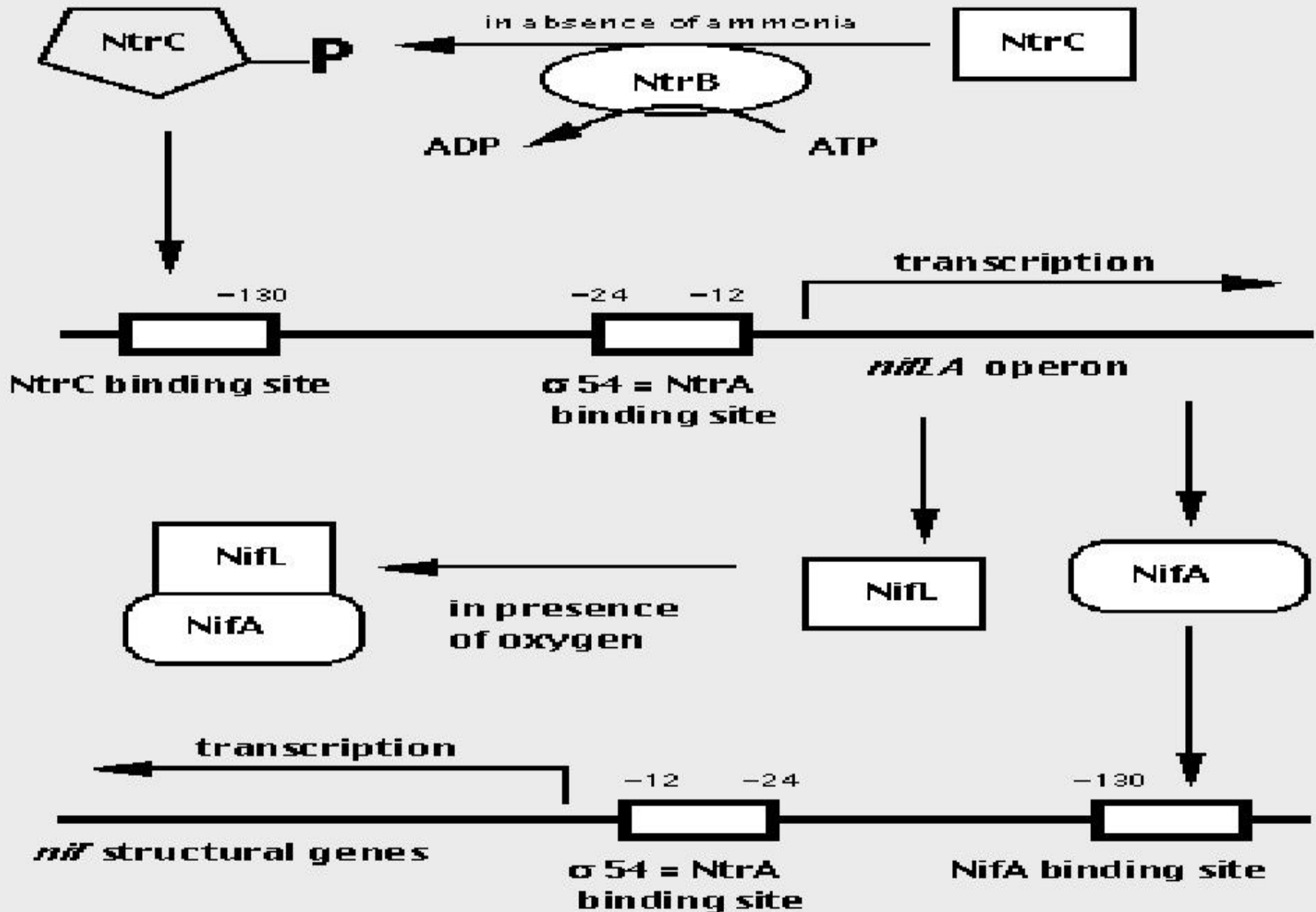
nif genes expression

- Gene expression in *nif* operons is dependent on the RNA polymerase σ_{54} factor and on the NifA transcriptional activator. The σ_{54} subunit (also known as RpoN, σ_N , or NtrA product of *ntrA* gene) recognizes a promoter-specific sequence located at positions -24 to -12 .
- In *nif* operons, transcription initiation by the σ_{54} -dependent RNA polymerase requires that the enhancer-binding-protein (EBP) and NifA binds to DNA regions known as upstream activator sequences.

Regulation of *nif* genes expression

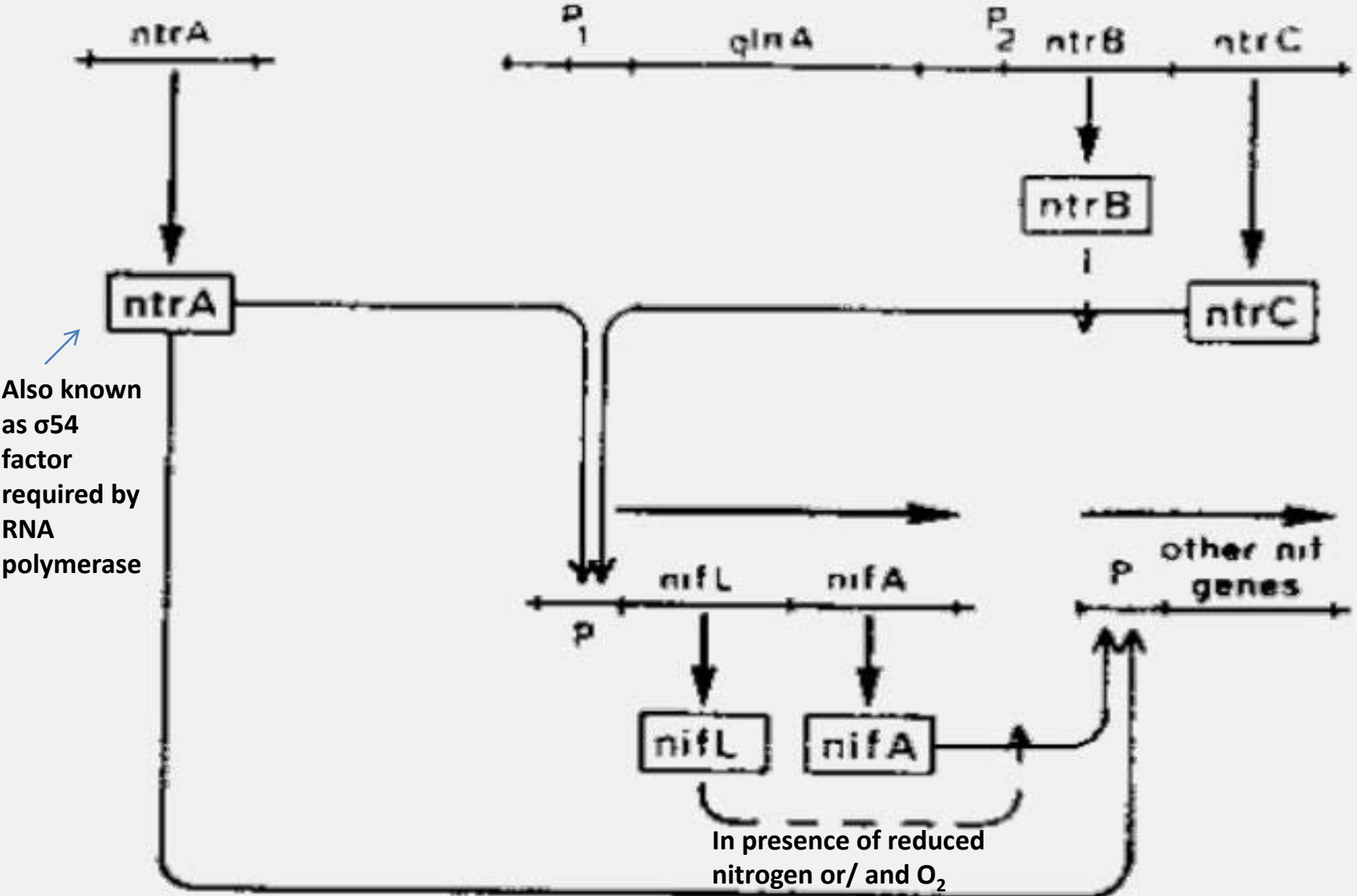
- Regulation of *nif* gene expression has two elements:
 - An external system designated *ntr* (nitrogen regulatory)
 - An internal system mediated by *nif A* and *nif L* gene products
- In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein.
- When there isn't enough fixed nitrogen available for the organism's use, NtrB the product of *ntrB* gene (function as protein kinase) phosphorylates NtrC. NtrC-P triggers NifA expression, and NifA activates the rest of the *nif* genes.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein NifL is activated.
- NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.
- NifL is regulated by the products of *glnD* and *glnK*.

REGULATION OF NITROGEN FIXATION



Regulation of nif gene-

Where P, P1 and P2 = Promoter region of operon



Questions

- Write in detail about nif genes and regulation of their expression.
- Write a short note on nif genes functions.
- Write a short note on regulation of nif genes expression.