

Chapter 13: Global Regulation of Nitrogen

All organisms require nitrogen for biological molecules including vitamins, nucleotides, and amino acids

Possible sources of nitrogen for bacteria

- NO_3^- : nitrate (must be reduced first)
- Degradation of bases in nucleotides and amino acids
- N_2 through nitrogen fixation
 - Only certain types of bacteria can perform this rxn

All biosynthetic reactions involve transferring nitrogen by:

- NH_3 directly

Assimilation if low NH_3 available: nucleotides and amino acids must be degraded

Involves two enzymes:

- **Glutamine synthetase (GS):** encoded by *glnA*
 - Enzymatic activity requires high energy input so transcription and activity are regulated
- **Glutamate synthase**



Assimilation if high NH_3 available

NH_3 is added directly to α -ketoglutarate by glutamate dehydrogenase (GD)



↳ If glutamate is efficiently produced then lower amounts of glutamine are needed

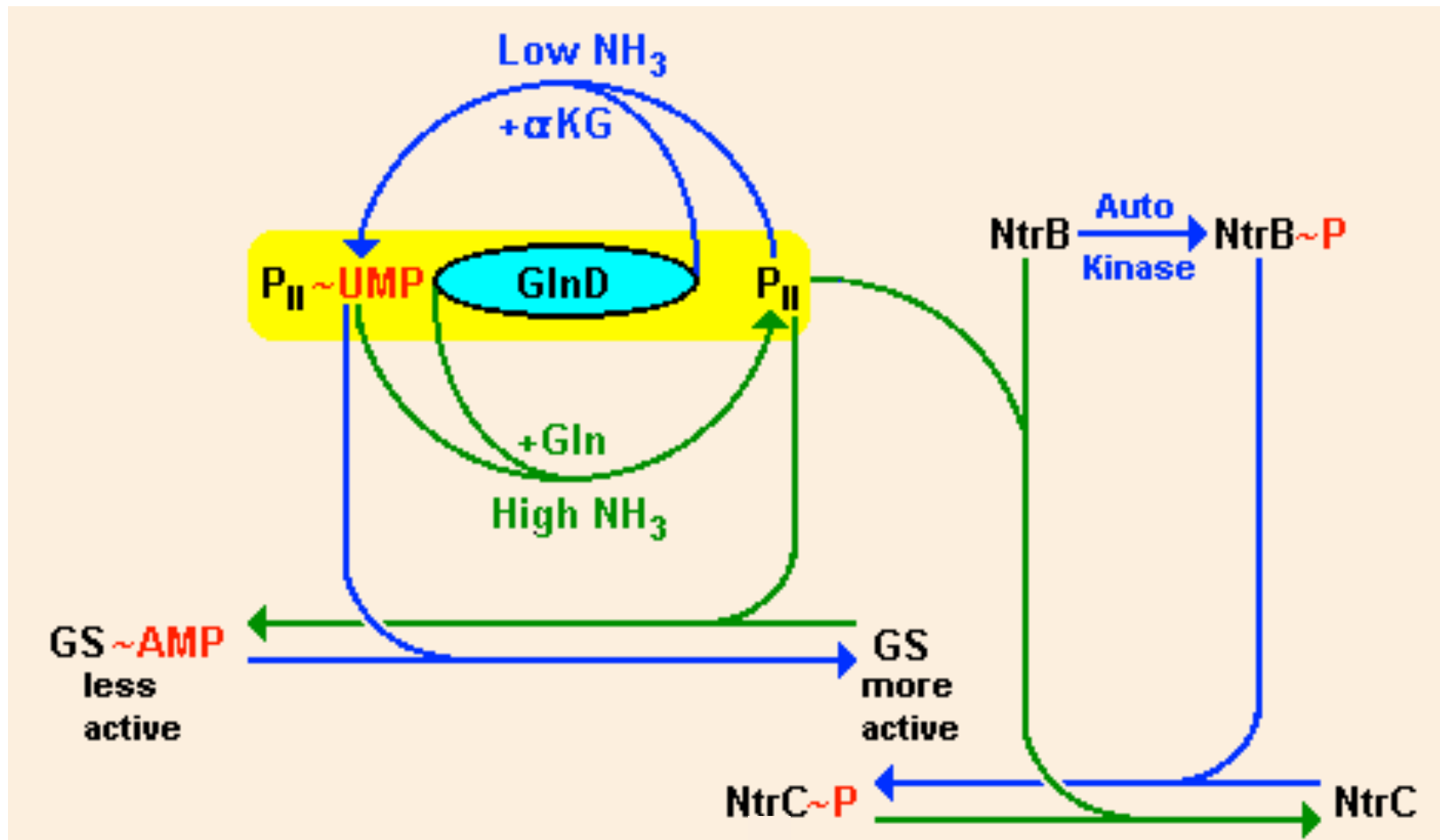
A portion of the glutamate above is converted by GS to glutamine needed for translation



Regulation of Nitrogen Assimilation

glnA-ntrB-ntrC operon and regulation by signal transduction

- If adenylylated by GlnD/ P_{ii} then enzyme is less active
- ***ntrB***: NtrB is the sensor kinase that can phosphorylate itself and donate the phosphate group to NtrC
 - Ability to autophosphorylate depends on the presence of the protein $P_{ii}\sim$ UMP
 - ↳
 - ↳ If glutamine level high, the GlnD enzyme will remove the UMP from P_{ii}
- ***ntrC***: NtrC is the response regulator that once phosphorylated activates txn of the *glnA-ntrB-ntrC* operon



If [NH₃] high then glutamine will predominate over α-KG

- P_{II} stimulates GlnD to add an AMP group to GS

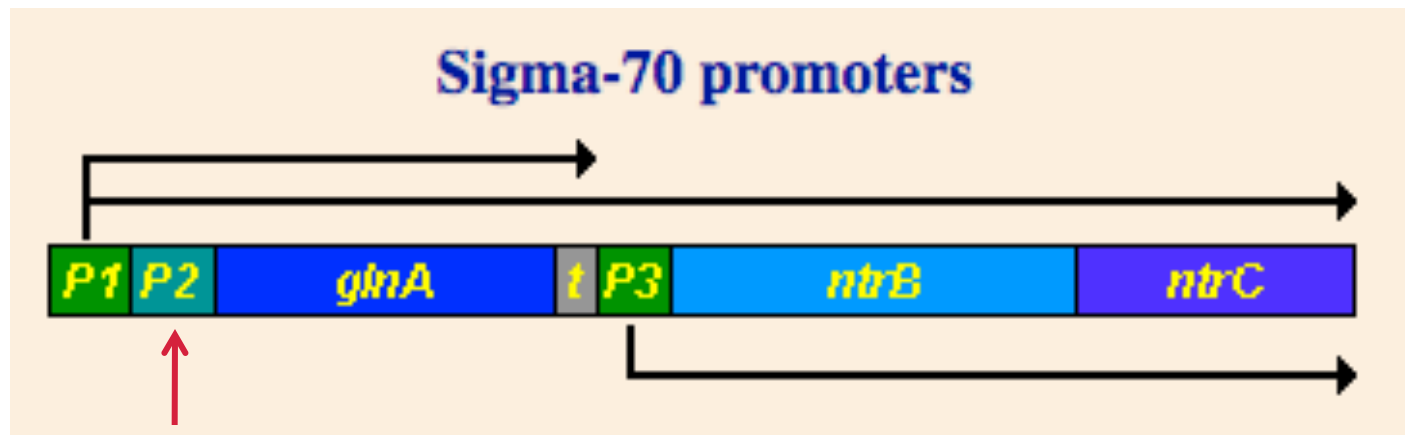
If [NH₃] low then α-KG will predominate over glutamine

- NtrC~P will activate txn of the *glnA-ntrB-ntrC* operon

Transcription of the *glnA-ntrB-ntrC* Operon

Operon is controlled by three separate promoters

- 1 promoter recognized by the nitrogen regulated sigma factor (σ^{54})
 - Only recognizes the promoter if NtrC~P is bound to an upstream element causing a conformation change in the DNA



Sigma-54 promoter

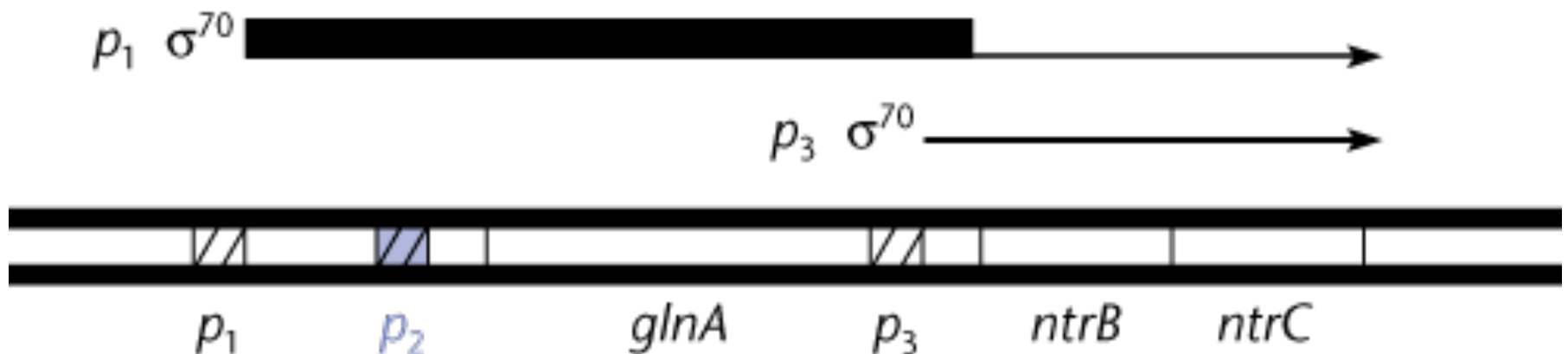
Transcription if [NH₃] high

Two transcripts from P1:

- some transcripts will contain *glnA*, *ntrB*, *ntrC*
- Most of the mRNAs will only contain *glnA*

One transcript from P3: only contains *ntrB* and *ntrC*

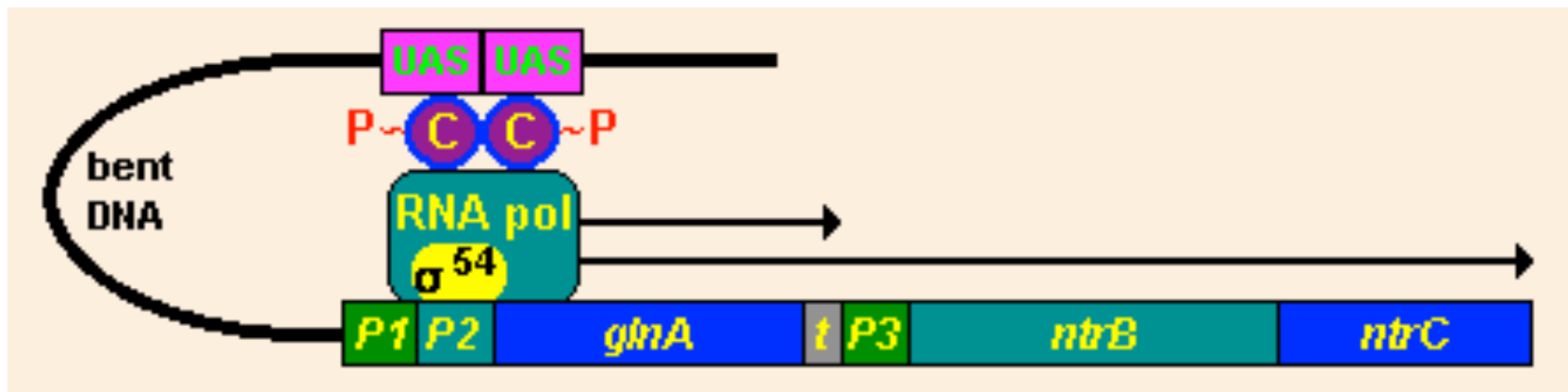
↳ Some glutamine synthetase is needed for amino acid synthesis



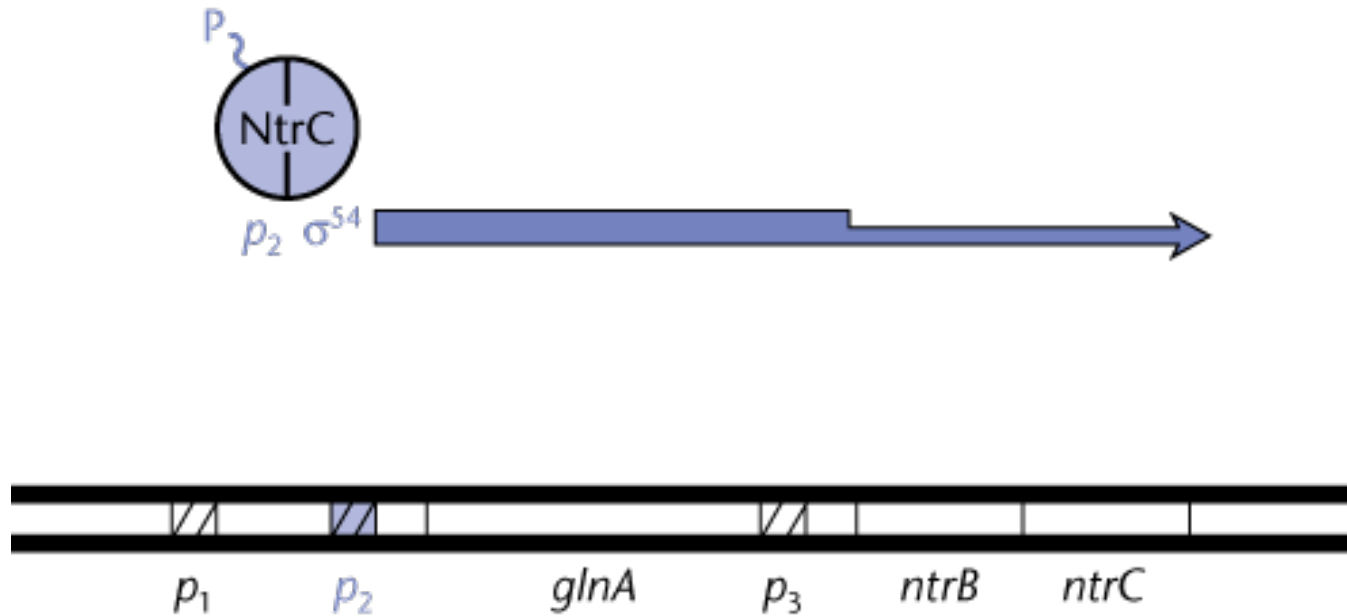
Transcription if [NH₃] low

NtrC becomes phosphorylated through signal transduction

- NtrC~P binds to UAS: upstream activator sequence
 - When UAS bound by two molecules of NtrC~P, triggers bending of DNA so that the UAS is in close proximity to P2
 - DNA Conformation allows σ -54 to recognize P2



Two transcripts result from P2



High levels of both *glnA* and *glnA-ntrB-ntrC* transcripts

- **Need more glutamine synthetase than NtrB and NtrC in low NH_3 conditions**

Sequence of Events if NH_3 Levels are Low

Ratio of glutamine to α -ketoglutarate is low

1) α -ketoglutarate stimulates GlnD to add UMP to P_{II}

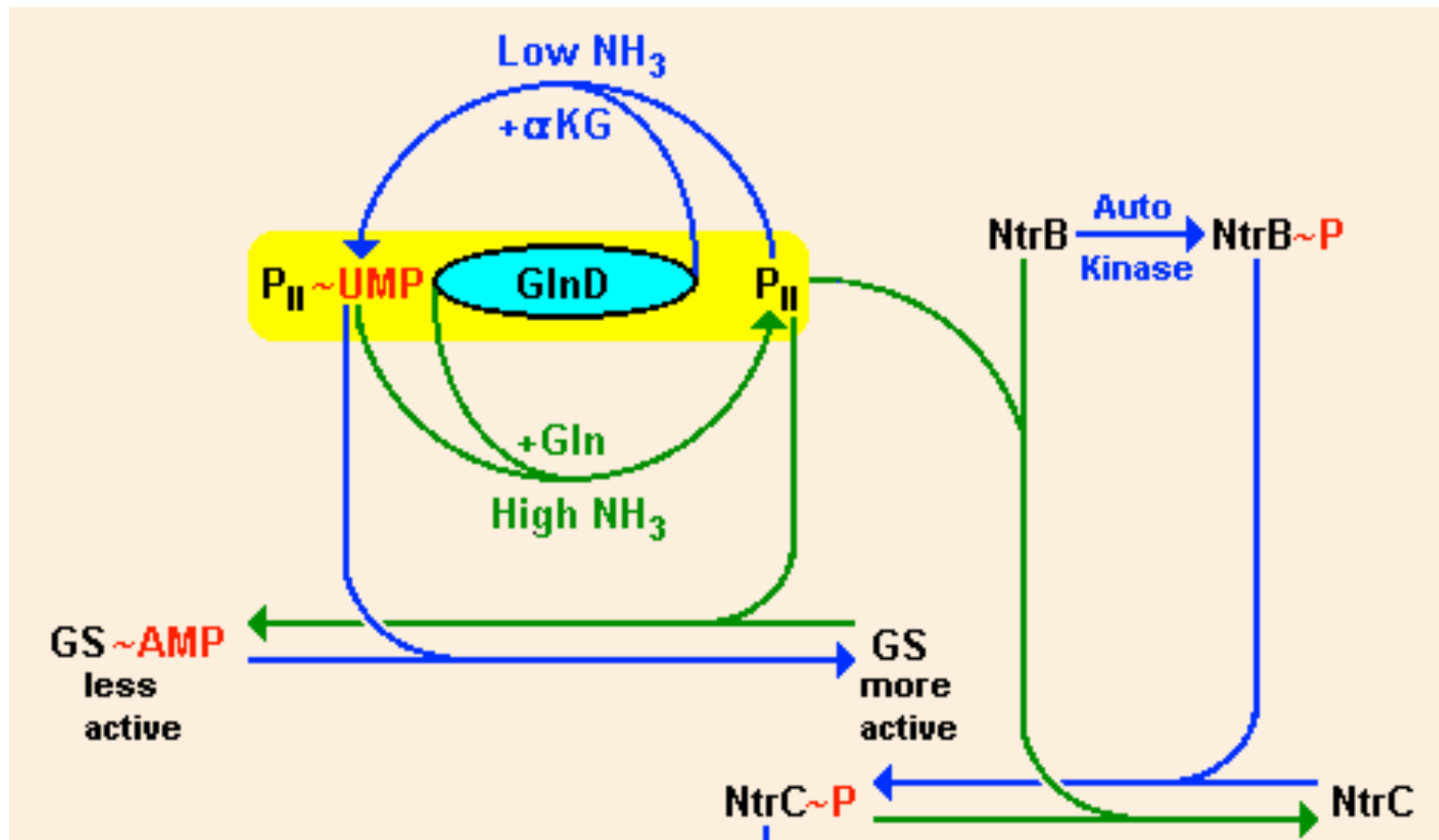
➤ P_{II} -UMP catalyzes the removal of AMP from GS~AMP



2) Presence of P_{II} -UMP stimulates NtrB to autophosphorylate itself

4) NtrC~P binds to the UAS and stimulates DNA bending

5) Bending of DNA recruits σ^{54} and transcription of *glnA-ntrB-ntrC* from P2 is activated



Sequence of Events if NH_3 Levels are High

Ratio of glutamine to α -ketoglutarate is high

1) Presence of glutamine stimulates GlnD to remove the UMP group from $\text{P}_{\text{II}}\sim\text{UMP}$

➤ Presence of P_{II} catalyzes the addition of AMP to GS

↳ **GS~AMP form is NOT very active and is susceptible to feedback inhibition**

3) Unphosphorylated form of NtrB removes phosphate group from any $\text{NtrC}\sim\text{P}$ present

4) NtrC is not phosphorylated so will not bind to UAS

Types of Regulation Involved in Nitrogen Assimilation