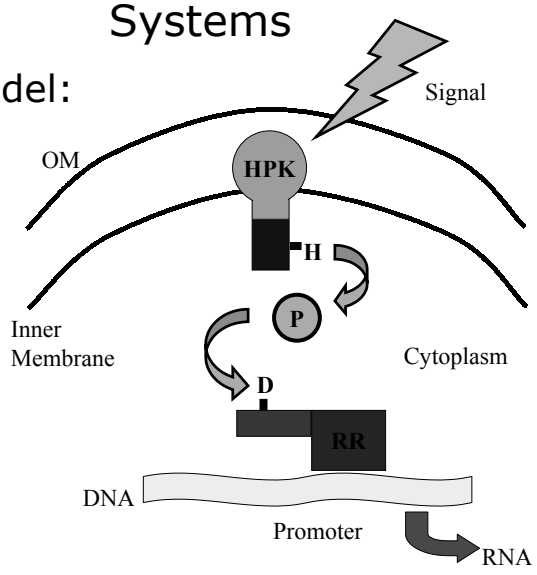
Stimulus-Response coupling by Two-component Regulator systems

- TCR act as a stimulus-response system.
  - detection of environmental signal
  - transduce signal to effector protein
  - initiate response
  - Most transcriptional regulators, but some affect protein activity
- Modular system
  - conserved signal transduction strategy
  - signal receiver and effector domains system specific
  - exact signal transduction pathway is system specific
- Simple paradigm is a two protein system

Two-Component Signal Transduction Systems

- Basic model:



The signal transduction pathway

- Must pass the signal detected on to a domain capable of affecting transcription
- Phosphorylation passes signal down the pathway

  HPK+signal $\rightarrow$ HPK-P

  HPK-P + RR $\rightarrow$ RR-P + HPK
- The RR cannot remain activated

  RR-P + HPK $\rightarrow$ RR + Pi

  RR-P + P'tase $\rightarrow$ RR + Pi
- At amino acid level:

  ATP+His $\leftrightarrow$ADP+His- P

  His- P+Asp $\leftrightarrow$His+Asp- P

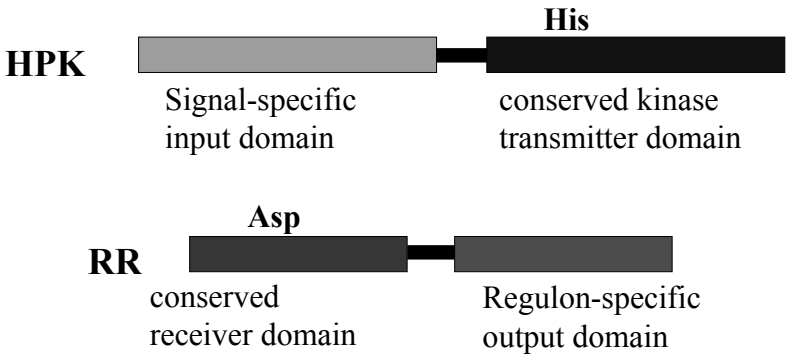
  Asp-P+H_2O $\leftrightarrow$ Asp+Pi

The TCR Superfamily

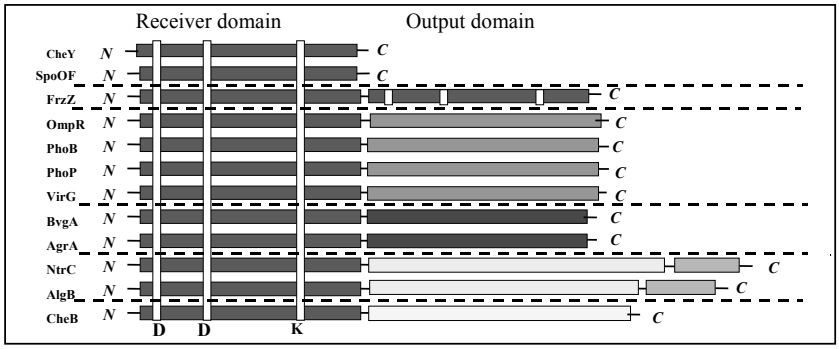
- Amino acid sequence comparisons show large numbers of TCRs (at least 300 known in bacteria)
- In virtually all bacteria adequately studied
- genome sequencing –only *Mycoplasma genitalium* and *Methanococcus jannaschii* TCRs not found
  - limited regulatory systems
  - 'life-style'
- Yeast –HPK and RR-like
- Arabidopsis –HPK-like
- Mitochorondria –HPK-like

TCR sub-families

- Modularity
- Domain organisation can be used to organise into sub-groups
- Comparison based on HPK or RR associated domain



RR domain comparisons



Domain organisation of RRs.
The *N*-terminal receiver domains are conserved among RRs and contain the highly conserved amino acids D, D, and K.
Based on similarity of C-terminal domains, RRs can be sub-divided into sub-groups.

Receiver domain
Winged HtH domain
HtH domain
Sigma-54 interaction domain
HtH domain
Methylesterase domain

Genetic Analysis of TCRs

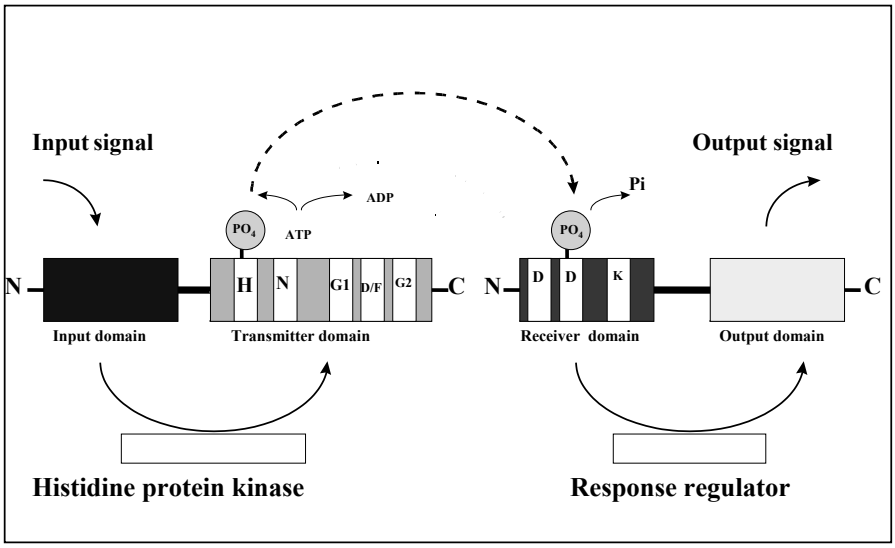
- Identification of TCR systems
- 'Classical' approaches:
  - Mutants (Transposons etc)
  - Mutant phenotype
  - Clone DNA flanking Tn mutant
    - Complementing library
    - Sub-clone, sequence, analysis.
- Utilise domain conservation
  - Southern probe
    - consider probe and stringency
  - PCRDOP
    - consider sequence conservation and codon usage
- Genome Sequencing
  - BLASTP comparisons of cds during annotation

Genetic analysis of mechanisms

- Delete component
  - ie remove HPK –is RR constitutively active?
- Over express component
  - increase RR –quench phosph of receiver?
  - increase HPK –in absence of signal is it in active state?
- Domain deletion
  - ie delete signal domain. Is kinase constitutively active? signal domain act negatively
  - ie delete receiver domain -what effect on transcription?
- Domain disconnection
  - ie 'separate' input and transmitter –disrupt signal pathw?
- Domain chimeras
  - use domains from different systems

Signal transduction mechanisms

- The transmitter and receiver are the common domains that define a TCR
- Consider general ideas about mechanisms before look at detailed example OmpR/EnvZ
- Highly conserved amino acids in transmitter and receiver are important in accepting phosphate and in kinase activity.

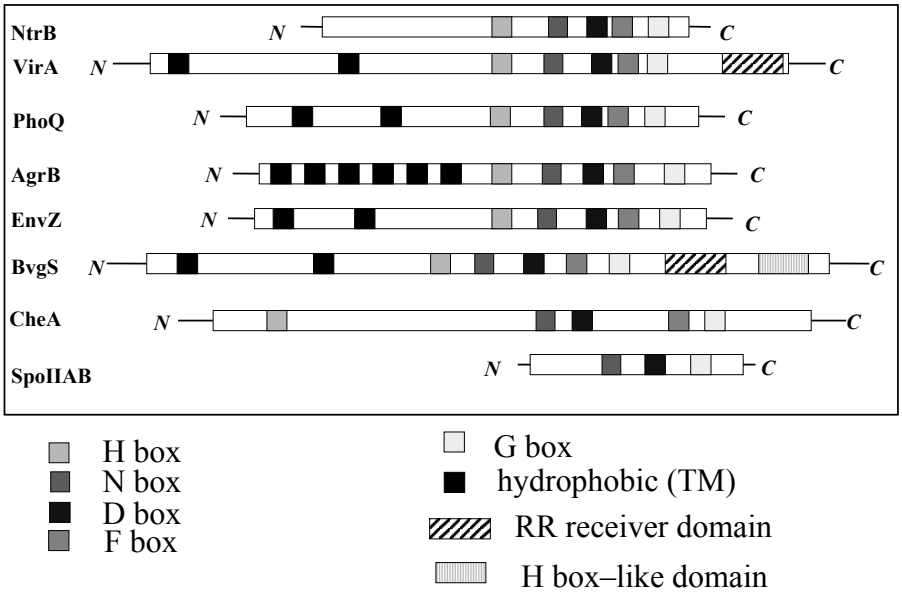


Histidine Protein Kinases

- The HPK sensor (input) domain is specific
  - EnvZ –osmolarity by membrane changes?
  - PhoQ –binding of divalent cations see diagram
- The transmitter has 2 sub-domains
  - H box subdomain –phosphorylated His
  - N-G-D/F-G box sub domain has ATP-binding activity and contains kinase activity see diagram
  - these are conserved AA in most transmitters

HPKs (cont.)

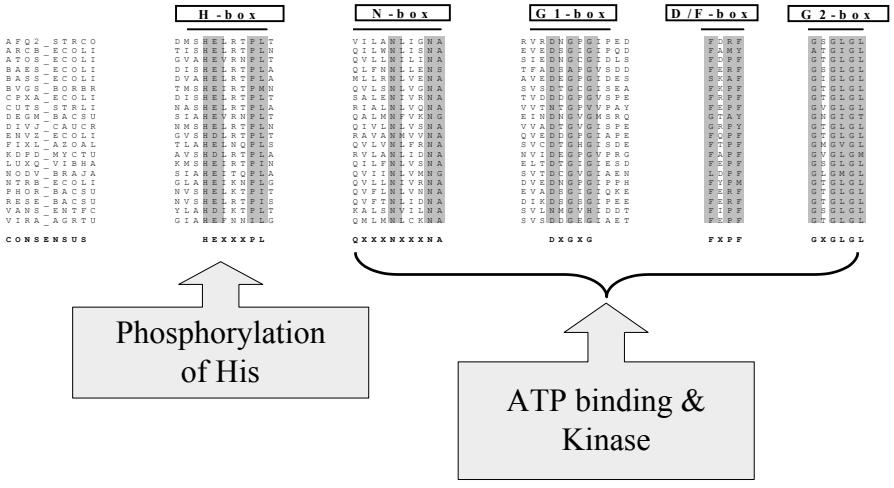
- Specificity between cognate HPK-RR systems
  - why does HPK-a interact with RR-a and not also RR-b, RR-c, RR-d…?
  - surrounding AA show conservation in sub-family members
  - each system will have specificity in conserved areas
- Cross-talk
  - evidence of interaction between some systems



2001-2002

C: 13

HPK sub-domains



2001-2002

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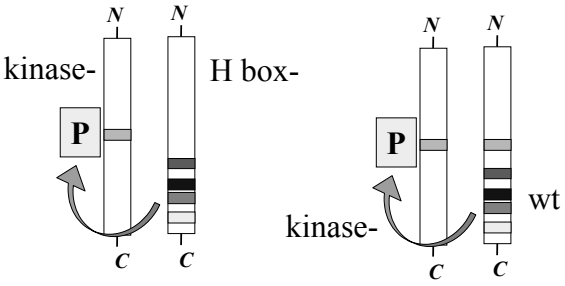
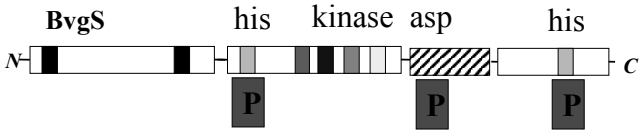
HPKs

- The ND/FG boxes acts as kinase
  - bind ATP
- HPKs act as dimer
  - intermolecular phosphorylation
  - kinase sub domain involved in dimerisation (CheA mutants)
  - mix mutants and domain deletions:



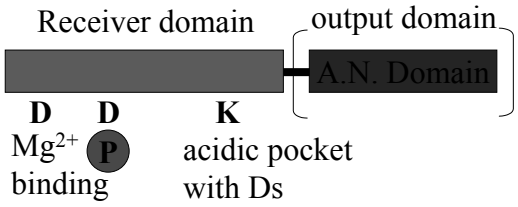
HPKs

- Rate of autophosphorylation determined by input signal
  - influence dimerisation
  - or influence dimer structure
- Kinase has dephosphorylation activity
  - need to dephosphorylate RR receiver so response terminates when signal finishes
- Domain structure in HPKs can be complicated:

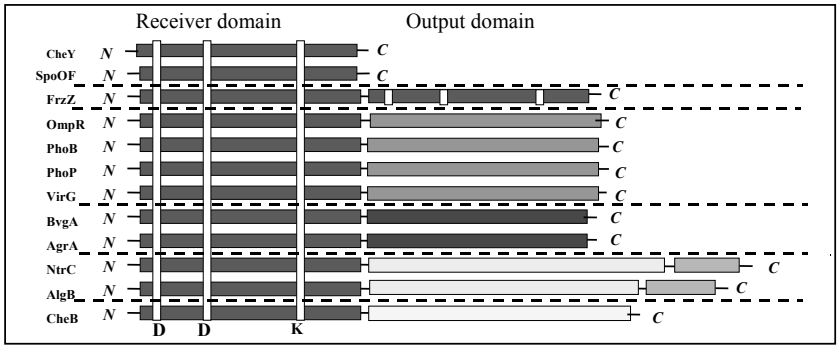


Response Regulators

- Receiver domain is conserved
- output domain sub family & system specific –CheY *protein interaction*
- Transfer of P requires transmitter-receiver interaction
- Many (not all –OmpR) RR receiver domains inhibit output domain
  - delete receiver –constitutive active output domain
- ½ life of Asp-P affect response
  - dephosphorylation –HPK + others



RR domain comparisons



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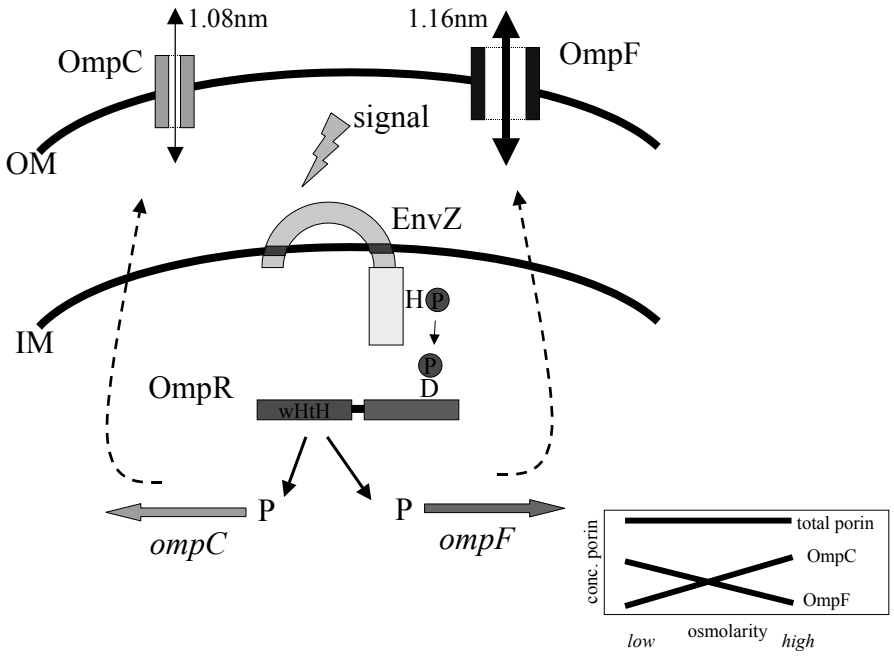
TCR example: EnvZ/OmpR

- Background
  - response/sensitivity to high/low osmolarity in Ec.
  - conc of small molecules –salts/sugars
  - reciprocal changes in two OMP
  - Mutant studies id 3 loci –*ompC*, *ompF*, *ompB*.
  - conjugation mapping! –*ompC* (21 min), *ompF* (48min), *ompB* (74 min).
  - *ompB* locus has two genes *envZ* & *ompR*
  - gene fusions show *ompB* involved transcriptional regulation
  - EnvZ –450aa 10 copies/cell inner Memb
  - OmpR –239aa 1000 copies/cell cytoplasmic



The signal

- responds to concentration of small molecules: not molecule specific
- Stimulus periplasmic
  - trucated mutant (EnvZ115) not respond
  - Tn*phoA* study show mutant not in periplasm
  - mutant partially compensates for EnvZ null mutant
- Stimulus assoc. with low osmol.
  - sensor domain mutants result in constitutive high osmol phenotype –OmpC>OmpF
- stimulus complex
  - unlikely specific molecules
  - not cytoplasmic
  - membrane/cell wall changes?

HPK: EnvZ

- EnvZ
  - activities:
    - autophosphorylation
    - OmpR phosphorylation
    - OmpR phosphatase
  - 2 membrane spanning domains
  - 2 domains –transmitter conserved
- How show N-domain is sensor?
  - Gene fusions show N-domain is periplasmic
  - sequence show TM regions
  - EnvZ115 cytoplasmic
    - in 'right place'
  - mutations in sensor give constitutive F-C+ phenotype

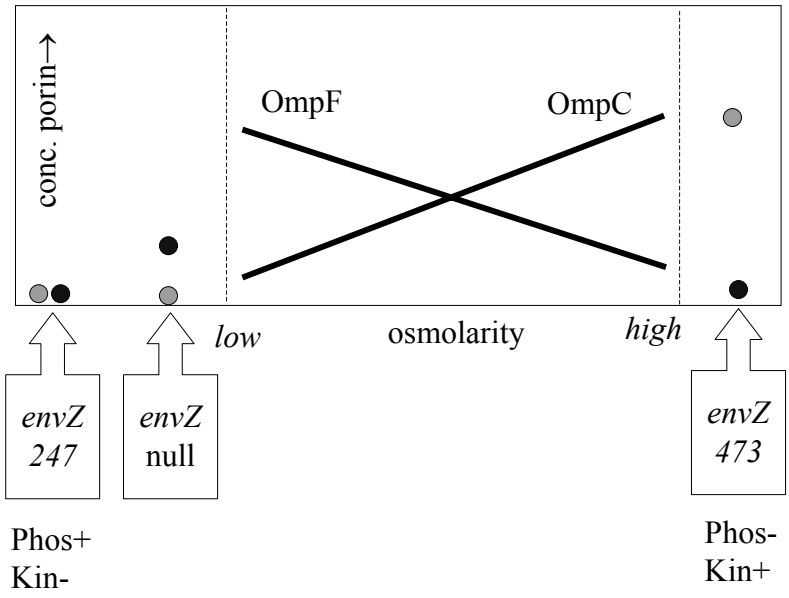
EnvZ (cont.)

- Is N-domain a sensor? cont…
  - Domain chimeras:
  - chemotaxis sensor (respond to AA) attached to transmitter. OmpC/F controlled wrt [AA]
- phosphate transfer
  - H243V –not autoph. *vivo*& *vitro*
  - EnvZ-P→OmpR-P *in vitro*
  - EnvZ+OmpR-P→dephos *in vitro*
  - balance of phos vs dephos activity?
- EnvZ signalling states
  - *envZ* null allele →F+/- C-
  - not= to low or high osmolarity phenotype
  - two active states: not simply On or Off.

EnvZ mutants cont.

- kinase vs phosphatase activity
  - EnvZ kinase- mutants *envZ247* and *envZ250*
  - both confer F-/C- phenotype
  - merodiploid of phosphatase- (*envZ473*) and either kinase-mutant gives constitutive low osmolarity phenotype
  - co-dominant
  - opposite extremes in signalling state: ph'tase dominant or kinase dominant
  - *in vitro* w/ mutants:
    - low is > phosphatase activity
    - high is > kinase activity
- stimulus (auto-P) modulates:

  kinase↔phosphatase

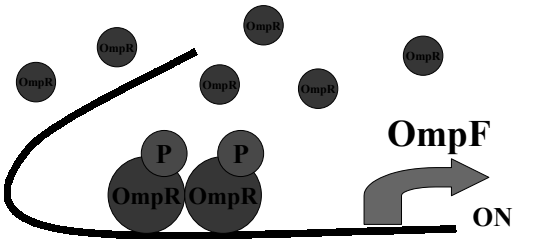


RR: OmpR

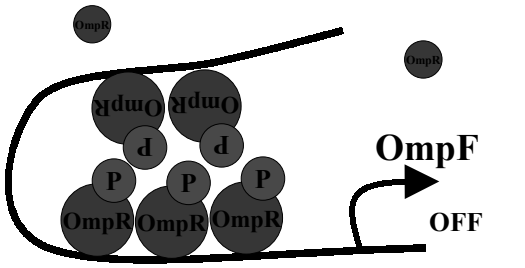
- transduces signal from sensor to changes in transcription
  - reciprocal regulation of porins OmpC and OmpF
  - likely to have at least 2 states associated with low and high osmolarity
- Has receiver domain and DNA binding domain
  - Crystal structure of DNA binding domain show has "winged HtH" domain
  - bind DNA and interact with RNAP

Phosphorylation states of OmpR

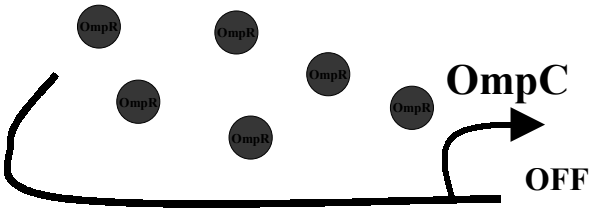
- Two classess of *ompR* mutants:
  - constitutive F+C- (low)
  - constitutive F-C+ (high)
  - F-C+ (high) dominant to F+C-
  - thus F-C+ allele stops F+C- allele activating F transcription
- Due to simple OmpR/OmpR-P?
  - No: envZ null or kinase- are F-C- when OmpR not phos
- Not OmpR-p/OmpR-PP (NMR)
- Ratio of OmpR to OmpR-P?
  - current model based on this
  - DNA protection studies show OmpR binds to large regions around OmpF/C promoters
  - binding change with OmpR phosphorylation
  - mutant OmpR bind different places



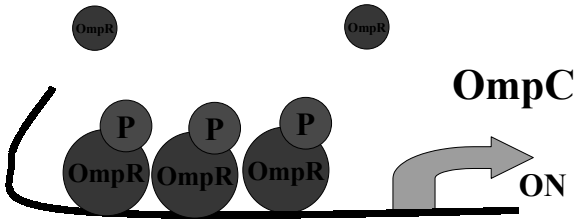
Low Osmolarity. Low OmpR-P and high OmpR



High Osmolarity. High OmpR-P and low OmpR



Low Osmolarity. Low OmpR-P and high OmpR

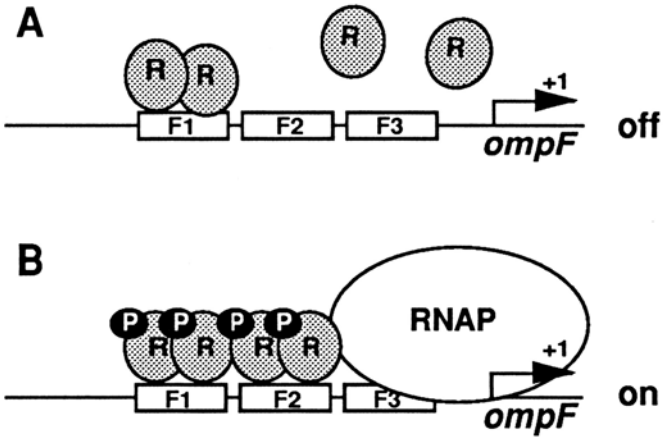


High Osmolarity. High OmpR-P and low OmpR

OmpR interaction with RNAP

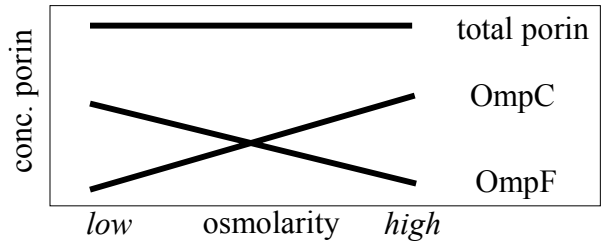
- RNAP α-subunit (C-terminal) required for OmpR regulation
  - *rpoA* mutants can suppress *envZ/ompR* mutants
  - *rpoA* mutants can affect porin expression
  - mutants map to C-terminal
- Mutations in *ompR* that affect interaction with RNAP map to 20 amino acid exposed loop in OmpR C-terminal part of HtH domain

Model based on promoter binding studies for OmpR interaction with P*ompF*



Co-operative interactions between **OmpR-P** molecules

from: Huang *et al*. PNAS 1997 **94**: 2828



LOW
EnvZ:
p'tase>>kinase

OmpR:
OmpR>>OmpR-P

Porin:
OmpF>>OmpC

HIGH
EnvZ:
kinase >> p'tase

OmpR:
OmpR-P>>OmpR

Porin:
OmpC>>OmpF

Summary TCR

- Modular signal transduction systems
- Super-family/sub-family
- conservation of transmitter and receiver domains
- transduction by phosphorylation
- many systems regulate transcription
- detailed example in EnvZ/OmpR
- Differences from OmpR paradigm in other TCR systems