Molecular Microbiology (1991) 5(11), 2575–2580

MicroReview

The role of the NAC protein in the nitrogen regulation of *Klebsiella aerogenes*

R. A. Bender
Department of Biology, University of Michigan, Ann Arbor, Michigan 48109–1048, USA.

Summary

The NAC (nitrogen assimilation control) protein from *Klebsiella aerogenes* is a LysR-like regulator for transcription of several operons involved in nitrogen metabolism, and couples the transcription of these σ70-dependent operons to regulation by the σ54-dependent NTR system. NAC activates expression of operons (e.g. histidine utilization, *hut*), allowing use of poor nitrogen sources, and represses expression of operons (e.g. glutamate dehydrogenase, *gdh*) allowing assimilation of the preferred nitrogen source, ammonium. NAC is both necessary and sufficient to activate transcription, but the expression of the *nac* gene is totally dependent on the central nitrogen regulatory system (NTR) and RNA polymerase carrying the σ54 sigma factor (RNAPσ54). Nitrogen starvation signals the NTR system to transcribe *nac*, and NAC activates the transcription of *hut*, *put* (proline utilization), and urease. NAC does not affect the transcription of RNAPσ54-dependent operons like *glnA* or *nifLA*, which respond directly to the NTR system, but activates transcription of RNAPσ70-dependent operons. Thus NAC acts as a bridge between RNAPσ70-dependent operons like *hut* and the RNAPσ54-dependent NTR system. The activation of operons like *hut* by NAC in response to nitrogen starvation is at least superficially similar to their activation by CAP–cAMP in response to carbon and energy starvation.

Nitrogen regulation

Thirty-five years ago, Neidhardt and Magasanik (1957) found that expression of the histidine-utilization (*hut*) operons from *Klebsiella aerogenes* was increased under conditions of nitrogen starvation. This increased expression was specific for pathways that yielded ammonia and/or glutamate; pathways that yielded only carbon and/or energy were actually more 'catabolite repressed' under conditions of nitrogen starvation than under nitrogen excess. In *K. aerogenes*, this nitrogen regulation (N-Reg) affects a wide array of operons involved in the fixation of ammonia into glutamate, the catabolism of amino acids, the reduction of inorganic nitrogen compounds, and the catabolism of organic compounds (Macaluso *et al.*, 1990). A partial list of the N-Reg-responsive pathways for *K. aerogenes* is given in Table 1.

N-Reg was not studied further at that time because of the absence of a genetic system for *K. aerogenes*. The other enteric species for which the genetics were well established were inappropriate: *Escherichia coli* lacks the *hut* operons entirely and *Salmonella typhimurium* does not show N-Reg of *hut*. The discovery of a transducing phage for *K. aerogenes* led to a renewal of N-Reg studies (Prival and Magasanik, 1971) and to the discovery of the central role of the nitrogen regulatory system (NTR) system in N-Reg. Many groups around the world have contributed to our current understanding of the NTR system in the enteric bacteria, but three groups in particular have served as continuing foci for this study: those of Magasanik (working mainly with *E. coli*), Kustu (working mainly with *S. typhimurium*), and the Nitrogen Fixation Unit at Sussex, UK (working mainly with *K. pneumoniae*).

The GLN system

The preferred nitrogen source for *K. aerogenes* is ammonium. All other nitrogen compounds result in slower growth (when glucose is the carbon source) and thus are growth-rate-limiting. However, *K. aerogenes* cannot respond directly to ammonium itself. Ammonium has no effect on N-Reg unless it can be converted to glutamine (Bender and Magasanik, 1977). Thus, a discussion of N-Reg must begin with the regulation of glutamine metabolism described by Stadtman and his colleagues (Ginsburg and Stadtman, 1973). The GLN system, the regulatory network that controls the rate of assimilation of ammonium into the amide position of glutamine, is built around a small regulatory protein, P_{II} (encoded by *glnB*), which can exist in two forms — unmodified (P_{II}) or modified by the covalent attachment of one UMP residue to each subunit (P_{II}–UMP). The uridylyltransferase

Received 25 July, 1991; revised 7 August, 1991. Tel. (313) 936 2530; Fax (313) 747 0884.

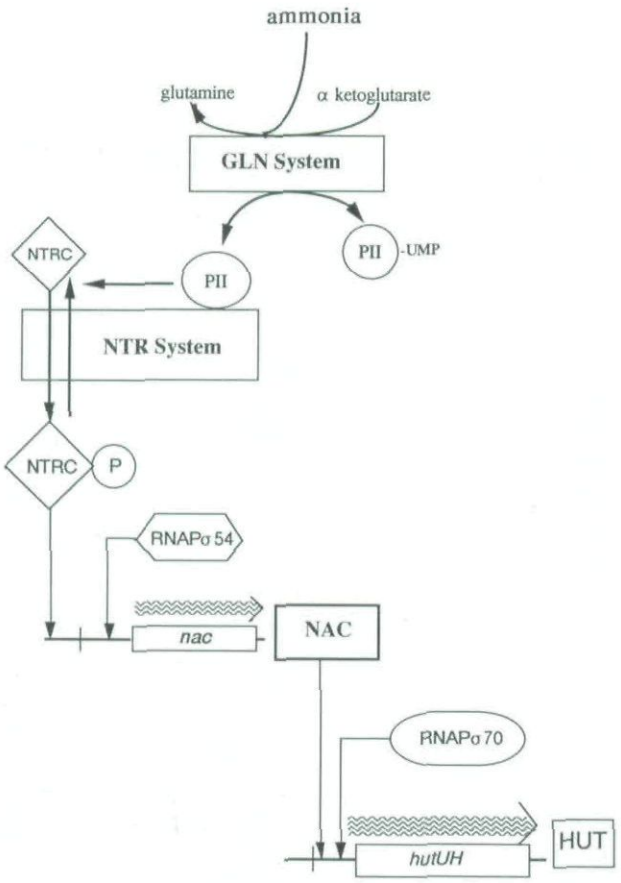

Fig. 1. The role of the NAC protein in nitrogen regulation of HUT (histidine utilization) enzyme formation. Three levels of control can be distinguished. (i) Addition of ammonia leads to accumulation of glutamine, which in turn causes P_{II} to accumulate in its unmodified form. (ii) Unmodified P_{II} greatly stimulates dephosphorylation of NTRC-phosphate (NTRC-P) to its inactive form (NTRC), thus eliminating expression of the *nac* gene by RNA Polymerase carrying the σ54 subunit (RNAPσ54). (iii) In the absence of NAC, *hut* transcription by RNAPσ70 is not activated. Thus addition of ammonia leads to loss of *hut* expression. Conversely, in the absence of ammonia, the glutamine pool declines and P_{II} is modified to P_{II}–UMP. P_{II}–UMP does not stimulate dephosphorylation of NTRC-P so active NTRC-P accumulates and stimulates transcription of *nac* by RNAPσ54. NAC protein accumulates and stimulates transcription of the *hut* operons by RNAPσ70. Thus when ammonia (glutamine) is removed, P_{II} is inactivated to P_{II}-UMP, NTRC is activated to NTRC-P, NAC is produced, and *hut* operon expression is activated.

(encoded by *glnD*) responsible for modifying P_{II} (reversibly) is regulated by the intracellular concentrations of glutamine (indicating N excess) and 2-ketoglutarate (indicating N starvation). When nitrogen is limiting, UMP residues are added and P_{II}–UMP accumulates. When nitrogen is abundant, UMP residues are removed and P_{II} accumulates. When nitrogen is in excess, P_{II} signals an inactivation (by adenylylation) of glutamine synthetase, thus reducing the rate of glutamine formation. When nitrogen is in short supply, P_{II}–UMP signals the reactivation (by deadenylylation) of glutamine synthetase, thus increasing the rate of glutamine formation. Thus P_{II} plays a homeostatic role in the GLN system, keeping the rate of glutamine synthesis balanced against the supply of ammonia and the biosynthetic demand for glutamine. But P_{II} also plays a role in regulating transcription by interaction with the NTR system.

The NTR system

The NTR of enteric bacteria is the subject of numerous recent reviews (e.g. Kustu *et al.*, 1986; Magasanik and Neidhardt, 1987; Merrick, 1988), so only a simplified outline is presented here. RNA polymerase carrrying the 54 kDa sigma subunit (RNAPσ54) differs from the more common RNAPσ70 in two ways: RNAPσ54 recognizes a very different promoter sequence from RNAPσ70, and RNAPσ54 seems unable to form open complexes at its promoters unless it interacts with a specific activator, i.e. an enhancer-binding protein. The NTR system itself consists of such an activator protein (called variously NTRC or NR_I) and a specific phosphotransferase (called variously NTRB or NR_{II}) capable of phosphorylating and dephosphorylating the activator. Although both the phosphorylated and non-phosphorylated forms of the activator (NTRC-P and NTRC in Fig. 1) can recognize enhancer sequences on the DNA and bind there, only NTRC-P can interact with RNAPσ54 to cause open complex formation. In the absence of other proteins, the phosphotransferase

Table 1. Pathways known to be responsive to N-Reg in *K. aerogenes*.

		Nac?[a]
Ammonia assimilation		
Glutamine synthetase (*glnA*)	(NH_4→glutamine)	–
Glutamate synthase	(glutamine→glutamate)	+
Glutamate dehydrogenase (*gdh*)	(NH_4→glutamate)	+
Amino acid catabolism		
Histidine utilization (*hut*)	(histidine→NH_4+glutamate)	+
Proline utilization (*put*)	(proline→glutamate)	+
Tryptophan utilization	(tryptophan→NH_4)	–
Asparagine utilization	(asparagine→NH_4)[b]	–
Ornithine utilization	(ornithine→glutamate)	–
Inorganic nitrogen compounds		
Dinitrogen fixation (*nifL*)	(N_2→NH_4)	–
Nitrate reduction (assimilatory)	(NO_3→NH_4)	–
Organic nitrogen compounds		
Urea utilization (*ure*)	(urea→NH_4)	+

a. A plus in this column indicates that NAC is required for N-Reg; every system known to be responsive to N-Reg requires the NTR system for N-Reg.

b. It is not known how *K. aerogenes* derives NH_4 from the aspartate that is also formed from this reaction; nor is it known how glutamate is catabolized to NH_4.

(NTRB) can catalyse the phosphorylation of NTRC as well as allow the slow autodephosphorylation of NTRC-P; thus NTRC-P (the active form) accumulates. The connection between the NTR proteins and N-Reg comes through P_{II}. Unmodified P_{II} (signalling N excess) greatly stimulates the dephosphorylating activity of NTRB; P_{II}–UMP (signalling N starvation) may be inert with respect to NTRB. Thus when P_{II} is unmodified, inactive NTRC accumulates; when P_{II} is modified to P_{II}–UMP, active NTRC-P accumulates and RNAPσ54 transcribes actively.

This model of NTR control has been tested using purified components *in vitro* at both *glnA* (glutamine synthetase) promoter and *nifL* (dinitrogen fixation) and found to be sufficient to explain N-Reg of these promoters. It was tempting to extend the model to explain N-Reg of all the systems in Table 1, particularly in view of the fact that mutants lacking either the activator (NTRC-P) or the σ54 subunit of RNAPσ54 also lacked N-Reg of every system is Table 1 (Macaluso *et al.*, 1990). However, although the NTR system in necessary for N-Reg of all the pathways in Table 1, it is sufficient only for some. The remaining pathways, e.g. *hut* , also require the NAC (nitrogen assimilation control) protein for their N-Reg.

The NAC system

Mutants that lack NAC because of insertions in the *nac* gene of *K. aerogenes* have lost N-Reg of some, but not all, pathways (Bender *et al.*, 1983; Macaluso *et al.*, 1990). In particular, the activation of *hut* and the proline utilization (*put)* system is lost in NAC-defective strains, but the activation of *glnA* and *nifL* operons remains intact in NAC-defective strains. Consistent with this difference, both the *hut* and *put* operons are transcribed by RNAPσ70 (Nieuwkoop *et al.*, 1988; Chen and Maloy, 1991) whereas the *glnA* and *nifL* operons are transcribed by RNAPσ54. Thus NAC is necessary for N-Reg of *hut, put,* and perhaps other operons transcribed by RNAPσ70.

NAC is also sufficient for activation of *hut* and *put* expression. When an isopropyl-β-D-thiogalactoside (IPTG)-inducible *tac* promoter (Chow and Berg, 1988) was inserted between the *nac* promoter and the *nac* coding sequences, NAC-dependent operons like *hut* and *put* were activated by IPTG, not by N starvation (A. Schwacha and R. A. Bender, unpublished). This IPTG induction of *hut* and *put* occurred even in the presence of excess ammonia, showing that if NAC was formed, *hut* and *put* were activated no matter what the metabolic state of the cell with respect to nitrogen. In fact, when NAC was produced from this IPTG-inducible promoter, even cells lacking the NTR system (because of mutations eliminating NTRC or σ54) showed activation of *hut* and *put.* Thus NAC appears sufficient for activation and the only role of the NTR system is to control expression of the *nac* gene.

NAC formation is controlled by the NTR system

Fusion of the *nac* promoter with the *lacZ* gene allowed us to monitor β-galactosidase as a measure of *nac* gene expression (Macaluso *et al.*, 1990). Strains with a wild-type NTR system showed strong N-Reg of *nac* expression. Strains lacking NTRC or σ54 were unable to derepress *nac*, even under severe N starvation. Strains constitutive for the NTR system were constitutive for *nac* as well. The sequence of the DNA region upstream of the *nac* coding sequence shows a strong match to the consensus RNAPσ54-dependent promoters (A. Schwacha and R. A. Bender, unpublished). About 120 bp further upstream is a match to a consensus NTRC-binding site.

NTR control of *nac* expression has been directly demonstrated by *in vitro* transcription of *nac* with purified components (A. Ninfa and R. A. Bender, unpublished). A cloned *nac* promoter was very efficiently transcribed by RNAPσ54 if NTRC-P was present. Omission of NTRC-P or substitution of NTRC for NTRC-P eliminated transcription of *nac.* As expected, the amount of NTRC-P required for *nac* transcription was somewhat higher than that required for *glnA* transcription. Thus we assume that *nac* is an operon like *nifLA* whose transcription is directly regulated by the NTR system and whose function is the regulation of a subfamily of nitrogen-responsive operons, either directly or (as is the case with NIFL) in response to other regulatory signals.

What do we know about NAC?

Studies of the *nac* gene (A. Schwacha and R. A. Bender, unpublished) and the NAC protein (T. J. Goss and R. A. Bender, unpublished) have given us much information about NAC. The *nac* gene was cloned and sequenced. The deduced amino acid sequence of NAC immediately identified NAC as a member of a family of regulatory proteins known as the LysR family (Henikoff *et al.*, 1988), supporting the notion that NAC is a regulatory protein.

The NAC protein was purified to near homogeneity (T. J. Goss and R. A. Bender unpublished) and is probably a dimer in solution. Purified NAC bound to *hutUp* DNA in gel mobility shift assays. Purified NAC also stimulated RNAPσ70-mediated transcription from *hutUp*, the promoter of the *hutUH* operon, just as CAP–cAMP does. In contrast to other LysR family regulators, NAC may have only one conformation. NAC produced *in vivo* from a *tac* promoter activated *hut, put* and urease gene transcription whether the cells were starved for nitrogen or not. Moreover, NAC made under N starvation and N excess were equally able to bind *hutUp* DNA, and the presence of such traditional N-Reg signals as 2-ketoglutarate and glutamine did not seem to affect NAC's ability to activate RNAPσ70-mediated transcription in a purified system.

To summarize, we know that NAC is necessary and sufficient to explain the transcriptional activation of the *hut* operons (and several others) in response to N-Reg. Three clear questions remain unanswered: (i) do other organisms have the NAC; (ii) how does NAC activate transcription; and (iii) why does *K. aerogenes* need NAC when NTR seems able to do the job?

NAC in other bacteria

Clearly *K. aerogenes* has NAC. Equally clearly, *S. typhimurium* does not. *S. typhimurium* cannot activate *hut* expression in response to N-Reg (Magasanik, 1978), even though it has a normal NTR system (Kustu *et al.*, 1986). When the *hut* genes from *S. typhimurium* were moved to *K. aerogenes*, they responded to N-Reg (Goldberg *et al.*, 1976) as long as the *K. aerogenes* used was *nac*$^+$ (Best and Bender, 1990). In a complementary experiment, the cloned *nac* gene from *K. aerogenes* was moved into *S. typhimurium*, and the resulting strain showed N-Reg of *hut* expression (Best and Bender, 1990). Thus wild-type *S. typhimurium* lacks an active NAC product. The situation with *E. coli* is less clear, but it appears that *E. coli* has an active NAC. Wild-type *E. coli* lacks the *hut* operons, but when the *hut* genes from *K. aerogenes* were moved into *E. coli* they responded to N-Reg in *E. coli*, albeit less dramatically (Goldberg *et al.*, 1976). Moreover, introduction of the cloned *nac* gene from *K. aerogenes* into *E. coli* did not increase *hut* expression (our unpublished results). Preliminary Southern blots detected an *E. coli* DNA sequence with similarity to the *K. aerogenes nac* gene but found no such sequence in *S. typhimurium* (W. R. Muse and R. A. Bender, unpublished). Thus *S. typhimurium* appears to have lost *nac*. It also could be argued that *E. coli* is in the process of losing the NAC system, since the operons known to be NAC-dependent in *K. aerogenes* are either absent from *E. coli* (*hut, ure*) or have lost their ability to respond to the N-Reg signal (totally for *gdh* and almost totally for *put*). Non-enteric bacteria (e.g. the pseudomonads and the rhizobia) have *hut* operons that are quite similar to *hut* from *K. aerogenes* both in sequence similarity and in their response to N-Reg. To date, we have no information on the existence of a NAC analogue outside the enterics, but the overall sequence similarity between the *hut* operons of *Pseudomonas putida* and those of *K. aerogenes* leads one to speculate that NAC might also be found outside the enterics, particularly since LysR family members are common outside the enterics.

How does NAC activate transcription?

We have few data that address this question. We know that NAC binds specifically to the promoter regions of *hut*, *put* and *ure*, the three operons whose transcription is activated by NAC (T. J. Goss and R. A. Bender, unpublished). For *hut*, the NAC-binding site is centered at about –64 relative to the start of transcription (Fig. 2), a position that mimics the site of CAP–cAMP binding in the *lac* operon of *E. coli* (Reznikoff and Abelson, 1978). The NAC-binding site in *put* (T. J. Goss and R. A. Bender, unpublished) is in a similar position relative to the transcriptional start of the *putP* gene (Chen and Maloy, 1991); thus we assume that position may be important. The NAC-binding site in *ure* is about 900 bp upstream from the *ureA* coding region, but the transcription start of the operon has not yet been identified.

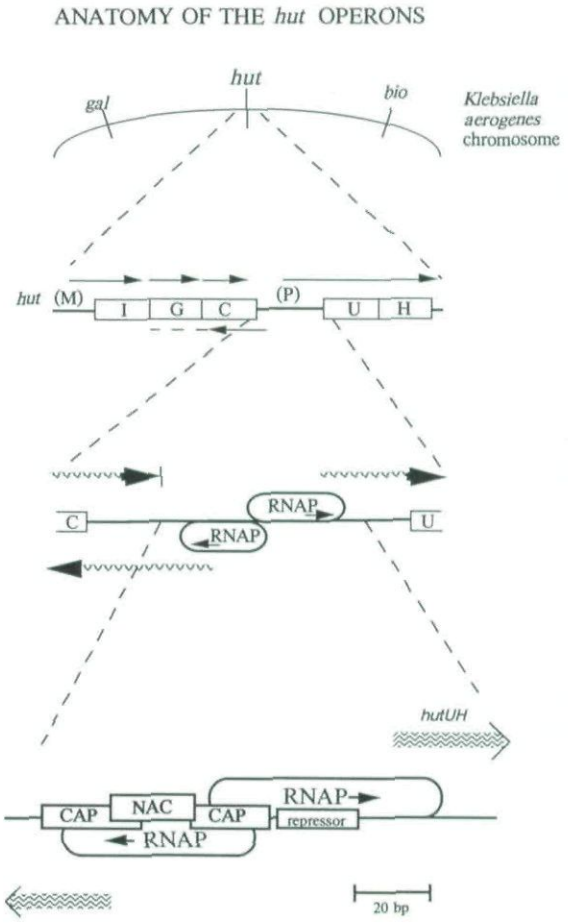

Fig. 2. The 'anatomy' of the *hut* operons. Line 1 indicates that the *hut* gene cluster lies between *gal* and *bio*. Line 2 (not to scale) shows the locations of the genes in the cluster. The transcription units (Schwacha *et al.*, 1990) are indicated by arrows. Line 3 shows the overlap between the promoter of the *hutUH* operons and a promoter of unknown function reading opposite *hut* transcription. Line 4 shows the location of the known binding sites within the *hutUH* promoter region. The extent of the sites was determined by deletion analysis using a functional assay (RNA Polymerase), gel mobility shift assay (two CAP–cAMP sites and a NAC site) or an *in vivo* titration assay (repressor). The RNAP sites have been confirmed by an exonuclease III 'footprint' assay (Nieuwkoop and Bender, 1988).

Returning to *hut*, we note that the two CAP–cAMP sites centered at –42.5 and –81.5 (R. Osuna and R. A. Bender, manuscript in preparation) would cause a double bend in the promoter that would be expected to perturb at least the –35 region of the *hutUH* promoter. Since the NAC site is almost exactly in the middle of these two CAP sites, it is tempting to speculate that the binding of a single NAC molecule at –64 might bend the DNA into a structure resembling that formed by two CAP–cAMP complexes bound at –42 and –81 (Fig. 2). We have not yet demonstrated that CAP–cAMP can bend DNA in the *hutUH* promoter, much less that NAC can. Still, the model is attractive for three reasons. (i) It explains how two different positive regulators, bound at two different sites, can activate the same RNAPσ70. (ii) Several of the known NAC-binding sites have a run of five or more A residues in their central region interrupted by a single base pair. If this base pair were eliminated, one might see a 'natural bend' and it is tempting to view NAC as 'neutralizing' this base pair. (iii) The bending model is the easiest model to disprove: either NAC bends the DNA or it does not.

Besides activating operons involved in catabolism of poor nitrogen sources, NAC also represses transcription of operons involved in the assimilation of ammonium. For example, glutamate dehydrogenase (*gdh*) is strongly repressed by NAC (Bender *et al.*, 1983; Macaluso *et al.*, 1990). NAC bound to a DNA fragment from a cloned *gdh* gene (T. J. Goss and R. A. Bender, unpublished), but neither the precise binding site nor the location of the *gdh* promoter has yet been determined. It is easy to imagine that NAC could interfere with the binding of RNAP or some other factor essential for *gdh* transcription.

Why does K. aerogenes *need NAC?*

The paradox is simple: the NTR system regulates the expression of several operons directly in response to N-Reg. Interposing another regulator between the NTR system and (e.g.) *hut* makes sense if another degree of regulation is added, but we have been unable to find any such regulation. When NAC is made, *hut* is made; when NAC is not made, *hut* is not made. Why not just regulate *hut* directly with NTR? Two features of the system offer a hint — one physiological and one structural. The IPTG-inducible *nac* gene allowed us to vary the level of *nac* expression and showed clearly that *ure*, *gdh*, and *hut* have different sensitivities to NAC (A. Schwacha and R. A. Bender, unpublished). *ure* is almost fully derepressed before *gdh* repression becomes appreciable, and *gdh* is almost fully repressed before *hut* derepression becomes appreciable. Thus NAC may serve as a signal amplifier in a cascade control, conceptually similar to the cascade control described for glutamine synthetase regulation (Ginsburg and Stadtman, 1973).

The structural argument for NAC follows from the observation that *hut* and *put* are transcribed by RNAPσ70 and are arranged in clustered operons with *hutC* immediately upstream of *hutUH* (Schwacha and Bender, 1990) and *putA* immediately upstream of *putP* (Chen and Maloy, 1991). Adding an extra (RNAPσ54-specific) promoter to either the *hutUH* or the *putP* promoter region might not interrupt any essential arrangement, but the need to put an enhancer 100–200 bp upstream of the RNAPσ54-specific promoter would require insertions into coding regions. Thus attempts to recruit RNAPσ70-dependent operons into an RNAPσ54-dependent regulatory system might prove too complex. Adding a NAC site might enlarge the promoter somewhat, but would not require alterations in nearby coding regions. Thus, in evolutionary terms, we view NAC as an agent for recruiting RNAPσ70-dependent operons into an RNAPσ54-dependent regulatory system.

All the NAC-dependent operons known to date have secondary roles. *hut* and *put* are used to provide carbon and energy and are regulated by CAP–cAMP in all three enterics. Glutamate dehydrogenase is important both for amino acid biosynthesis and for osmoregulation; it is regulated in response to both these signals in all three enterics. Even urease has a secondary role (agmatine degradation in *K. aerogenes)* but its regulation (in *K. aerogenes)* is strictly by N-Reg. Even where the NAC regulation is absent (e.g. *S. typhimurium)* these other controls remain active. Thus it is attractive to imagine that these operons originally evolved for other functions and that the development of a NAC system allowed them to be co-opted into the nitrogen regulatory response of *K. aerogenes*. In summary, we would argue that NAC is a transcriptional activator that couples RNAPσ70-dependent operons to the RNAPσ54-dependent NTR system.

Acknowledgements

This work was supported by a Public Helath Service (GM47156) from the National Institutes of Health. I am especially grateful to my colleagues Thomas J. Goss and Anthony Schwacha (this laboratory) and Alex Ninfa (Wayne State University) for allowing me to discuss their unpublished work.

References

Bender, R.A., and Magasanik, B. (1977) *J Bacteriol* **132:** 100–105.

Bender, R.A. *et al.* (1983) *J Bacteriol* **156:** 444–446.

Best, E.A., and Bender, R.A. (1990) *J Bacteriol* **172:** 7043–7048.

Chen, L.-M., and Maloy, S. (1991) *J Bacteriol* **173:** 783–790.

Chow, W.-Y., and Berg, D.E. (1988) *Proc Natl Acad Sci USA* **85:** 6468–6472.

Ginsburg, A., and Stadtman, E.R. (1973) In *The Enzymes of*

Glutamine Metabolism. Prusiner, S., and Stadtman, E.R. (eds). New York: Academic Press, pp. 9–43.

Goldberg, R.B. *et al.* (1976) *J Bacteriol* **127:** 114–119.

Henikoff, S. *et al.* (1988) *Proc Natl Acad Sci USA* **85:** 6602–6606.

Kustu, S. *et al.* (1986) In *Regulation of Gene Expression. Symposium of the Society for General Microbiology*. Booth, I.R., and Higgins, C.R. (eds). Cambridge: Cambridge University Press, pp.139–154.

Macaluso, A. *et al.* (1990) *J Bacteriol* **172:** 7249–7255.

Magasanik, B. (1978) In *The Operon*. Miller, J.H., and Reznikoff, W.S. (eds). Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 373–387.

Magasanik, B., and Neidhardt, F.C. (1987) In *Escherichia coli and Salmonella typhimurium*. Neidhardt, F.C. (ed.). Washington, D.C., American Society for Microbiology, pp. 1318–1325.

Merrick, M. (1988) In *The Nitrogen and Sulphur Cycles*. Cole, J.A., and Ferguson, S.J. (eds). Cambridge: Cambridge University Press, pp. 331–361.

Neidhardt, F.C., and Magasanik, B. (1957) *J Bacteriol* **73:** 253–259.

Nieuwkoop, A.J., and Bender, R.A. (1988) *J Bacteriol* **170:** 4986–4990.

Nieuwkoop, A.J. *et al.* (1988) *J Bacteriol* **170:** 2240–2246.

Prival, M.J., and Magasanik, B. (1971) *J Biol Chem* **246:** 6288–6296.

Reznikoff, W.S., and Abelson, J.N. (1978) In *The Operon*. Miller, J.H., and Reznikoff, W.S. (eds). Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 221–243.

Schwacha, A., and Bender, R.A. (1990) *J Bacteriol* **172:** 5477–5481.

Schwacha, A. *et al.* (1990) *J Bacteriol* **172:** 5991–5998.