Nitrogen fixation genes (*nif* K,D,H) in the filamentous nonheterocystous cyanobacterium *Plectonema boryanum* do not rearrange

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Abstract. The organisation of the structural genes for nitrogen fixation (*nif* K,D and H) in a nonheterocystous, filamentous cyanobacterium *Plectonema boryanum* has been examined in comparison with a heterocystous cyanobacterium, *Anabaena torulosa*. DNA from repressed (fix⁻) cultures of *A. torulosa* showed a discontinuous *nif* region spread over approximately 18 kb, an arrangement typical of the vegetative cells of heterocystous cyanobacteria. The region contained a contiguous *nif* DH separated from *nif* K by nearly 11 kb DNA. The intervening 11 kb DNA harboured the gene *xis* A involved in the rearrangement of *nif* K,D,H to form a cluster during differentiation of heterocysts. DNA from *Plectonema boryanum* had a small, contiguous *nif* KDH cluster spanning a region of approximately 4 kb. DNA homologous to the 11 kb excison with its resident *xis* A was not present. *Nif* hybridisation patterns of restriction digests of the DNA isolated from repressed (fix⁻) or induced (fix⁺) cultures of *P. boryanum* were completely identical. These results unequivocally demonstrate that in the nonheterocystous cyanobacterium, unlike in the heterocystous strains, no gene rearrangement, either within the *nif* KDH cluster or in its vicinity, accompanies the expression of nitrogenase activity.

Keywords. Filamentous nonheterocystous cyanobacteria; nitrogen fixation genes; *P. boryanum*; gene rearrangement.

1. Introduction

The ability to reduce atmospheric dinitrogen is restricted to only a few groups of prokaryotes. The reduction of dinitrogen to ammonia is brought about by an oxygen labile enzyme complex consisting of two proteins – nitrogenase and nitrogenase reductase (Eady and Postgate 1974). Nitrogenase is an $\alpha_2 \beta_2$ tetramer encoded by *nif* K (β subunits) and *nif* D (α subunits), while the reductase is a α_2 dimer encoded by *nif* H (Dixon 1984). Nitrogenase and nitrogenase reductase from different N_2-fixing microbes appear to have been fairly conserved and exhibit considerable homology and cross reactivity both at the level of proteins and their corresponding genes (Orme-Johnson 1985). The organisation of *nif* structural genes (i.e. *nif* K,D,H) in various diazotrophs is, however, by no means uniform. For example, the enterobacterium *Klebsiella pneumoniae* (Merrick *et al* 1980), the photosynthetic bacterium *Rhodopseudomonas capsulata* (Avtges *et al* 1983) and the fast growing rhizobia, *Rhizobium meliloti* (Corbin *et al* 1982), *R. leguminosarum* (Downie *et al* 1983) or species of *Azotobacter* (Jones *et al* 1984) all possess a contiguous *nif* KDH cluster. In contrast, in the slow growing rhizobia *R. japonicum* (Hahn *et al* 1984), and in the *Rhizobium* which nodulates the non-legume *Parasponia* (Weinman *et al* 1984), *nif* KD and *nif* H are separated. The functional significance of these arrangements is not yet understood.

Many cyanobacteria, which are the unique photoautotrophic diazotrophs with an oxygenic mode of photosynthesis, show both contiguous and discontinuous type of *nif*K,D,H organisation (Haselkorn 1986; Hallenbeck 1987). Thus, in *Gloeothece* PCC 6909-1, a unicellular, aerobic, nitrogen-fixing cyanobacterium, *nif*K,D,H occurs in a cluster (Kallas *et al* 1983) while in the vegetative cells of the filamentous, heterocystous cyanobacterium *Anabaena* 7120 yet another discontinuous arrangement has been reported with *nif*K being separated from *nif*DH by an 11 kb intervening sequence (Rice *et al* 1982). More interestingly, in heterocysts this intervening sequence (excison) is precisely deleted and *nif*K and *nif*DH get juxtaposed together in a cluster and are transcribed, as in *Klebsiella*, from a single *nif*H promoter (Golden *et al* 1985). This rearrangement is apparently linked to N₂ fixation since it also occurs in het⁻ mutants of heterocystous cyanobacteria (Damerval *et al* 1985) and even in *Escherichia coli* wherein a relevant portion of *Anabaena* DNA has been cloned (Lammers *et al* 1986).

These findings have raised important questions regarding the organisation of the *nif* region in filamentous, nonheterocystous cyanobacteria, especially the possibility of a rearrangement of the *nif* structural genes. It is interesting to know whether such cyanobacteria (i) have a clustered or a discontinuous arrangement of *nif*K,D,H, (ii) rearrange *nif*K,D,H during N₂ fixation, and (iii) possess excision mechanisms similar to those (*xis*A) responsible for *nif*K,D,H rearrangement during heterocyst differentiation in *Anabaena*. A previous work has shown a contiguous *nif*K,D,H arrangement in repressed (fix⁻) cultures of two filamentous, nonheterocystous forms (Kallas *et al* 1985) and in one of these strains the possibility of a *nif* rearrangement has been reported (Barnum and Gendel 1985). But these reports do not provide definitive information on all the three essential points cited above. In the present study these questions are investigated in a filamentous, nonheterocystous cyanobacterium *Plectonema boryanum*-594.

2. Materials and methods

2.1 *Organisms and growth conditions*

Anabaena torulosa, a filamentous, heterocystous, sporulating strain of brackish waters was isolated in this laboratory (Fernandes and Thomas 1982) and used in axenic condition. *Plectonema boryanum*-594, a filamentous, nonheterocystous strain was obtained from Prof R Haselkorn, University of Chicago. Fifteen-litre cultures of both strains were grown in five-fold diluted cyanophycean medium (CM/5) (David and Thomas 1979), with or without 10 mM KNO₃, under continuous illumination (2·5 mW per square cm) and aeration (2 litres per min) to the stationary phase. *P. boryanum* was induced to fix N₂ microaerobically by growing in combined N-free CM/5 and continually sparging with N₂ for 40 hr as described previously (Apte and Thomas 1984).

2.2 *Extraction of chromosomal DNA, restriction digestion and preparation of Southern blots*

High molecular weight chromosomal DNA was prepared from approximately 15 g fresh weight of cells harvested from each culture. DNA was extracted by a slight

modification of the procedure described by Mazur *et al* (1980). Lysozyme concentration used and the period of lysis at 37°C was as follows: *A. torulosa*, 5 mg per ml, 45 min; *P. boryanum*, 10 mg per ml, 2·5–3 hr. Repeated (2–3 times) spooling of DNA on to glass rods was carried out to obtain a high purity of DNA. In addition sometimes DNA was further purified on NACS columns (Bethesda Research Labs, Gaithersburg, Maryland) using the procedure recommended by the manufacturers. DNA was hydrolysed with *Eco*RI, *Cla*I, *Bgl*II and *Hind*III (all from Bethesda Research Labs.) for 5 hr under buffer conditions and temperature recommended by the supplier. The reactions were terminated by heating for 10 min at 65°C. Five μg DNA was electrophoresed per lane of a 0·7% agarose gel (20 × 15 × 0·8 cm). Denaturation, neutralisation and unidirectional Southern transfer of DNA to Millipore (HAHY 304, FO HA, 0·4 μm) were performed in 20 × SSC as described by Maniatis *et al* (1982).

2.3 *Gene probes and hybridisations*

All the probes used were recombinant plasmids constructed by the laboratory of Prof R Haselkorn and contained clones of fragments from the *nif* region of *Anabaena* 7120. These gene probes have been described in table 1. A nick translation kit (N-5000, Amersham International plc., Amersham, England) and α-[^{32}P]dCTP (Isotope Division, BARC, Bombay) were used to radiolabel probes to $1–2 \times 10^8$ cpm per μg DNA. Prehybridisation (6 hr) and hybridisation (20 hr) were performed in 6 × SSC at 65°C as described by Maniatis *et al* (1982). Blots were washed in the following sequence: (i) 2 × SSC + 0·2% SDS, three times for 10 min

Table 1. *Anabaena* 7120 DNA probes used in this study.

Recombinant plasmid used[a]	*Anabaena* DNA insert	Reference
pAn 207·8[b]	0·7 kb *Hind*III segment from within *nif*K	Rice *et al* (1982)
pAn 207·65[c]	A ≃ 2·4 kb *Hinc*II-*Kpn*I fragment containing 0·185 kb of *nif*K, ≃ 1·14 kb of *xis*A and the rest of the DNA corresponding to the 11 kb excison	R Haselkorn, personal communication (see Lammers *et al* 1986, for a restriction map of the excison)
pAn 207·3[b]	2 kb *Hind*III fragment located on the 11 kb excison close to *nif*D	Golden *et al* (1985)
pAn 256[b]	A *Hind*III fragment with most of *nif*D (1·4 kb) and non-*nif* DNA (1·3 kb)	Rice *et al* (1982)
pAn 154·3[b]	A *Hind*III segment with all of *nif*H (0·9 kb), 0·15 kb of *nif*D and non-*nif* DNA (0·75 kb)	Mevarech *et al* (1980)

[a] All the plasmids were provided by Prof Robert Haselkorn;
[b] these *Anabaena* inserts have been cloned into *Hind*III site of pBR 322;
[c] the *Anabaena* insert was cloned into *Sma*I site of pUC19.

each at 25°C, (ii) 1 × SSC + 0·1% SDS, four times for 30 min each at 65°C, (iii) 0·1 × SSC, two times for 15 min each at 25°C. Dried blots were exposed to X-ray films at −70°C using intensifying screens, for 2–7 days.

3. Results

Figure 1 shows the hybridisation of various *Anabaena* 7120 probes to Southern blots of *Hind*III digests of *A. torulosa* DNA. *A. torulosa* was used here as a representative of heterocystous cyanobacteria and as a positive control for the various *Anabaena* probes employed. All the probes hybridised well to *A. torulosa* DNA but showed a *Hind*III restriction pattern quite distinct from that of *Anabaena* 7120 (Golden *et al* 1985). *Eco*RI and *Bgl*II did not hydrolyse *A. torulosa* DNA properly. *Hind*III sites in the *nif* region of *A. torulosa* were less frequent than in *Anabaena* 7120 thus yielding larger *nif* positive fragments (2·8–3·2 kb) than those observed in *Anabaena* 7120. In spite of this, no single fragment hybridised to all three probes of the *nif* structural genes (*nif*K,D as well as *nif*H; figure 1) thus indicating that they were not clustered. Two fragments hybridised to *nif*H probe. We have evidence now that the larger fragment (3·2 kb) is involved in the rearrangement in heterocysts (data not included) and is therefore associated with *nif*D while the 1·2 kb fragment probably indicates a second copy. Two probes internal to the 11 kb intervening sequence (excison) between *nif*K and *nif*D of *Anabaena* 7120 were used to locate homologous sequences in *A. torulosa*. To our knowledge the data in figure 1 is the first evidence of occurrence of genes homologous to fragments 207·3 and 207·65 outside *Anabaena* 7120. Interestingly, a summation of fragment sizes hybridising to these two probes yield 11·2 kb suggesting that the dimensions of such excisons may be identical in many heterocystous cyanobacteria. The overall results suggest a discontinuous *nif*K,D,H arrangement similar to other heterocystous cyanobacteria (Hallenbeck 1987).

Figure 2 shows the hybridisation of *Anabaena* 7120 *nif*K (2b), *nif*D (2c) and *nif*H (2d) probes to Southern blots of *Cla*I, *Eco*RI and *Hind*III single and double digests of DNA isolated from repressed (fix⁻) cultures of *P. boryanum* (2a). All these probes hybridised to a single *Cla*I fragment of 12·1 kb. In *Hind*III digests both *nif*D and *nif*H were found located on the same 3·3 kb fragment indicating a close linkage between these two genes. No evidence for an association between *nif*K and *nif*D genes was found in these digests.

P. boryanum DNA did not hybridise to the probes pAn207·3 and pAn207·65 (data not shown) even at a much lower stringency of washing (5 × SSC + 0·5% SDS) although positive bands were observed with *A. torulosa* DNA digests. Clearly, therefore, DNA homologous to the sequence intervening between *nif*K and D of *Anabaena* 7120 and more specifically to *xis*A was absent in *P. boryanum*.

Figure 3 provides a comparison of the hybridisation of *Anabaena* 7120 *nif*K,D,H probes to DNA isolated from repressed (fix⁻) or induced (fix⁺) cultures of *P. boryanum*. All the three probes hybridised to either a single 12·1 kb *Cla*I fragment (3a) or a single 3·4 kb *Bgl*II fragment (3b). This pattern of hybridisation was completely identical both in fix⁻ as well as fix⁺ cultures and no evidence of a gene rearrangement within (3b) or nearby (3a) to the *nif*KDH cluster was detected. *Bgl*II data clearly showed that all the three genes (*nif*K,D,H) were contiguous.
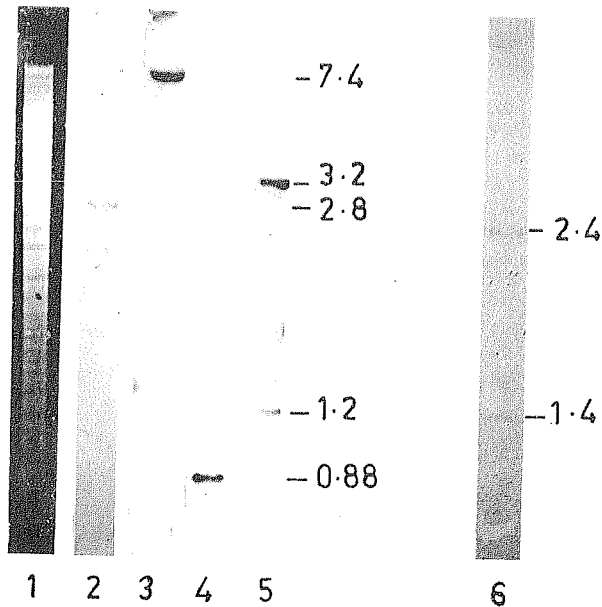
Figure 1. Hybridisation of *Anabaena* 7120 gene probes to DNA obtained from the vegetative cells of the heterocystous cyanobacterium *Anabaena torulosa*. The lanes from left to right are: a stained agarose gel showing electrophoretic fractionation of *A. torulosa* vegetative cell DNA digested with *Hind*III (lane 1); autoradiograms showing hybridisation of replicate blots of lane 1 to pAn207·8 (*nif*K, lane 2), pAn207·3 (fragment internal to the 11 kb excison located close to *nif*D, lane 3), pAn256 (*nif*D, lane 4) and pAn154·3 (*nif*H, lane 5). Lane 6 shows hybridisation of pAn207·65 (*xis*A) to a separate blot. DNA fragment sizes (in kb) shown to the right of lane 5 correspond to lanes 2–5 and those shown to the right of lane 6 correspond to lane 6 only.

4. Discussion

The hybridisation patterns described in figures 2 and 3 have allowed the construction of a restriction map for the *nif* region of *P. boryanum*-594 (figure 4a). The map is complete for a region of approximately 8 kb spanning the *Eco*RI site to the left of *nif*K to the *Eco*RI site to the right of *nif*H. The two *Cla*I sites which lie outside this region cannot be mapped and their proposed location is tentative (indicated by dotted line). Based on the *Bgl*II data, the *nif*KDH cluster should span 3·4 kb which cannot possibly accommodate all the information necessary to make active nitrogenase proteins. In *K. pneumoniae*, the *nif*KDH cluster spans 4 kb (Roberts and Brill 1981) and it is likely that the physical limits of *P. boryanum* *nif*KDH cluster are similar to or greater than those described by figure 4a. A similar restriction map of the *nif* region of *A. torulosa* could not be determined due to incomplete hydrolysis and the absence of overlapping restriction fragments. Yet a tentative *Hind*III map has been prepared by lining up *Hind*III fragments in the order in which they are found in *Anabaena* 7120 (Golden *et al* 1985). This is presented in figure 4b for comparison. A total span of 18 kb appears to accommodate the *nif*K,D,H and intervening sequence (with *xis*A) of *A. torulosa*. This is in agreement with the dimensions of corresponding DNA in *Anabaena* 7120 (Haselkorn
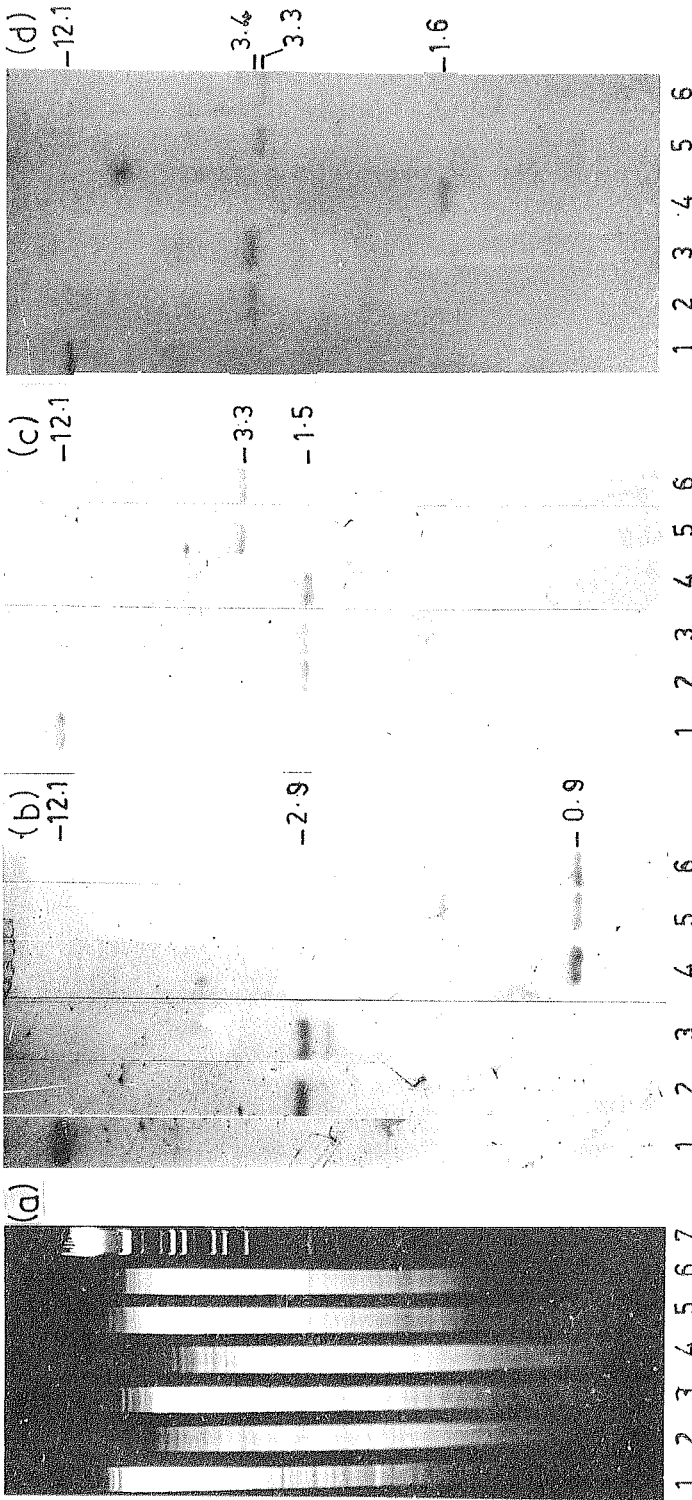
Figure 2. Localisation of *nif*K,D and H genes in the repressed (fix⁻) cultures of the nonheterocystous cyanobacterium *Plectonema boryanum*-594. (a) Stained agarose gel showing electrophoretic fractionation of *P. boryanum* DNA digested with *Cla*I (lane 1), *Cla*I + *Eco*RI (lane 2), *Eco*RI (lane 3), *Eco*RI + *Hind*III (lane 4), *Hind*III (lane 5) and *Hind*III + *Cla*I (lane 6). Lane 7 contains size markers; a mixture of bacteriophage lambda DNA digested with *Xho*I and *Bst*EII. To the right are autoradiograms showing hybridisation of replicate blots of the gel shown in (a) to *Anabaena* 7120 probes pAn207-8 (b), pAn256 (c) and pAn1543 (d). DNA fragment sizes in kb are shown to the right of each autoradiogram.
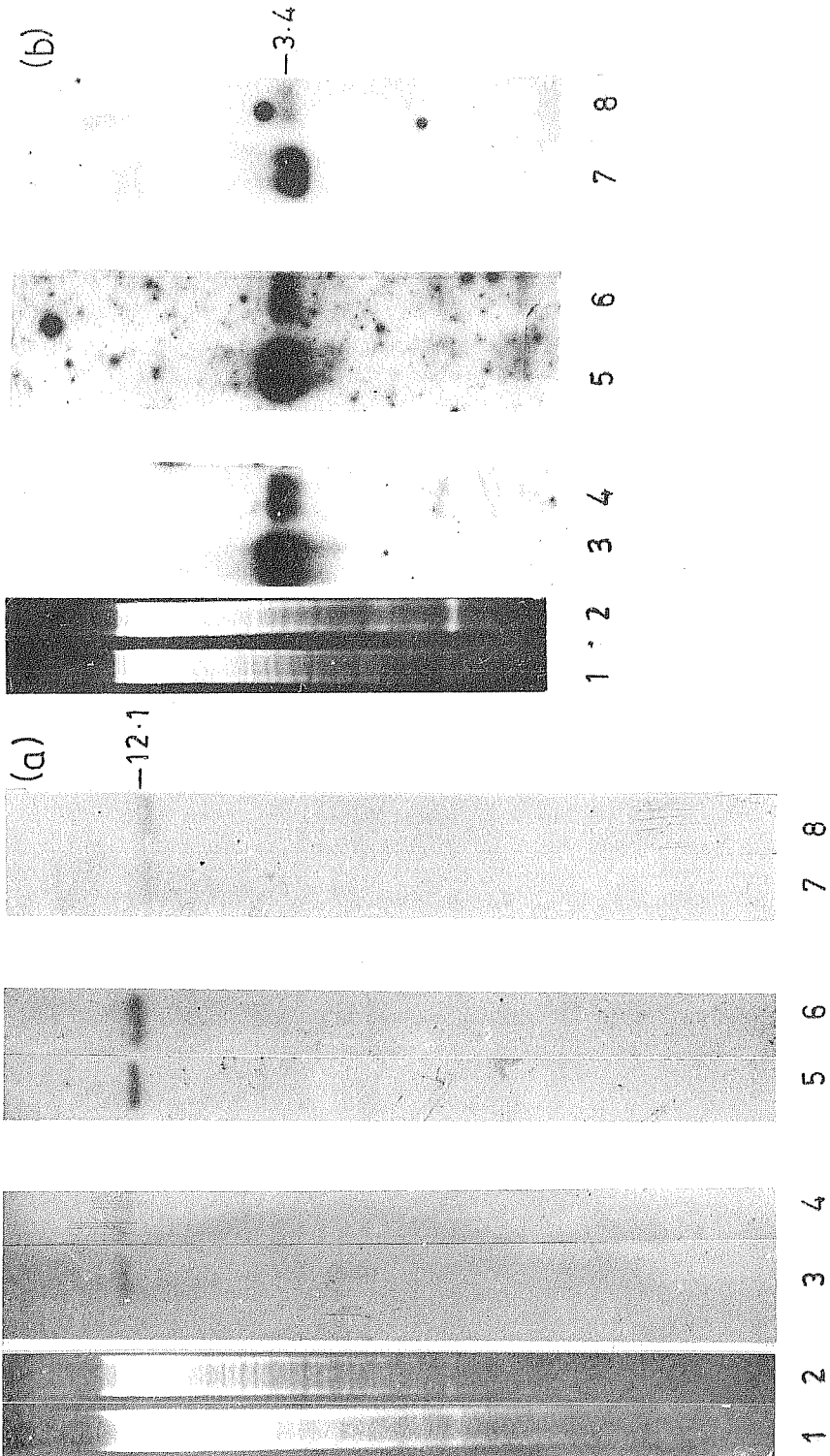
Figure 3. Hybridisation of *Anabaena nif*K,D and H probes to DNA isolated from repressed (fix⁻) or induced (fix⁺) cultures of *P. boryanum*-594. DNA from fix⁻ (lanes 1,3,5 and 7) and fix⁺ (lanes 2,4,6 and 8) cultures was digested with either *ClaI* (a) or *BglII* (b). Lanes 1 and 2 show electrophoretic fractionation of the restriction digests on agarose gels. Lanes 3 to 8 show autoradiograms of hybridisation of replicate blots of lanes 1 and 2 to pAn207-8 (lanes 3,4), pAn256 (lanes 5,6) and pAn154·3 (lanes 7,8). The numbers indicate DNA fragment sizes in kb.
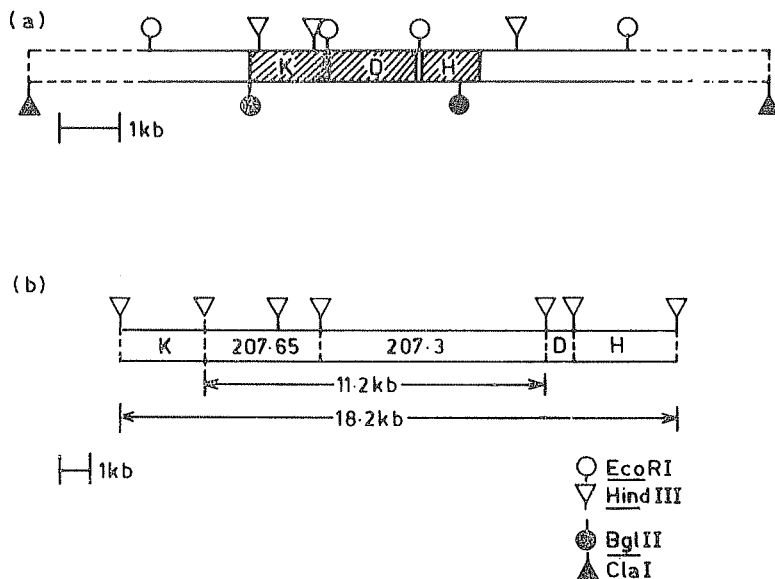
Figure 4. Restriction maps of *nif* region of *Plectonema boryanum* (a) and *Anabaena torulosa* (b). The *P. boryanum* map has been constructed from the data shown in figures 2 and 3 and is complete for an 8 kb region (indicated by solid lines). The location of *Cla*I sites is tentative and is indicated by dotted lines. The *Bgl*II sites are shown to be within *nif* cluster since 3·4 kb DNA cannot accommodate the information pertaining to all three *nif* genes. The physical sizes (shown as cross-hatched rectangles) of *nif*K and D are based on present data and that of *nif*H is based on known molecular weight of nitrogenase reductase. A tentative map of *A. torulosa* (b) obtained by lining up all *Hind*III fragments (figure 1) hybridising to various gene probes in the same order in which they are located on *Anabaena* 7120 chromosome. The fragments within each gene can be oriented either way. The map is given for comparison with *Plectonema* (a) and shows the important differences in *nif* organisation: i.e. a total span of ≃ 18 kb with an intervening sequence of ≃ 11 kb in *A. torulosa* as against a small, contiguous cluster of ≃ 4 kb in *P. boryanum*.

et al 1986) except that the latter has many more *Hind*III sites (13) than has *A. torulosa* (7) in this region.

It is quite clear now that the heterocystous and nonheterocystous cyanobacteria differ strikingly in their organisation of *nif* structural genes. In the heterocystous forms, the vegetative cell DNA shows a characteristic discontinuous arrangement (figure 4b) of *nif*K,D and H. An intervening sequence between *nif*K and D approximately 11 kb in size, with a resident *xis*A may also be a general feature of all heterocystous forms including their het⁻ mutants. In contrast, the filamentous, nonheterocystous cyanobacteria show a contiguous arrangement of *nif*K,D,H within a small cluster (figure 4a) similar to unicellular cyanobacteria (Kallas *et al* 1983) or *Klebsiella* (Merrick *et al* 1980). The total size of fragments hybridising to all the three *nif* structural genes in *P. boryanum* ranges from 3·4 kb (*Bgl*II) to 4·2 kb (*Hind*III) which cannot accommodate any more than the structural genes. Clearly, therefore, a rearrangement within the *nif* cluster is improbable and is proved to be so by the *Bgl*II data (figure 3b).

The possibility of a reorganisation close to the *nif*KDH cluster, if not within, was suggested by Barnum and Gendel (1985) who showed that the fix⁺ cultures of

P. boryanum showed a 4·5 kb *Cla*I fragment containing all three *nif* genes in addition to the 12 kb *Cla*I fragment found in fix⁻ cultures. The results shown in figure 3 discount this observation. It is possible that the 4·5 kb fragment was a result of some non-specific hybridisation at a relatively low stringency employed by the above workers. The present study unequivocally demonstrates that filamentous, nonheterocystous cyanobacteria exhibit no reorganisation of genes, either within the *nif*KDH cluster or in its close vicinity during the expression of nitrogenase activity.

Acknowledgements

References

Apte S K and Thomas J 1984 Effect of sodium on nitrogen fixation in *Anabaena torulosa* and *Plectonema boryanum*. *J. Gen. Microbiol.* 130: 1161–1168

Avtges P, Scolnik P A and Haselkorn R 1983 Genetic and physical map of the structural genes (*nif*H,D,K) coding for the nitrogenase complex of *Rhodopseudomonas capsulata*. *J. Bacteriol.* 156: 251–256

Barnum S R and Gendel S M Organisation of *nif*H,D,K in the nonheterocystous filamentous cyanobacterium *Plectonema boryanum*. In *Nitrogen fixation research progress* (eds) H J Evans, P J Bottomley and W E Newton (Dordrecht: Martinus Nijhoff) p. 516

Corbin D, Ditta G and Helinski D R 1982 Clustering of nitrogen fixation (*nif*) genes in *Rhizobium meliloti*. *J. Bacteriol.* 149: 221–228

Damerval T, Franche C, Rippka R and Cohen-Bazire G 1985 Rearrangement of *nif* structural genes in *Nostoc* PCC 7906. In *Nitrogen fixation research progress* (eds) H J Evans, P J Bottomley and W E Newton (Dordrecht: Martinus Nijhoff) p. 517

David K A V and Thomas J 1979 Extracellular polypeptides of *Anabaena* L-31: evidence for their role in regulation of heterocyst formation. *J. Biosci.* 1: 447–455

Dixon R A 1984 The genetic complexity of nitrogen fixation. *J. Gen. Microbiol.* 130: 2745–2756

Downie J A, Ma Q-S, Knight C D, Hombrecher G and Johnston W B 1983 Cloning of the symbiotic region in *Rhizobium leguminosarum*. the nodulation genes are between nitrogenase genes and a *nif*A-like gene. *EMBO J.* 2: 947–952

Eady R R and Postgate J R 1974 Nitrogenase. *Nature (London)* 249: 805–810

Fernandes T and Thomas J 1982 Control of sporulation in the filamentous cyanobacterium *Anabaena torulosa*. *J. Biosci.* 4: 85–94

Golden J W, Robinson S J and Haselkorn R 1985 Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium *Anabaena*. *Nature (London)* 314: 419–423

Hahn M, Meyer L, Studier D, Regensburger B and Hennecke H 1984 Insertion and deletion mutations within the *nif* region of *Rhizobium japonicum*. *Plant Mol. Biol.* 3: 159–168

Hallenbeck P C 1987 Molecular aspects of nitrogen fixation by photosynthetic prokaryotes. *CRC Crit. Rev. Microbiol.* 14: 1–48

Haselkorn R 1986 Organisation of the genes for nitrogen fixation in photosynthetic bacteria and cyanobacteria. *Annu. Rev. Microbiol.* 40: 525–547

Haselkorn R, Golden J W, Lammers P J and Mulligan M E 1986 Developmental rearrangement of cyanobacterial nitrogen fixation genes. *Trends Genet.* 2: 255–259

Jones R, Woodley P and Robson R 1984 Cloning and organisation of some genes for nitrogen fixation from *Azotobacter chroococcum* and their expression in *Klebsiella. Mol. Gen. Genet.* 197: 318–327

Kallas T, Coursin T and Rippka R 1985 Different organisation of *nif* genes in nonheterocystous and heterocystous cyanobacteria. *Plant Mol. Biol.* 5: 321–329

Kallas T, Rebiere M C, Rippka R and Tandeau de Marsac N 1983 The structural *nif* genes of the cyanobacteria *Gloeothece* sp. and *Calothrix* sp. share homology with those of *Anabaena* sp., but the *Gloeothece* genes have a different arrangement. *J. Bacteriol.* 155: 427–431

Lammers P J, Golden J W and Haselkorn R 1986 Identification and sequence of a gene required for a developmentally regulated DNA excision in *Anabaena. Cell* 44: 905–911

Maniatis T, Fritsch E F and Sambrook J 1982 *Molecular cloning: a laboratory manual* (Cold Spring Harbor: Cold Spring Harbor Laboratory)

Mazur B J, Rice D and Haselkorn R 1980 Identification of blue-green algal nitrogen fixation genes by using heterologous DNA hybridisation probes. *Proc. Natl. Acad. Sci. USA* 77: 186–190

Merrick M, Filser M, Dixon R, Elmerich C, Sibold L and Houmard J 1980 The use of translocatable genetic elements to construct a fine structure map of the *Klebsiella pneumoniae* nitrogen fixation (*nif*) gene cluster. *J. Gen. Microbiol.* 117: 509–520

Mevarech M, Rice D and Haselkorn R 1980 Nucleotide sequence of a cyanobacterial *nif*H gene coding for nitrogenase reductase. *Proc. Natl. Acad. Sci. USA* 77: 6476–6480

Orme-Johnson W H 1985 Molecular basis of biological nitrogen fixation. *Annu. Rev. Biophys. Chem.* 14: 419–459

Rice D, Mazur B J and Haselkorn R 1982 Isolation and physical mapping of nitrogen fixation genes from the cyanobacterium *Anabaena* 7120. *J. Biol. Chem.* 257: 13157–13163

Roberts G P and Brill W J 1981 Genetics and regulation of nitrogen fixation. *Annu. Rev. Microbiol.* 35: 207–235

Weinman J J, Fellows F F, Gresshoff P M, Shine J and Scott K F 1984 Structural analysis of the genes encoding the molybdenum iron protein in the *Parasponia rhizobium* strain ANU 289. *Nucleic Acid Res.* 12: 8329–8344