JOURNAL OF BACTERIOLOGY, May 1984, p. 404–410
0021-9193/84/050404-07$02.00/0

Vol. 158, No. 2

Mapping of *Rhodopseudomonas capsulata nif* Genes†

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Received 21 October 1983/Accepted 1 February 1984

The endogenous gene transfer system of *Rhodopseudomonas capsulata* was used to analyze mutations which block the ability to use molecular nitrogen as the sole nitrogen source (*nif*). With this fine-structure mapping tool, linkage of *nif* mutations could be reliably established if separated by 2,700 base pairs or less. Eleven independent mutations were analyzed, and five linkage groups were found. The overall chromosomal arrangement of these groups awaits conjugational or physical analysis. A candidate for the inactive subunit of *R. capsulata* Fe protein was located in gels at a position of about 38,000 molecular weight, 5,000 more than that of the presumed active subunit.

Nitrogen fixation is a widespread procaryotic process carried out by the highly conserved enzyme complex nitrogenase (3). This conservation is also reflected in the homology between the nitrogenase structural genes from *Klebsiella pneumoniae* and those from many other diazotrophs (15, 22). Although the genes for the additional functions necessary for nitrogen fixation do not share notable homology (22), the transcriptional signals for free-living diazotrophs appear to be similar across species lines (2). The purple photosynthetic bacteria share these conserved properties (2, 5) but also manifest an unusual regulation of nitrogenase activity by covalent modification and inactivation of the Fe protein (11, 12) in response to NH_4^+ or glutamine (18). This additional form of control suggests that the genetics of this system might be somewhat more elaborate than that for the well-studied *K. pneumoniae* system (20). Because *Rhodopseudomonas capsulata* was the first phototrophic species in which a facile endogenous genetic transfer system had been demonstrated (14), it was the organism selected for genetic investigation of nitrogen fixation among the *Rhodospirillaceae*.

To initiate this study, we isolated mutants of *R. capsulata* that were unable to grow with N_2 as the sole nitrogen source (Nif^-). Eleven mutations have been shown to be arranged in five linkage groups separated by at least 2,700 base pairs (bp). Representatives of these five groups are now being used to screen newly isolated spontaneous Nif mutants for additional linkage groups (J. D. Wall and J. Love, manuscript in preparation) and for complementation of cloned DNA fragments (1).

MATERIALS AND METHODS

Bacterial strains. Bacterial strains and mutants used are described in Table 1.

Media and growth conditions. The minimal medium was RCVB as described by Wall et al. (27) except that biotin was omitted and the designation was changed to RCV. This defined medium routinely contained 30 mM sodium DL-malate and 7.5 mM $(NH_4)_2SO_4$ as the carbon and nitrogen sources, respectively. For derepression of nitrogenase, RCV medium was modified by omission of $(NH_4)_2SO_4$ and addition of 7.5 mM sodium L-glutamate or by reduction of the $(NH_4)_2SO_4$ concentration to 2 mM. G buffer (32) was 10 mM Tris-hydrochloride, pH 7.8, containing NaCl, $MgCl_2$, and $CaCl_2$, each at 1 mM. Growth medium for the production of gene transfer agent was 0.3% (wt/vol) yeast extract (Difco Laboratories)–0.3% (wt/vol) Bacto-Peptone (Difco) (YP medium). For agar plates, liquid media were solidified with 1.2% (wt/vol) agar (Difco).

Photosynthetic cultures were grown anaerobically in completely filled screw-capped tubes of 17- or 20-ml capacity in a glass-sided water bath maintained at 30 to 32°C and illuminated with a bank of three 60-W Lumiline incandescent lamps (ca. 6,000 lx).

Nif mutant isolation. *Rhodopseudomonas capsulata* B10 cells were irradiated with UV light to 0.02% survival or were mutagenized with ethyl methane sulfonate (EMS) by the method of Meynell and Meynell (16). Mutagenized cells were allowed to grow heterotrophically or photoheterotrophically for UV- and EMS-treated cells, respectively, in RCV medium overnight; the cells were then harvested and suspended in RCV medium lacking ammonium salts. To starve for nitrogen, the cells were incubated aerobically in darkness for 4 h at 32°C. This culture was diluted to contain a concentration of cells such that turbidity was just visible to the unaided eye, and penicillin G (Parke, Davis & Co., Inc.) was added to a final concentration of 10 U/ml. The flask containing the culture was placed in a GasPak (BBL Microbiology Systems) jar made anaerobic with an H_2-plus-CO_2 gas generator (H_2-CO_2-N_2 atmosphere), and the jar was incubated in the light at 32°C overnight. The surviving cells were harvested, penicillinase (Penase; Difco) was added to the cells to 100 U/ml, and the mixture was incubated for 30 min at 35°C. Subsequently, the cells were inoculated into RCV, a permissive medium, for overnight growth. By using this procedure, we carried out two or three rounds of penicillin enrichment before spreading survivors on plates containing solidified RCV medium modified to contain 0.01% (wt/vol) $(NH_4)_2SO_4$. After incubation in anaerobic jars in the light, small colonies, presumably those unable to use N_2 to supplement growth, were picked and retested for growth with N_2 as the sole nitrogen source.

Gene transfer procedure. Gene transfer was carried out with the gene transfer agent (GTA) described by Marrs (14), according to procedures previously described (28, 32), with the following modifications. Samples of 0.1 ml each of harvested recipient cells and of donor cell-free filtrates were mixed in 0.4 ml of G buffer containing 500 μg of bovine albumin per ml (fraction V; Sigma Chemical Co.). After incubation for 60 min at 35°C, 0.2-ml samples were plated

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† Contribution 9510 of the Missouri Agricultural Experiment Station journal series.

TABLE 1. Bacterial strains

Strain	Genotype	Source
Rhodopseudomonas capsulata		
B10	Wild type	Marrs (12)
W15	*nif-4*	(25)
J41	*nif-41*	This work[a]
J43	*nif-43*	This work[a]
J56	*nif-56*	This work[b]
J57	*nif-57*	This work[b]
J58	*nif-58*	This work[b]
J59	*nif-59*	This work[b]
J60	*nif-60*	This work[b]
J61	*nif-61*	This work[b]
J62	*nif-62*	This work[b]
J1	*rif-1*	Spontaneous Rif^r derivative of strain B10
J441	*nif-44 rif-1*	This work[c]
Rhodospirillum rubrum	Wild type	Ludden

[a] From strain B10 after UV irradiation.
[b] From strain B10 after EMS treatment.
[c] From strain B10, *nif* after UV irradiation, and *rif* from strain J1 by GTA.

either on RCV plates lacking $(NH_4)_2SO_4$ for selection of transductants capable of growing on N_2 or on YP plates containing 2.0 mM $MgSO_4$ and 2.0 mM $CaCl_2$ for subsequent selection of rifampin resistance. Rifampin (Sigma) was prepared as a sterile solution in dimethyl sulfoxide, and the final concentration on plates was ca. 80 μg/ml.

Analysis of genetic data. The distance between two markers causing the same phenotype can be estimated by the ratio test (6) described in detail by Yen and Marrs (32). In the present study, the marker used for normalization of Nif$^-$ mutant crosses was *rif-1*. The method of Kimball (8) was used to calculate 95% confidence intervals for the ratios of the Nif$^+$/Rif^r recombinants. Cotransfer frequencies (ϕ) were calculated as follows: $\phi = 1 - [(Nif_2^+/Rif_2^r)/(Nif_1^+/Rif_1^r)]$, where the subscript 2 indicates recombinants from mutant × mutant crosses and 1 indicates those from wild-type × mutant crosses. Map distances (d) were calculated from the empirical function $d = 1 - \phi^{1/2}$ (32).

Crude extracts. *Rhodopseudomonas capsulata* B10 and *Rhodospirillum rubrum* were grown photoheterotrophically to the early stationary phase in RCV medium lacking ammonium, supplemented with 7.5 mM L-glutamate. For *R. rubrum*, 15 μg of biotin per ml was also added. Under a stream of O_2-free argon, cells were transferred to 250-ml centrifuge bottles containing 1 ml of 10 mM sodium dithionite in 100 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 8.0. After centrifugation (16,000 × *g* for 30 min), cells were lysed by passage through a French pressure cell (16,000 lb/in^2), and the lysate was collected into an argon-filled, serum-stoppered centrifuge tube containing 1 ml of the same dithionite solution. The lysate was centrifuged to remove whole cells and debris (12,000 × *g* for 1 h), and the supernatant was frozen (−20°C) until used as crude extract.

PAGE. Denaturing polyacrylamide gel electrophoresis (PAGE) was carried out by the method of Laemmli (9) in 10% (wt/vol) acrylamide slab gels in a Hoefer apparatus. For samples of whole cells, small portions of cultures were mixed with equal volumes of sample buffer and placed in a boiling water bath for 1.5 min. Samples containing 50 to 100 μg of protein were loaded onto the gel. For nondenaturing PAGE, the buffer systems described by Laemmli (9) were used with the sodium dodecyl sulfate (SDS) omitted. To achieve anaerobiosis, 0.1 g of sodium dithionite was added per liter of upper-electrode buffer, and the gels were prerun for 20 min at 10 mA.

Crossed immunoelectrophoresis. Crossed immunoelectrophoresis was carried out essentially as described by Randall et al. (19) except that the first dimension was SDS-PAGE (10% wt/vol) of crude extracts. Lyophilized antiserum to purified *Rhodospirillum rubrum* Fe protein was generously provided by P. Ludden and T. Dowling and was suspended in 5 mM phosphate buffer, pH 7.5. Preliminary Ouchterlony tests confirmed cross-reactivity between this antiserum and *Rhodopseudomonas capsulata* extracts derepressed for nitrogenase activity.

An appropriate track was cut from an unprocessed SDS-polyacrylamide gel and affixed to the electrophoresis plate (Gel Bond; FMC Corp.) with a few drops of molten 0.9% LE agarose (Miles Laboratories, Inc.) in Gelman high-resolution barbital buffer. Antiserum was added to the remaining agarose, and this mixture was poured uniformly over the plate. Horizontal electrophoresis was carried out for 2 h at 3 mA/cm (width) on an LKB Multiphor apparatus at 10°C. Processing of the gel was as described by Wang (29).

Nitrogenase activities. Nitrogenase activities of whole cells were determined by the method of Wall and Gest (26). For the determination of in vitro activities, 165-ml cultures were grown photoheterotrophically (34°C) to the stationary phase in RCV medium modified to contain limiting ammonium (4 mM). Cells were concentrated about fivefold by centrifugation. The reaction mixture, which was used both for lysis and for C_2H_2 reduction, was placed in a nominally 15-ml serum vial and contained the following in a final volume of 1 ml: 25 mM HEPES (pH 8.0), 5 mM ATP, 20 mM creatine phosphate, 0.1 mg of creatine phosphokinase, 5 mM $MgCl_2$, 0.8 mM $MnCl_2$, 0.15 mg of lysozyme, and 0.04 mg of polymyxin B. After 0.3 to 0.4 ml of cells was added and the contents of the vial were flushed for 10 min with O_2-free Ar, the mixture was incubated for 30 min at 33°C. $Na_2S_2O_4$ (0.1 ml of a 100 mM solution) and acetylene (0.5 ml) were added to begin the assay, and ethylene production over time was monitored by flame ionization gas chromatography (model 5320 detector fitted with a Chromasorb 108 80- to 100-mesh column operated at 65°C; Nuclear-Chicago Corp.).

Alternatively for in vitro nitrogenase determinations, cells were cultured in RCV medium containing only 0.75 mM $(NH_4)_2SO_4$, and all processing steps were performed under O_2-free N_2 in a glove box. Crude extracts were prepared as described above. The reaction mixture for acetylene reduction contained, in a final volume of 1 ml, 20 mM HEPES (pH 8.0), 5 mM ATP, 30 mM creatine phosphate, 0.05 mg of creatine phosphokinase, 25 mM $MgCl_2$, 0.5 mM $MnCl_2$, 10 mM $Na_2S_2O_4$, and 0.2 ml of crude extract (1.4 to 2.4 mg of protein). After 5 min at 33°C, 0.5 ml of acetylene was added, and ethylene production was monitored.

Protein. Whole-cell or crude-extract protein was determined by the Lowry method (10) after digestion of the samples with 0.2 M NaOH for 1 min at 100°C.

Chemicals. Acrylamide and protein molecular weight standards were from Bio-Rad Laboratories; 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonated salt was from G. Fredrick Smith Chemical Co. Acetylene was generated from carbide with water, and other gases were obtained locally. Other chemicals and enzymes were from Sigma Chemical Co.

RESULTS

Genetic analysis. Eleven independently isolated mutants unable to grow with dinitrogen as the sole nitrogen source were genetically analyzed to determine the relative chromosomal position of the mutation in each. All of the mutants grew well in unsupplemented RCV medium and reverted at frequencies consistent with single-site mutations. The fine-structure mapping tool used was the endogenous GTA which is similar to a small phagelike particle (31). DNA extracted from the GTA particle is a linear, double-stranded fragment of about 4,600 bp that is randomly packaged from the chromosome. Cotransfer of markers of the same phenotype by this agent requires the application of the ratio test (32) such that the distance over which linkage was reliably demonstrated for the Nif$^-$ mutations was ca. 2,700 bp.

The number of Nif$^+$ and Rif^r recombinant CFUs was determined for each mutant with an Nif$^+$ Rif^r donor (strain J1 crosses, Table 2). Approximately equal numbers of recombinants were obtained in each case, confirming that both markers were equally represented in the GTA particles, and that transfer and expression of Nif$^+$ and Rif^r phenotypes were not vastly different.

After the construction of Rif^r derivatives of each of the mutants for use as donors, most of the possible pairwise crosses between mutants were carried out (Table 2). When the ratio of Nif$^+$/Rif^r recombinants from the mutant × mutant cross was much less than that of the corresponding wild type × mutant cross, the mutations were considered linked; when the ratios were similar, the mutations were considered unlinked. From the ratios obtained, conservative cotransfer frequencies and map distances were calculated, and those showing linkage are shown in Table 3. In general, the map distances can be interpreted in physical distances as that fraction of the length of DNA packaged by GTA, 4,600 bp (25). Most reciprocal crosses gave similar map distances; however, several exceptions were found (e.g., J41 × J62 crosses) for which there was no explanation. Reciprocal

TABLE 2. Crosses between Nif$^-$ *Rhodopseudomonas capsulata* strains: Nif$^+$ recombinants/Rif^r recombinants[a]

Donor[b]	No. of Nif$^+$ transferants / No. of Rif$^+$ transferants for the recipient strains									
	W15	J41	J43	J56	J57	J58	J59	J60	J61	J62
J1	1591 / 1475	1248 / 1112	968 / 775	1287 / 1311	2534 / 2963	2505 / 2899	378 / 374	2264 / 2131	1384 / 1579	2151 / 2054
W151		ND	696 / 845	442 / 884	108 / 668	436 / 458	519 / 554	6 / 590	378 / 392	409 / 406
J411	885 / 830		559 / 544	47 / 54	549 / 463	417 / 394	267 / 319	91 / 78	514 / 694	148 / 643
J431	1819 / 1599	9594 / 7819		723 / 784	1429 / 1270	965 / 990	950 / 1145	2122 / 1808	1841 / 2018	859 / 737
J441	505 / 490	734 / 718	5520 / 3822	1721 / 2554	1116 / 1066	1518 / 2938	625 / 1191	532 / 564	1148 / 1018	1152 / 1267
J561	1289 / 2854	ND	ND		594 / 1163	1167 / 1197	425 / 439	1457 / 2735	946 / 1002	1725 / 1732
J571	65 / 2743	ND	ND	2293 / 4630		5410 / 4958	601 / 601	620 / 4611	5616 / 6192	378 / 397
J581	ND	ND	ND	495 / 486	484 / 434		108 / 1769	366 / 338	855 / 651	1766 / 1530
J591	ND	ND	ND	619 / 603	361 / 306	24 / 334		584 / 470	408 / 453	493 / 487
J601	37 / 804	ND	ND	757 / 1223	1376 / 6000	848 / 924	535 / 545		853 / 915	339 / 301
J611	ND	71 / 83	ND	1453 / 1532	881 / 893	528 / 538	558 / 588	1201 / 964		1526 / 1327
J621	ND	2483 / 5600	ND	1006 / 858	864 / 836	4801 / 5148	ND	663 / 661	3497 / 3547	

[a] The numbers of recombinant CFUs (corrected for reversion and spontaneous mutation) are the sums of the averages of duplicate plates from two or more experiments. ND, Not determined; no Nif$^+$ recombinants were obtained for mutants crossed with themselves.

[b] Donor strains are rifampin resistant derivatives of Nif$^-$ mutants containing *rif-1* donated from strain J1 by GTA. The acquisition of this marker was denoted by adding the number 1 to the strain designation; e.g., J411, J41 Rif^r.

TABLE 3. Crosses between Nif⁻ mutants of *Rhodopseudomonas capsulata* showing linkage

Donor	Recipient	Nif⁺/Rif^r ratio[a]	95% Confidence interval	Cotransfer frequency	Map distance
J1	J62	1.05	0.99–1.16	—[b]	—
J411	J62	0.23	0.20–0.26	0.83–0.73	0.09–0.14
J1	J41	1.12	1.04–1.21	—	—
J621	J41	0.52	0.42–0.45	0.64–0.57	0.20–0.25
J1	J58	0.86	0.81–0.91	—	—
J441	J58	0.52	0.49–0.55	0.46–0.32	0.32–0.43
J591	J58	0.07	0.04–0.10	0.96–0.88	0.02–0.06
J1	J59	1.01	0.88–1.17	—	—
J441	J59	0.52	0.48–0.58	0.59–0.34	0.23–0.42
J581	J59	0.06	0.05–0.07	0.96–0.92	0.02–0.04
J1	J56	0.98	0.91–1.06	—	—
J571	J56	0.50	0.47–0.53	0.56–0.42	0.25–0.35
J601	J56	0.62	0.56–0.68	0.47–0.25	0.31–0.50
W151	J56	0.50	0.44–0.56	0.58–0.39	0.24–0.38
J441	J56	0.67	0.67–0.68	0.37–0.25	0.39–0.50
J1	J57	0.86	0.81–0.91	—	—
J561	J57	0.51	0.46–0.56	0.49–0.31	0.30–0.44
J601	J57	0.23	0.22–0.24	0.76–0.70	0.13–0.16
W151	J57	0.16	0.13–0.20	0.88–0.78	0.06–0.12
J1	J60	1.06	1.04–1.16	—	—
J561	J60	0.53	0.50–0.57	0.57–0.46	0.25–0.33
J571	J60	0.13	0.12–0.14	0.90–0.86	0.05–0.07
W151	J60	0.01	0.00–0.02	1.00–0.98	0.00–0.01
J1	W15	1.08	1.01–1.16	—	—
J561	W15	0.45	0.42–0.48	0.64–0.52	0.20–0.28
J571	W15	0.02	0.02–0.03	0.98–0.97	0.01–0.02
J601	W15	0.05	0.03–0.07	0.97–0.94	0.02–0.03

[a] Ratios derived from numbers in Table 2.
[b] —, Not applicable for the wild-type donor.

crosses with strain J441 were not carried out since the rifampin-sensitive Nif⁻ mutant was lost soon after isolation.

As a result of this analysis, five linkage groups were identified (Fig. 1). The map distances were the averages of values from reciprocal crosses (Table 3). The linkage shown with the J441 × J56 cross suggested that groups IV and V might be near each other. However, a streptomycin-resistant derivative of strain J56 was constructed, and the ratio of Nif⁺/Str^r recombinants was determined with strain J441 as the recipient. A ratio of 1.18 (5,882 Nif⁺ CFU/4,972 Str^r CFU) was obtained, which indicated that groups IV and V were not linked, a conclusion supported by complementation analysis of the cloned fragments (1).

Biochemical studies. The in vivo nitrogenase activities of the mutants were determined by acetylene reduction (Table 4). Six of the mutants lacked detectable activity. All mutants in linkage group V had residual activity which correlated positively with the observation of a slow growth on N_2. Although strains J43 and J57 had measurable activity, no growth on N_2 was visible. In vitro assays showed similar results without dramatic increases in activity, which might be expected if one or more of the mutations interrupted electron transport (23). The mutants had ≤6% of the in vitro activity of the wild-type strain, which was found to be 6.7 to 10.0 nmol · min^{-1} · mg of protein^{-1}.

The nitrogenase enzyme complex constitutes a significant percentage of the protein in a derepressed diazotrophic cell (4, 21). Therefore, by comparing electrophoretic patterns of proteins of the mutants with that of the wild-type strain, we could determine the presence or absence of the polypeptides of the MoFe or Fe proteins (Table 4). Denaturing PAGE was carried out on whole-cell proteins from wild-type cultures

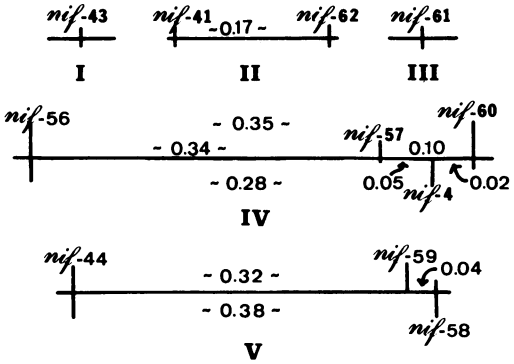

FIG. 1. Linkage groups of mutations conferring a Nif⁻ phenotype on *Rhodopseudomonas capsulata*. The numbers above the lines are averages of map distances from reciprocal crosses.

TABLE 4. Nitrogenase in Nif⁻ mutants of *Rhodopseudomonas capsulata*

Strain	Linkage group	In vivo[a] activity	Protein[b]	
			MoFe	Fe
B10	None	5.93	++	++
J43	I	0.043	±	+
J41	II	0.002	++	+
J62	II	0.002	+	+
J61	III	0.002	−	−
J441	V	0.267	++	++
J58	V	0.140	±	++
J59	V	0.132	±	++
J56	IV	0.002	−	−
J57	IV	0.055	+	++
J60	IV	0.002	−	++
W15	IV	0.002	−	−

[a] In vivo nitrogenase activity expressed as micromoles of ethylene produced per hour per milligram of whole-cell protein. The level of detection was 0.002.

[b] Identified on denaturing polyacrylamide gels. The relative scale for decreasing amounts of protein was ++, +, and ±, with − indicating no visible protein band.

grown on nitrogenase-repressing (ammonium as the N source) and -derepressing (glutamate as the N source) media. Three prominent ammonium-repressible polypeptides were observed of ca. 61,000, 57,500, and 32,500 molecular weights, which were similar to those reported for purified nitrogenase (4). The MoFe polypeptides, the larger two, were further verified by coincident electrophoresis with the subunits of a protein giving a purple band in anaerobic native polyacrylamide gels when acidified in the presence of the Fe stain 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonated salt (data not shown). The Fe protein was independently identified by crossed immunoelectrophoresis against antiserum to purified *Rhodospirillum rubrum* Fe protein (Fig. 2).

Those strains exhibiting some residual activity either in vivo or in vitro had small amounts of both MoFe and Fe proteins (Table 4). None of the mutants completely lacked the Fe protein alone, although strain J41 had a reduced Fe protein content. Unlike strain J62, which is in the same linkage group, strain J41 was unable to grow anaerobically with glutamate as the nitrogen source, and the majority of revertants were restored for growth both on N_2 and glutamate. Although mutants of this phenotype have been previously isolated (30), their biochemical nature remains unresolved.

Linkage group IV has recently been shown to include the structural genes (*nifH, D, K*) coding for the nitrogenase complex (1). The functions impaired in other groups are not known. None of the lesions was linked to an *his* mutation or to a previously described mutation which eliminates glutamine synthetase activity (26).

The crossed immunoelectrophoresis experiment (Fig. 2A) revealed the presence of a second minor band which shared antigenic determinants with the Fe protein. It was present in derepressed *Rhodopseudomonas capsulata* cells at a position corresponding to a molecular weight of 38,000, i.e., 5,000 greater than that of the putative Fe protein. Unlike the active and inactive subunits of *Rhodospirillum rubrum*, which were found in nearly equal quantities in these experiments (Fig. 2B), the amount of the larger polypeptide in the *Rhodopseudomonas capsulata* extract was, at most, 20% of that of the smaller. In a comparison of one-dimensional SDS-PAGE protein patterns of repressed versus derepressed wild-type cells, an ammonium-repressible protein of 38,000 molecular weight was occasionally observed when glutamate was the derepressing nitrogen source but not when cultures were starved for nitrogen. *Rhodospirillum rubrum* Fe protein is also readily inactivated after growth on glutamate (24).

DISCUSSION

Fine-structure mapping of 11 *nif* mutations has shown that five linkage groups were represented among the mutations. An uncertainty occurred in the interpretation of the data from the J441 × J56 cross which supported linkage of groups IV and V. This was not supported by reciprocal crosses. In addition, Avtges et al. (1) have recently shown that an 11.8-kilobase pair (kbp) DNA fragment, which codes for the *nif* structural genes and includes about 7 kbp upstream from *nif-56*, does not complement *nif-58*, a member of group V. In fact, transposon mutagenesis of that 7-kbp region showed the absence of genes for nitrogen fixation.

The order *nifH, D, K* established by Avtges et al., (1) with the mutation of strain J56 in the *nifH* region, that of strain J57 in *nifD*, and that of strain J60 in *nifK*, correlates well with the genetic map (Fig. 1). Although the map distances were not additive in this group, the physical distances calculated from these genetic data could be roughly correlated with those obtained by molecular techniques. A major exception was the mapping involving strain W15. The *nif* mutation in strain W15 was derived after nitrosoguanidine mutagenesis (28). Although it reverts at a frequency consistent with a single-site alteration, the mutant did not always grow well, and it has an anomalous protein pattern (5) with respect to its map position.

Mutant J56 did not appear to contain significant amounts of MoFe protein. This result was in contrast to the demonstration that strain J56 carries a nonpolar mutation which could complement mutations in *nifD* and *nifK* (1). In *K. pneumoniae*, the product of *nifH* has been suggested to play

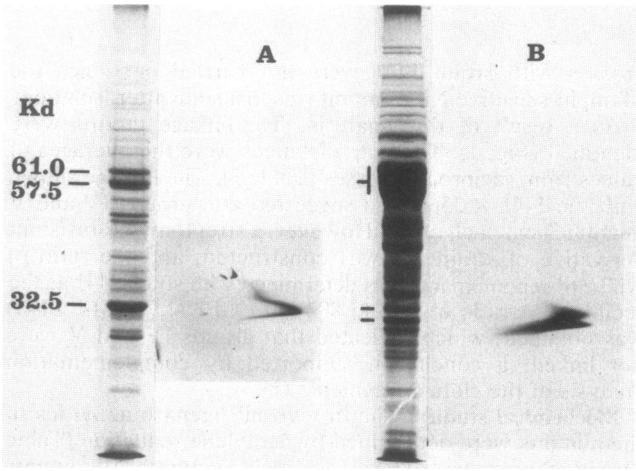

FIG. 2. Crossed immunoelectrophoresis of nitrogenase-derepressed extracts for the identification of Fe protein. (A) Track to the left is an SDS-acrylamide gel of crude extract proteins from *Rhodopseudomonas capsulata* identical to that used for electrophoresis into antiserum. The rocket is the protein-stained antigen-antibody precipitin line. Molecular weights (10^3) of MoFe (61.0 and 57.5) and Fe (32.5) polypeptides are indicated. (B) Track to the left is an SDS-acrylamide gel of crude extract proteins from *Rhodospirillum rubrum*. The regions of MoFe and Fe polypeptides are indicated by the lines at the left of the track. No additional cross-reacting material was present in either of the gels.

a role in the stability of the *nifK* and *nifD* products (21). If this were also the case for *Rhodopseudomonas capsulata*, both observations would be consistent.

The determination of five linkage groups among 11 mutations suggested that the overall arrangement of *nif* genes in *Rhodopseudomonas capsulata* might be different from the single cluster found in *K. pneumoniae*. The physical mapping of the structural genes *nifH, D, K* by Avtges et al. (1) supports that supposition. However, it is still possible that all essential *nif* genes are clustered to the right of those genes in a region of 25 kbp or longer. Conjugational mapping and extended physical analysis will soon distinguish these possibilities.

The regulation of nitrogenase activity in the phototrophs has been shown to be correlated with a covalent modification of the Fe protein in response to the availability of reduced nitrogen (11, 12, 18, 24). When inactive, one subunit of the dimer from *Rhodospirillum rubrum* is modified and migrates more slowly on denaturing polyacrylamide gels (13). In contrast, Michalski et al. (17) implied that the modified subunit of *Rhodopseudomonas capsulata* Fe protein migrated to a position corresponding to a molecular weight of 33,000, the same as that of the unmodified subunit. The observation of a 38,000-molecular weight polypeptide in *Rhodopseudomonas capsulata* extracts which shares antigenic determinants with the putative Fe protein suggests that distinguishable forms occur. In addition, Hallenbeck et al. (4) reported finding an ammonium-repressible polypeptide of a similar molecular weight during the two-dimensional PAGE of soluble proteins of *Rhodopseudomonas capsulata*. Recently, a preliminary report has confirmed that the slower-migrating subunit of Fe protein appears in response to ammonium shock (7). It is of interest that in *Rhodopseudomonas capsulata* the modification results in a much larger difference in apparent molecular weights of the subunits than in *Rhodospirillum rubrum*.

ACKNOWLEDGMENTS

We thank Howard Gest, in whose laboratory this work was initiated, and Jean Love for excellent technical assistance.

This work was supported by grant PCM-7904223 from the National Science Foundation.

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