JOURNAL OF BACTERIOLOGY, Mar. 2011, p. 1477–1480 Vol. 193, No. 6
0021-9193/11/$12.00 doi:10.1128/JB.01230-10

The *Escherichia coli* K-12 MntR Miniregulon Includes *dps*, Which Encodes the Major Stationary-Phase DNA-Binding Protein▽§

Kaneyoshi Yamamoto,[1] Akira Ishihama,[1] Stephen J. W. Busby,[2] and David C. Grainger[3]*

Department of Frontier Bioscience and Research Center for Micro-Nano Technology, Hosei University, Koganei, Tokyo 184-8584, Japan[1]; School of Biosciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom[2]; and School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom[3]

Received 13 October 2010/Accepted 4 January 2011

***Escherichia coli* MntR protein is the Mn^{2+}-responsive transcriptional repressor of the MntH manganese transporter. We have used chromatin immunoprecipitation to determine the distribution of Mn^{2+}-MntR across the entire *E. coli* chromosome *in vivo*, and we report that MntR binds to only four targets, adjacent to the *mntH*, *mntR*, *yebN*, and *dps* genes. Unexpectedly, we found that *dps* expression is directly repressed by Mn^{2+}-MntR.**

Mn^{2+} homeostasis in *Escherichia coli* is regulated by MntR, a Mn^{2+}-sensitive transcription factor (15). Mn^{2+} stimulates the binding of MntR to the promoter of the *mntH* gene, which encodes the sole Mn^{2+} transporter of *E. coli*. Thus, in Mn^{2+}-rich environments, MntR represses the *mntH* promoter, thereby shutting down the synthesis of MntH and preventing accumulation of toxic levels of Mn^{2+}. Similar systems control Mn^{2+} uptake in *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium, and related bacteria (3, 11, 12, 16). To determine the extent of the MntR regulon in *E. coli* we used chromatin immunoprecipitation and DNA microarrays (ChIP-chip) (7) to identify sites of Mn^{2+}-MntR binding *in vivo*. Strain BW25113 (6) was grown aerobically in LB medium supplemented with either 0.2 mM $MnCl_2$ (to stimulate DNA binding by MntR) or 0.1 mM EDTA (as a negative control). At an optical density at 650 nm (OD_{650}) of 0.3 to 0.4, cells were treated with formaldehyde, and cellular DNA was extracted and sonicated, yielding DNA fragments of 500 to 1,000 bp. After immunoprecipitation with anti-MntR antibodies, DNA fragments from $MnCl_2$ or control EDTA-treated cells were purified and labeled with Cy5 and Cy3, respectively. The two DNA samples were then mixed and hybridized to the 43,450-feature DNA microarray (Oxford Gene Technology, Oxford, United Kingdom). After washing and scanning, the Cy5/Cy3 signal intensity ratio was calculated for each probe. The experiment was done in duplicate, and an average of the two Cy5/Cy3 ratios was used for further analysis. Complete data sets are shown in Table S1 in the supplemental material. Figure 1A gives an overview of the profile for MntR binding. Four distinct MntR binding peaks are easily distinguishable from the background signal and correspond to intergenic regions of the chromosome adjacent to the *dps*, *mntR*, *yebN*, and *mntH* genes (note that, because they are located only ~4,000 bp apart, the separate *dps* and *mntR* peaks are not resolved in Fig. 1A). To identify the precise MntR binding sequences, we used W-AlignACE (5) to search for short, overrepresented DNA sequences in 400-bp segments centered on each peak. This process identified the 26-bp sequence motif shown in Fig. 1B, which matches the previously proposed consensus MntR-binding sequence (15). A detailed view of the binding signal for each target is shown in Fig. 1Ci to Civ. Note that two motifs were found at the *yebN* promoter and one motif was found at each of the other targets (Table 1). Intriguingly, the MntR binding sites at the *yebN* promoter are centered ~400 bp upstream of the *yebN* start codon. Thus, it is unlikely that MntR acts as a transcriptional repressor at this locus.

We expected that the binding targets for MntR would be adjacent to genes encoding proteins involved in the response to Mn^{2+}. Thus, MntH is the Mn^{2+} transporter, MntR is the Mn^{2+} sensor (11, 15, 16), and the YebN protein is a putative Mn^{2+} efflux pump induced by excess Mn^{2+} (G. Storz, personal communication). However, at first sight, Dps has no direct connection to manganese but is a well-characterized stationary-phase nucleoid-associated protein that stimulates supercompaction of the chromosome in response to starvation and oxidative stress (1). Structural analysis of Dps has shown that it has a fold similar to that of bacterial ferritin and can sequester excess Fe^{2+} ions (9). Expression of Dps is known to be regulated by OxyR, in response to oxidative stress (2), and three nucleoid proteins, IHF, Fis, and H-NS, in response to growth phase (2, 8). The organization of transcription factor binding sites in the *dps* regulatory region is shown in Fig. 2A. IHF and OxyR both function as activators, while Fis and H-NS repress transcription. Note that the MntR binding site identified by our analysis (Table 1) overlaps with the Fis binding sites (Fig. 2A).

The dps100, -200, -300, and -400 promoter fragments encode a series of nested deletions in the *dps* regulatory region (Fig. 2A). Previously, we used these fragments, cloned in the low-copy-number *lac* expression plasmid pRW50, to measure *dps* expression in response to growth rate (8). Here we have examined expression from each *dps* promoter::*lac* fusion in the presence or absence of Mn^{2+} or EDTA in either log-phase or

* Corresponding author. Mailing address: School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom. Phone: 44 (0)24 765 22509. Fax: 44 (0)24 7652 3701. E-mail: d.c.grainger@warwick.ac.uk.

§ Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 14 January 2011.

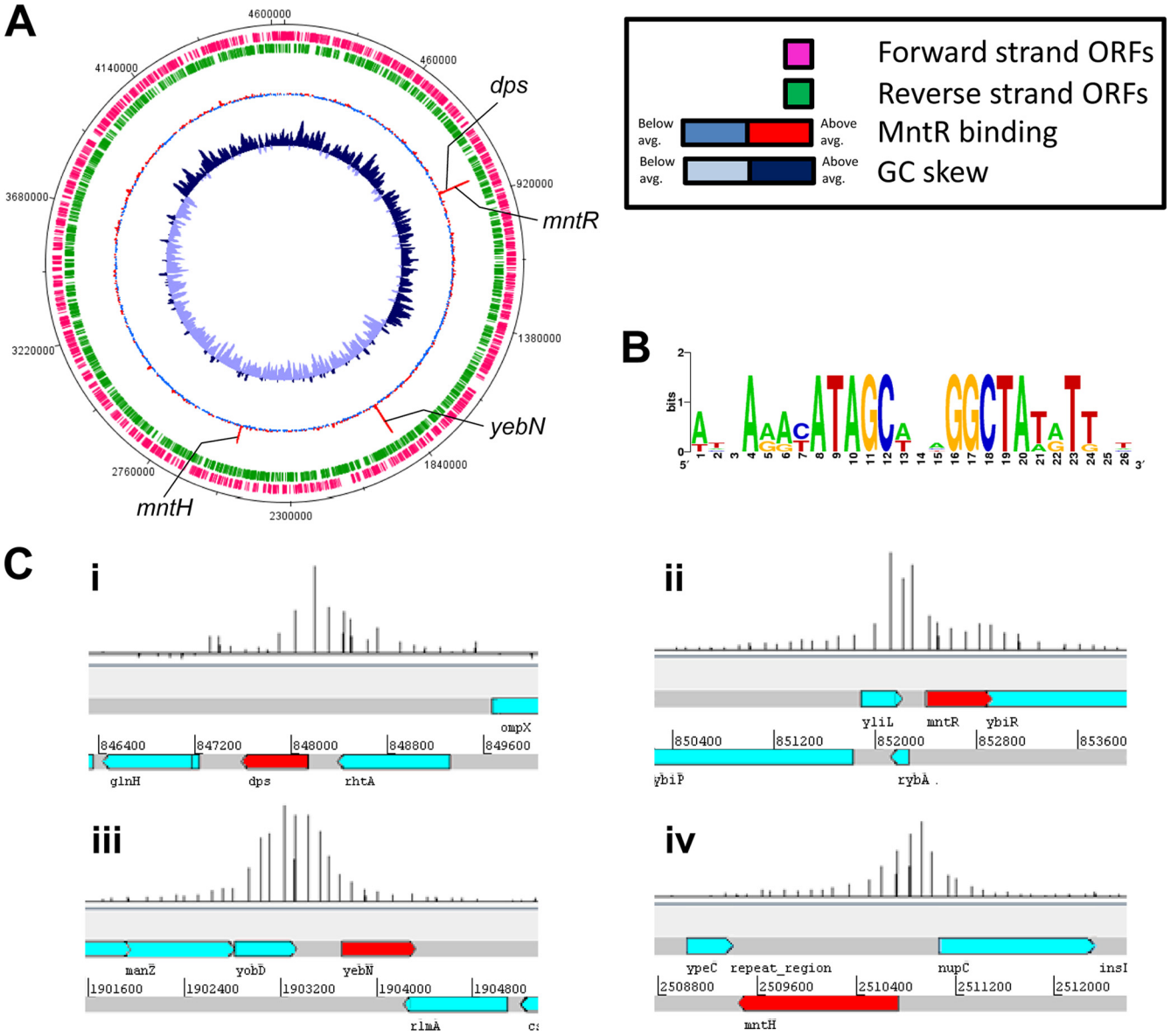

FIG. 1. Chromosome-wide distribution of the *Escherichia coli* MntR protein. (A) Location of MntR binding sites. The panel shows ChIP-chip data for MntR plotted against the features of the *E. coli* chromosome. Binding peaks for MntR are labeled with the name of the adjacent gene. Note that the separate peaks for MntR binding at the *dps* and *mntR* loci are too close together to be distinguished from each other at this resolution. The asymmetry in nucleotide composition between the leading and the lagging strand is shown as GC skew. (B) DNA sequence content of MntR binding sites. The panel shows a DNA sequence motif overrepresented in DNA fragments immunoprecipitated with anti-MntR. (C) The panel shows detailed MntR binding data from ChIP-chip experiments at the *dps* (i), *mntR* (ii), *yebN* (iii), and *mntH* (iv) genomic loci. The Cy5/Cy3 ratio for individual probes on the DNA microarray are shown as single lines plotted against the corresponding genome coordinates on the *E. coli* chromosome. Genes are shown as arrows, with putative MntR targets highlighted in red.

stationary-phase JCB387Δ*lac* cells (14), grown in LB. The results of this experiment are shown in Fig. 2B. The data show that the addition of $MnCl_2$ or EDTA to JCB387 cultures had no effect on *dps* expression during rapid growth. Conversely, in stationary phase, the addition of $MnCl_2$ to cultures resulted in marked repression of *dps* expression. This repression required only the core *dps* promoter region containing the MntR binding motif and was not dependent on upstream promoter sequences. We reasoned that our failure to detect Mn^{2+}-dependent repression of *dps* expression during rapid growth might be due to the effects of Fis, which represses *dps* expression during mid-log phase but not stationary phase (8). Thus, we measured expression from the dps100 fragment in JCB3871Δ*lac*Δ*fis* cells (19). The results show that, in the absence of Fis, Mn^{2+} dependent repression of *dps* expression can be observed in mid-log-phase cells (Fig. 3A). Surprisingly, the addition of EDTA resulted in increased *dps* promoter activity only in stationary-phase JCB3871 cells. Finally, to confirm that repression of *dps* expression by Mn^{2+} was mediated by MntR, we repeated our analysis in KP7600 (13) cells and the *mntR* derivative JD17512

TABLE 1. MntR binding sites identified by ChIP-chip analysis[a]

Peak center (bp)	Gene	MntR binding motif[b]	Location of motif (bp)
848204	*dps*	ATTAAGT**ATAGC**ACCG**GCTAT**GTGTT	848189.5
852127	*mntR*/*rybA*	TACAGAT**ATAGC**ACAG**GCTAT**ATTAT	852286.5
1903240	*yebN*	ACTAAAC**ATAGC**TTTG**GCTA**AATTCA	1903300.5
		ATCAGAC**ATAGC**TTAG**GCTAT**ATTAC	1903338.5
2510830	*mntH*/*nupC*	ATGAAAC**ATAGC**AAAG**GCTAT**GTTTT	2510752.5

[a] The table lists genome coordinates of MntR binding peaks, the genes adjacent to the MntR binding peaks, and the sequence and location of the likely MntR binding sites.
[b] Matches to the MntR consensus sequence are shown in bold, and mismatches are underlined.

(National BioResource Project [NIG, Japan]). As expected, repression of *dps* expression by Mn^{2+} was abolished in the absence of MntR (Fig. 3B).

In conclusion, we report the first chromosome-wide analysis of MntR binding. We show that, as previously predicted (12), the MntR regulon is small and consists of just four discrete binding loci. This is in contrast to other recently analyzed repressor proteins that bind numerous targets, including non-canonical target sites and targets in coding DNA (17, 18). Three of the four MntR targets (*mntH*, *mntR*, and *yebN*) are genes directly involved in Mn^{2+} homeostasis, and the mode of MntR action at these loci is documented elsewhere (Storz, personal communication). Conversely, the *dps* gene encodes a protein that mediates chromosome compaction and binds Fe^{2+} to protect cells against oxidative stress. Interestingly, the *mntH*, *yebN*, and *dps* promoters are all thought be regulated by Fur and/or OxyR in addition to MntR. This suggests an intimate connection between cellular responses to oxidative stress and Mn^{2+}/Fe^{2+} homeostasis. Indeed, recent observations have shown that Mn^{2+} protects against oxidative stress and is a key component of the OxyR-mediated stress response (4). Since Dps also protects cells against oxidative stress by sequestering Fe^{2+}, it is logical that *dps* expression should be induced when manganese levels are low and the risk of oxidative stress due to excess Fe^{2+} is increased. Intriguingly, Dps from *Sulfolobus solfataricus* has been shown to bind Mn^{2+} (10) and we speculate that Dps proteins from other organisms could be promiscuous with respect to their metal binding properties. Finally, we note that, while the MntR binding sites at the *mntR*, *mntH*, and *yebN* loci are conserved in *E. coli* and *S. enterica* (12), the

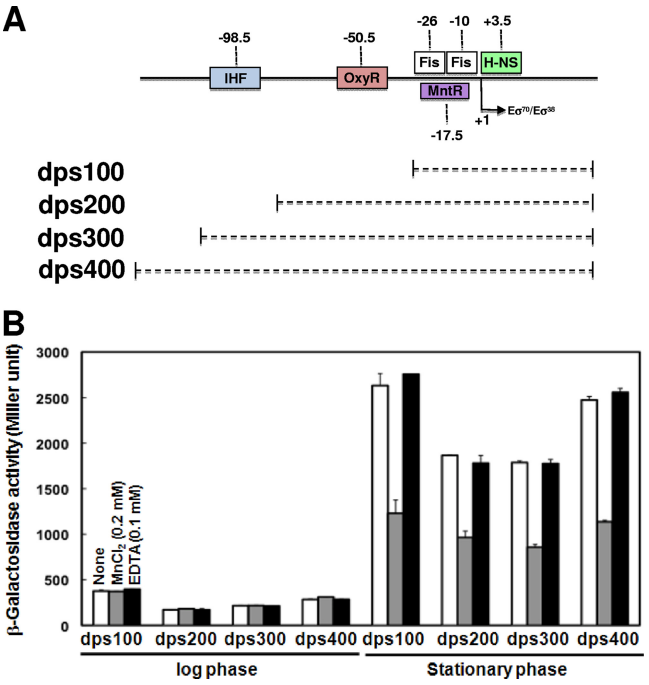

FIG. 2. Mn^{2+}-MntR represses transcription from the *dps* promoter. (A) The *dps* regulatory region and truncated derivatives. The panel shows a diagrammatic representation of the *dps* promoter (shown by an arrow) and the surrounding regulatory region of DNA. Binding sites for the transcriptional activators of *dps* transcription, IHF and OxyR, are shown by blue and pink boxes, respectively. Binding sites for the known repressors of *dps* transcription, Fis and H-NS, are shown by white and green boxes, respectively. The MntR binding site identified by our ChIP-chip analysis is shown as a purple box. Different regulatory region fragments used in this work (dps100, -200, -300, and -400) are also shown. (B) Dps expression is repressed by Mn^{2+} in stationary phase. The bar chart depicts levels of β-galactosidase activity in JCB387 cells carrying different-length *dps* regulatory region fragments fused to *lacZ*. Activities were measured in with and without $MnCl_2$ or EDTA in either log-phase (OD_{650} of 0.3 to 0.4) or stationary-phase (OD_{650} of −4.0) cells. Growth conditions were similar to those used for ChIP-chip experiments.

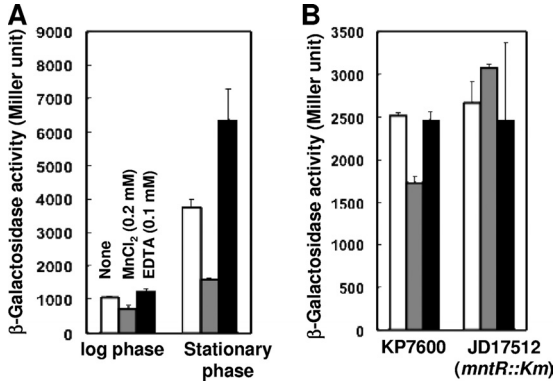

FIG. 3. Fis and MntR function redundantly at the *dps* promoter during logarithmic growth. (A) Fis and MntR function redundantly at the *dps* promoter. The panel shows levels of *lacZ* expression from the dps100::*lacZ* fusion in JCB3871Δ*fis* cells. The addition of $MnCl_2$ to cultures results in repression of *lacZ* expression regardless of growth phase. (B) Mn^{2+} repression of the dps100 promoter fragment requires MntR. The bar chart illustrates the results of β-galactosidase activity assays with either KP7600 (a parent strain) or JD17512 (*mntR*::Km^r) cells that have been transformed with the dps100::*lacZ* fusion construct. The data show that MntR is required to mediate repression by $MnCl_2$. Activities shown are for stationary-phase cells. Growth conditions were similar to those used for ChIP-chip experiments.

site at the *dps* promoter is found only in *E. coli*. Thus, there are likely to be subtle differences in the metal homeostasis strategies utilized by these closely related bacteria.

We are grateful to Shivani Singh and Kiran Chintakayala for advice and Joseph Wade, Taku Oshima, and Gigi Storz for helpful discussions.

This work was supported by a Wellcome Trust career development fellowship awarded to D.C.G. and a Royal Society international joint project award to S.J.W.B. and A.I.

REFERENCES

1. **Almirón, M., A. J. Link, D. Furlong, and R. Kolter.** 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. **6:**2646–2654.
2. **Altuvia, S., M. Almirón, G. Huisman, R. Kolter, and G. Storz.** 1994. The *dps* promoter is activated by OxyR during growth and by IHF and sigma S in stationary phase. Mol. Microbiol. **13:**265–272.
3. **Ando, M., et al.** 2003. Characterization of the role of the divalent metal ion-dependent transcriptional repressor MntR in the virulence of *Staphylococcus aureus*. Infect. Immun. **71:**2584–2590.
4. **Anjem, A., S. Varghese, and J. A. Imlay.** 2009. Manganese import is a key element of the OxyR response to hydrogen peroxide in *Escherichia coli*. Mol. Microbiol. **72:**844–858.
5. **Chen, X., L. Guo, F. Zhaocheng, and T. Jiang.** 2008. W-AlignACE: an improved Gibbs sampling algorithm based on more accurate position weight matrices learned from sequence and gene expression/ChIP-chip data. Bioinformatics **24:**1121–1128.
6. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. **97:**6640–6645.
7. **Grainger, D. C., et al.** 2004. Genomic studies with *Escherichia coli* MelR protein: applications of chromatin immunoprecipitation and microarrays. J. Bacteriol. **186:**6938–6943.
8. **Grainger, D. C., M. D. Goldberg, D. J. Lee, and S. J. Busby.** 2008. Selective repression by Fis and H-NS at the *Escherichia coli dps* promoter. Mol. Microbiol. **68:**1366–1377.
9. **Grant, R. A., D. J. Filman, S. E. Finkel, R. Kolter, and J. M. Hogle.** 1998. The crystal structure of Dps, a ferritin homolog that binds and protects DNA. Nat. Struct. Biol. **5:**294–303.
10. **Hayden, J. A., and M. P. Hendrich.** 2010. EPR spectroscopy and catalase activity of manganese-bound DNA-binding protein from nutrient starved cells. J. Biol. Inorg. Chem. **15:**729–736.
11. **Kehres, D. G., M. L. Zaharik, B. B. Finlay, and M. E. Maguire.** 2000. The NRAMP proteins of *Salmonella typhimurium* and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. Mol. Microbiol. **36:**1085–1100.
12. **Kehres, D. G., A. Janakiraman, J. M. Slauch, and M. E. Maguire.** 2002. Regulation of *Salmonella enterica* serovar Typhimurium *mntH* transcription by H_2O_2, Fe^{2+}, and Mn^{2+}. J. Bacteriol. **184:**3151–3158.
13. **Makinoshima, H., S.-I. Aizawa, H. Hayashi, T. Miki, A. Nishimura, and A. Ishihama.** 2003. Growth phase-coupled alterations in cell structure and function of *Escherichia coli*. J. Bacteriol. **185:**1338–1345.
14. **Page, L., L. Griffiths, and J. A. Cole.** 1990. Different physiological roles of two independent pathways for nitrite reduction to ammonia by enteric bacteria. Arch. Microbiol. **154:**349–354.
15. **Patzer, S. I., and K. Hantke.** 2001. Dual repression by Fe^{2+}-Fur and Mn^{2+}-MntR of the *mntH* gene, encoding an NRAMP-like Mn^{2+} transporter in *Escherichia coli*. J. Bacteriol. **183:**4806–4813.
16. **Que, Q., and J. D. Helmann.** 2000. Manganese homeostasis in *Bacillus subtilis* is regulated by MntR, a bifunctional regulator related to the diphtheria toxin repressor family of proteins. Mol. Microbiol. **35:**1454–1468.
17. **Shimada, T., A. Ishihama, S. J. Busby, and D. C. Grainger.** 2008. The *Escherichia coli* RutR transcription factor binds at targets within genes as well as intergenic regions. Nucleic Acids Res. **36:**3950–3955.
18. **Wade, J. T., N. B. Reppas, G. M. Church, and K. Struhl.** 2005. Genomic analysis of LexA binding reveals the permissive nature of the *Escherichia coli* genome and identifies unconventional target sites. Genes Dev. **19:**2619–2630.
19. **Wu, H., K. L. Tyson, J. A. Cole, and S. J. W. Busby.** 1998. Regulation of transcription initiation at the Escherichia coli nir operon promoter: a new mechanism to account for co-dependence on two transcription factors. Mol. Microbiol. **27:**493–505.