Supporting Information

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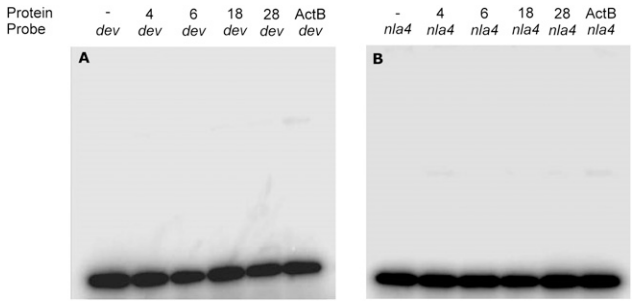

Fig. S1. EMSAs with EBP-DBDs and DNA fragments containing the putative *dev* and *nla4* operon promoters. The assays were performed with fragments of the *dev* (*A*) and *nla4* (*B*) operon promoters, as well as the following EBP-DBDs: none (lane 1), Nla4-DBD (lane 2), Nla6-DBD (lane 3), Nla18-DBD (lane 4), Nla28-DBD (lane 5), and ActB-DBD (lane 6).

Table S1. Alignment of tandem repeat half sites at EBP promoters

	Promoter fragment	1	2	3	4	5	6	7	8	9	10
EE1 sites*											
*nla28*EE1-1	*nla28*P1	C	C	A	T	G	C	G	G	T	T
*nla28*EE1-2	*nla28*P1	C	A	A	C	G	T	T	G	G	C
*act*EE1-1	*actB*P1	C	C	G	C	G	G	C	G	T	C
*act*EE1-2	*actB*P1	C	C	A	C	G	G	G	G	A	T
*nla6*EE1-1	*nla6*P1	C	A	A	C	G	C	C	A	T	C
*nla6*EE1-2	*nla6*P1	C	A	C	C	G	C	C	T	C	G
Consensus		C	C/A	A	C	G	C	C	G	T	C
%		100	100	67	83	100	50	50	67	50	50
EE2 sites†											
*nla28*EE2-1	*nla28*P2	C	T	C	C	G	C	A	G	T	T
*nla28*EE2-2	*nla28*P2	C	T	C	C	C	C	A	G	A	C
*act*EE2-1	*actB*P1	C	T	T	C	A	A	G	C	A	C
*act*EE2-2	*actB*P1	G	T	T	C	G	A	G	G	C	G
*nla6*EE2-1	*nla6*P1	C	C	T	C	G	C	C	T	T	G
*nla6*EE2-2	*nla6*P1	C	T	T	C	A	A	G	C	T	G
Consensus		C	T	T	C	G	C/A	G	G	T	G
%‡		83	83	67	100	50	100	50	50	50	50

*EE1 sites were those identified in promoter fragments that were found to bind the Nla6-DBD protein (*nla28*P1, *nla6*P1, and *actB*P1) using the program Consensus (1). The program is designed to find shared motifs and patterns in DNA among separate sequences. The six individual enhancer elements were aligned to get the consensus for Nla6-DBD binding.

†EE2 sites were those identified in promoter fragments that were found to bind the Nla28-DBD protein (*nla28*P2, *nla6*P1, and *actB*P1) using Consensus. The six individual enhancer elements were aligned to get the consensus for Nla28-DBD binding.

‡Numbers shown represent the percentage of instances that a particular nucleotide was found at a particular location.

1. van Helden J (2003) Regulatory sequence analysis tools. *Nucleic Acids Res* 31:3593–3596.

Table S2. Developmentally regulated operons with putative σ^{54} promoters*

	Preaggregation			Aggregation	
Activation time (hours of development)	1	2	4	6	12
Number of operons with putative σ^{54} promoters	135	15	4	75	8

To test our method experimentally, we randomly selected four putative σ^{54} promoters from this dataset and performed site-directed mutagenesis on the conserved dinucleotides in their −12 and −24 regions. We also created a 1-bp deletion in the spacer between these regions. The in vivo developmental activities of mutant promoters were as follows: promoters carrying mutations in their −12 region had activities ranging from 18–35% of WT, promoters carrying mutations in their −24 region had activities ranging from 5–15% of WT, and promoters with a 1-bp deletion in their spacer region had activities ranging from 2–7% of WT.

*Developmentally regulated operons contain genes whose expression increased 1.5-fold or greater at any time point during the preaggregation or aggregation stage of development compared with vegetative growth (0 h). To be designated a developmentally regulated operon with a σ^{54} promoter, DNA upstream of the first gene in the operon had to contain a potential binding site for σ^{54}-RNA polymerase (1) and a potential EBP binding site (2–5). The numbers of operons so defined that are activated during the preaggregation or aggregation stage of fruiting body development are shown. To test the error rate in the method used, we analyzed 11 known σ^{54} promoters and 24 known non-σ^{54} promoters via Promscan (6–33). We also analyzed *M. xanthus* intragenic regions via Promscan to confirm that Promscan does not find σ^{54} promoters in locations where they are unlikely to reside. Based on these tests and those described below, we estimate that our analysis had a false-positive rate of about 4% and a false-negative rate of about 19%. With this detection sensitivity, we found that 237 (28%) of the developmentally regulated operons activated during preaggregation or aggregation have putative σ^{54} promoters.

1. Studholme DJ, Buck M, Nixon T (2000) Identification of potential $\sigma^{(N)}$-dependent promoters in bacterial genomes. *Microbiology* 146:3021–3023.
2. Reitzer LJ, Magasanik B (1986) Transcription of *glnA* in *E. coli* is stimulated by activator bound to sites far from the promoter. *Cell* 45:785–792.
3. Minchin SD, Austin S, Dixon RA (1988) The role of activator binding sites in transcriptional control of the divergently transcribed *nifF* and *nifLA* promoters from *Klebsiella pneumoniae*. *Mol Microbiol* 2:433–442.
4. Morett E, Cannon W, Buck M (1988) The DNA-binding domain of the transcriptional activator protein NifA resides in its carboxy terminus, recognises the upstream activator sequences of *nif* promoters and can be separated from the positive control function of NifA. *Nucleic Acids Res* 16:11469–11488.
5. Goldman BS, et al. (2006) Evolution of sensory complexity recorded in a myxobacterial genome. *Proc Natl Acad Sci USA* 103:15200–15205 and correction (2006) 103:19605.
6. Botella JA, Murillo FJ, Ruiz-Vázquez R (1995) A cluster of structural and regulatory genes for light-induced carotenogenesis in *Myxococcus xanthus*. *Eur J Biochem* 233:238–248.
7. Brandner JP, Kroos L (1998) Identification of the Ω4400 regulatory region, a developmental promoter of *Myxococcus xanthus*. *J Bacteriol* 180:1995–2004.
8. Cheng YL, Kalman LV, Kaiser D (1994) The *dsg* gene of *Myxococcus xanthus* encodes a protein similar to translation initiation factor IF3. *J Bacteriol* 176:1427–1433.
9. Downard JS (1987) Identification of the RNA products of the *ops* gene of *Myxococcus xanthus* and mapping of *ops and tps* RNAs. *J Bacteriol* 169:1522–1528.
10. Fisseha M, Gloudemans M, Gill RE, Kroos L (1996) Characterization of the regulatory region of a cell interaction-dependent gene in *Myxococcus xanthus*. *J Bacteriol* 178:2539–2550.
11. Fisseha M, Biran D, Kroos L (1999) Identification of the Ω4499 regulatory region controlling developmental expression of a *Myxococcus xanthus* cytochrome P-450 system. *J Bacteriol* 181:5467–5475.
12. Garza AG, et al. (1998) SdeK is required for early fruiting body development in *Myxococcus xanthus*. *J Bacteriol* 180:4628–4637.
13. Garza AG, Harris BZ, Greenberg BM, Singer M (2000) Control of *asgE* expression during growth and development of *Myxococcus xanthus*. *J Bacteriol* 182:6622–6629.
14. Gronewold TM, Kaiser D (2002) *act* operon control of developmental gene expression in *Myxococcus xanthus*. *J Bacteriol* 184:1172–1179.
15. Horiuchi T, Akiyama T, Inouye S, Komano T (2002) Analysis of *dofA*, a *fruA*-dependent developmental gene, and its homologue, *dofB*, in *Myxococcus xanthus*. *J Bacteriol* 184: 6803–6810.
16. Horiuchi T, Taoka M, Isobe T, Komano T, Inouye S (2002) Role of *fruA and csgA* genes in gene expression during development of *Myxococcus xanthus*. Analysis by two-dimensional gel electrophoresis. *J Biol Chem* 277:26753–26760.
17. Inouye S (1984) Identification of a development-specific promoter of *Myxococcus xanthus*. *J Mol Biol* 174:113–120.
18. Inouye S, Nariya H (2008) Dual regulation with Ser/Thr kinase cascade and a His/Asp TCS in *Myxococcus xanthus*. *Adv Exp Med Biol* 631:111–121.
19. Keseler IM, Kaiser D (1995) An early A-signal-dependent gene in *Myxococcus xanthus* has a σ^{54}-like promoter. *J Bacteriol* 177:4638–4644.
20. Komano T, Franceschini T, Inouye S (1987) Identification of a vegetative promoter in *Myxococcus xanthus*. A protein that has homology to histones. *J Mol Biol* 196:517–524.
21. Lee K, Shimkets LJ (1996) Suppression of a signaling defect during *Myxococcus xanthus* development. *J Bacteriol* 178:977–984.
22. Li S, Lee Bu, Shimkets LJ (1992) *csgA* expression entrains *Myxococcus xanthus* development. *Genes Dev* 6:401–410.
23. Licking E, Gorski L, Kaiser D (2000) A common step for changing cell shape in fruiting body and starvation-independent sporulation of Myxococcus xanthus. *J Bacteriol* 182:3553–3558.
24. McGowan SJ, Gorham HC, Hodgson DA (1993) Light-induced carotenogenesis in *Myxococcus xanthus*: DNA sequence analysis of the *carR* region. *Mol Microbiol* 10:713–735.
25. O'Connor KA, et al. (1996) Photolyase of *Myxococcus xanthus*, a Gram-negative eubacterium, is more similar to photolyases found in Archaea and "higher" eukaryotes than to photolyases of other eubacteria. *J Biol Chem* 271:6252–6259.
26. Ogawa M, Fujitani S, Mao X, Inouye S, Komano T (1996) FruA, a putative transcription factor essential for the development of *Myxococcus xanthus*. *Mol Microbiol* 22:757–767.
27. Romeo JM, Zusman DR (1991) Transcription of the myxobacterial hemagglutinin gene is mediated by a σ^{54}-like promoter and a cis-acting upstream regulatory region of DNA. *J Bacteriol* 173:2969–2976.
28. Trudeau KG, Ward MJ, Zusman DR (1996) Identification and characterization of FrzZ, a novel response regulator necessary for swarming and fruiting-body formation in *Myxococcus xanthus*. *Mol Microbiol* 20:645–655.
29. Viswanathan K, Viswanathan P, Kroos L (2006) Mutational analysis of the *Myxococcus xanthus* Ω4406 promoter region reveals an upstream negative regulatory element that mediates C-signal dependence. *J Bacteriol* 188:515–524.
30. Viswanathan P, Murphy K, Julien B, Garza AG, Kroos L (2007) Regulation of *dev*, an operon that includes genes essential for *Myxococcus xanthus* development and CRISPR-associated genes and repeats. *J Bacteriol* 189:3738–3750.
31. Ward MJ, Lew H, Zusman DR (2000) Social motility in *Myxococcus xanthus* requires FrzS, a protein with an extensive coiled-coil domain. *Mol Microbiol* 37:1357–1371.
32. Wu SS, Kaiser D (1997) Regulation of expression of the *pilA* gene in *Myxococcus xanthus*. *J Bacteriol* 179:7748–7758.
33. Zhang W, Inouye M, Inouye S (1996) Reciprocal regulation of the differentiation of *Myxococcus xanthus* by Pkn5 and Pkn6, eukaryotic-like Ser/Thr protein kinases. *Mol Microbiol* 20: 435–447.

Table S3. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source
M. xanthus strains		
DK1622	WT *M. xanthus*	(1)
AG304	pNBC4::*nla4*, Kan^r	(2)
AG306	pNBC6::*nla6*, Kan^r	(2)
AG318	pNBC18::*nla18*, Kan^r	(2)
AG328	pNBC::*nla28*, Kan^r	(2)
AG1114	pNBC6::*nla6*, Kan^r; pNBC32::*nla28*, Tet^r	This study
DK10603	Δ*actB*	(3)
Plasmids		
pET102/D-TOPO	Amp^r	Invitrogen
pMBP-parallel1	Amp^r	(4)
pCR 2.1-TOPO	Amp^r, Kan^r (Invitrogen)	
pREG1727	Kan^r	R. Gill (5)
pKG14	Nla6-DBD$_{90}$*/pMBP-parallel1	This study
pKG15	Nla28-DBD$_{81}$*/pMBP-parallel1	This study
pKG16	Nla4-DBD$_{85}$*/pMBP-parallel1	This study
pKG17	ActB-DBD$_{64}$*/pMBP-parallel1	This study
pNBC103	Nla18-DBD$_{76}$*/pET102D, Amp^r	This study
pNBC104	Nla28-DBD$_{76}$*/pET102D, Amp^r	This study
pKG20	pCR 2.1-TOPO::*nla28*p418†	This study
pKG21	pCR 2.1-TOPO::*nla6*p207†	This study
pKG22	pKG20, g401t_g402t	This study
pKG23	pKG20, g413t_c414t	This study
pKG24	PKG20, g404del	This study
pKG25	pREG1727::*nla28*p418	This study
pKG26	pKG25, g401t_g402t	This study
pKG27	pKG25, g413t_c414t	This study
pKG28	pKG25, g404del	This study
pKG29	pKG21, g173t_g174t	This study
pKG30	pKG21, g185t	This study
pKG31	pKG21, g180del	This study
pKG32	pREG1727::*nla6*p207	This study
pKG33	pKG32, g173t_g174t	This study
pKG34	pKG32, g185t	This study
pKG35	pKG32, a180del	This study

*Numbers shown correspond to the number of amino acids comprising the DBD of the indicated EBP.
†Number of bases in the promoter fragment used.

1. Kaiser D (1979) Social gliding is correlated with the presence of pili in *Myxococcus xanthus. Proc Natl Acad Sci USA* 76:5952–5956.
2. Caberoy NB, Welch RD, Jakobsen JS, Slater SC, Garza AG (2003) Global mutational analysis of NtrC-like activators in *Myxococcus xanthus:* Identifying activator mutants defective for motility and fruiting body development. *J Bacteriol* 185:6083–6094.
3. Gorski L, Gronewold T, Kaiser D (2000) A $\sigma^{(54)}$ activator protein necessary for spore differentiation within the fruiting body of *Myxococcus xanthus. J Bacteriol* 182:2438–2444.
4. Sheffield P, Garrard S, Derewenda Z (1999) Overcoming expression and purification problems of RhoGDI using a family of "parallel" expression vectors. *Protein Expr Purif* 15:34–39.
5. Fisseha M, Gloudemans M, Gill RE, Kroos L (1996) Characterization of the regulatory region of a cell interaction-dependent gene in *Myxococcus xanthus. J Bacteriol* 178:2539–2550.

Table S4. Bacterial primers used in this study

Primers	Sequence	Amplicon size
Primers used to make EBP-DBD fragments		
actB-DBD (MBP) fwd	5′gagctgtcgctgaaggacatcgg3′	
actB-DBD (MBP) rev	5′cgcgagcgcggagctgtag3′	192 bp
nla4-DBD (MBP) fwd	5′agtgacctgcctgaatccgtg3′	
nla4-DBD (MBP) rev	5′cccgacaccgacgactga3′	255 bp
nla6-DBD (MBP) fwd	5′cacaccttccggctgccctt3′	
nla6-DBD (MBP) rev	5′ctcgttcgtcatccgctga3′	270 bp
nla18-DBD up	5′caccctgccgccgccttcggacgac3′	
nla18-DBD down	5′cgccggtgacgtgtccaggcc3′	229 bp
nla28-DBD up	5′caccacgggcgtcctggggccccag3′	
nla28-DBD down	5′cgactcggcctccggggcctc3′	229 bp
nla28-DBD (MBP) fwd	5′ctggcgctcaacgtgacggg3′	
nla28-DBD (MBP) rev	5′gccccggaggccgagtcgtag3′	243 bp
Primers used in RT-QPCR		
16s up	5′caagggaactgagagacagg3′	220 bp
16s down	5′ctctgtaccggccattgtagc3′	
actB up	5′gatgaagatgggcgccagcga3′	
actB down	5′gtgaatctcccgcgcgatgac3′	301 bp
nla4 up	5′gacgtggaagtggtgctgatg3′	
nla4 down	5′ctttcccgtgccggactcacc3′	318 bp
nla6 up	5′ctgaaggaggagctgccggac3′	
nla6 down	5′ctgatggacacgctggccttg3′	305 bp
nla18 up	5′gcgccagcacctcatcctcga3′	
nla18 down	5′gatgagcacgtccgactcgcc3′	295 bp
nla28 up	5′cgtggtggtggtgacagcctt3′	
nla28 down	5′caccatctccttccccgtgc3′	307 bp
MXAN4899 up	5′aaggtcgagctgggcaaga3′	
MXAN4899 down	5′agatgaggttgcagtccacg3′	262 bp
Primers used to generate promoter region fragments		
*actB*P1 up	5′ccgccgtcgtggagtc3′	
*actB*P1 down	5′cggctgcatgaggtcaaa3′	185 bp
*actB*P2 *up*	5′ggcatcgtcgagaagtcg3′	
*actB*P2 *down*	5′ccccttcgagctgcggat3′	157 bp
dev up	5′gcatgcatcagcgaa3′	
dev down	5′ccaacatgcgcaggt3′	70 bp
MXAN4899P1 up	5′tgctggaaacactggcgcgg3′	
MXAN4899P1 down	5′cagtcgaagcgcgcgttcaa3′	169 bp
MXAN4899P2 up	5′agccacctgaagccaccc3′	
MXAN4899P2 down	5′atgcagcgcctgcgctt3′	179 bp
*nla4*P1 up	5′atgcaggccagcgcccgg3′	
*nla4*P1 down	5′gcaggaggttgcgcccaac3′	134 bp
*nla4*P2 up	5′tgctggagctggacgacgc3′	
*nla4*P2 down	5′aagccggacttcgacgccac3′	148 bp
nla6P1 up	5′tgacgtgggccacatgatggagga3′	
nla6P1 down	5′tgttcggcgagttctaccggga3′	201 bp
nla6P2 up	5′tacatccgccggactgga3′	
nla6P2 down	5′tccaccggctacaacggcgc3′	166 bp
*nla28*P1 up	5′′gcgtcatctggttgcagggt3′	
*nla28*P1 down	5′gctcgagcggaatacccgt3′	151 bp
*nla28*P2 up	5′agggattgagcaggcggc3′	
*nla28*P2 down	5′gctcaagtgctccgtcatcgtc3′	159 bp
Primers used for RT-PCR		
*nla28*operon fwd	5′agggaacgcacgtggtggt3′	174 bp
*nla28*operon rev	5′agcaagcgctggagcaaat3′	
*nla6*operon fwd	5′tgttcacagttgggcgttgacct3′	198 bp
*nla6*operon rev	5′tcacacgtgtcgcgttcgtcat3′	
Primers used to generate mutations in the *nla6* and *nla28* promoter regions		
nla6 promoter		
g173t_g174t	5′atcgccaacgcgggccttttgcgcatcgtg3′	
g173t_g174t-anti	5′cacgatgcgcaaaaggcccgcgttggcgat3′	
g185t	5′cctggtgcgcatcgttttcggcgagttctac3′	
g185t-anti	5′gtagaactcgccgaaaacgatgcgcaccagg3′	
a180del	5′ggcctggtgcgctcgtgttcggcg3′	

Table S4. Cont.

Primers	Sequence	Amplicon size
a180del-anti	5′cgccgaacacgagcgcaccaggcc3′	
nla28 promoter		
g401t_g402t	5′ccaactggagtccgcgctttagcgggtgct3′	
g401t_402t-anti	5′agcacccgctaaagcgcggactccagttgg3′	
g413t_c414t	5′gagcgggtgcttttggagcgcgc3′	
g413t_c414t-anti	5′gcgcgctccaaaagcacccgctc3′	
g414del	5′gagtccgcgctggacgggtgctgc3′	
g414del-anti	5′gcagcaccctgccagcgcggactc3′	

fwd, forward; rev, reverse.