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How McrA regulates secondary metabolism

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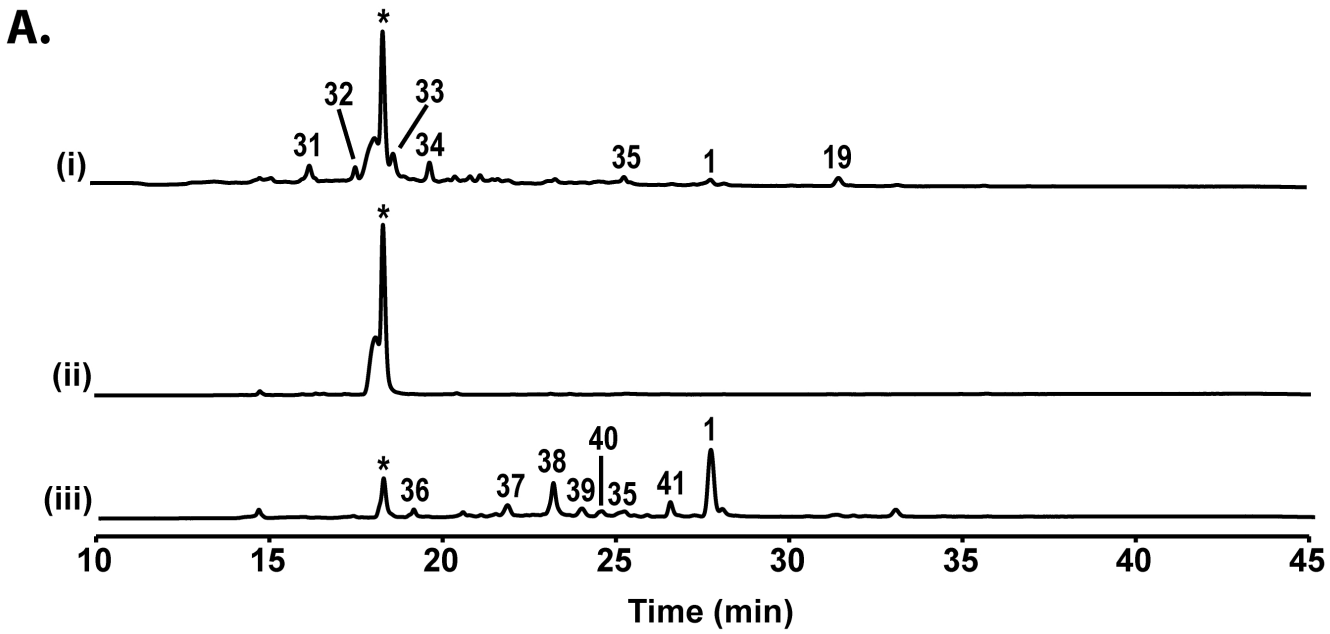
What is McrA (AN8694)?

- It was identified in a screen for negative regulators of secondary metabolism in *Aspergillus nidulans* (Oakley et al., 2017, Molecular Microbiology 103:347-365).

- It is strongly predicted to be a transcription regulator with a zinc-finger DNA binding domain.

- It is conserved among ascomycetes (1 or 2 strong homologs/genome).

McrA regulates production of many secondary metabolites

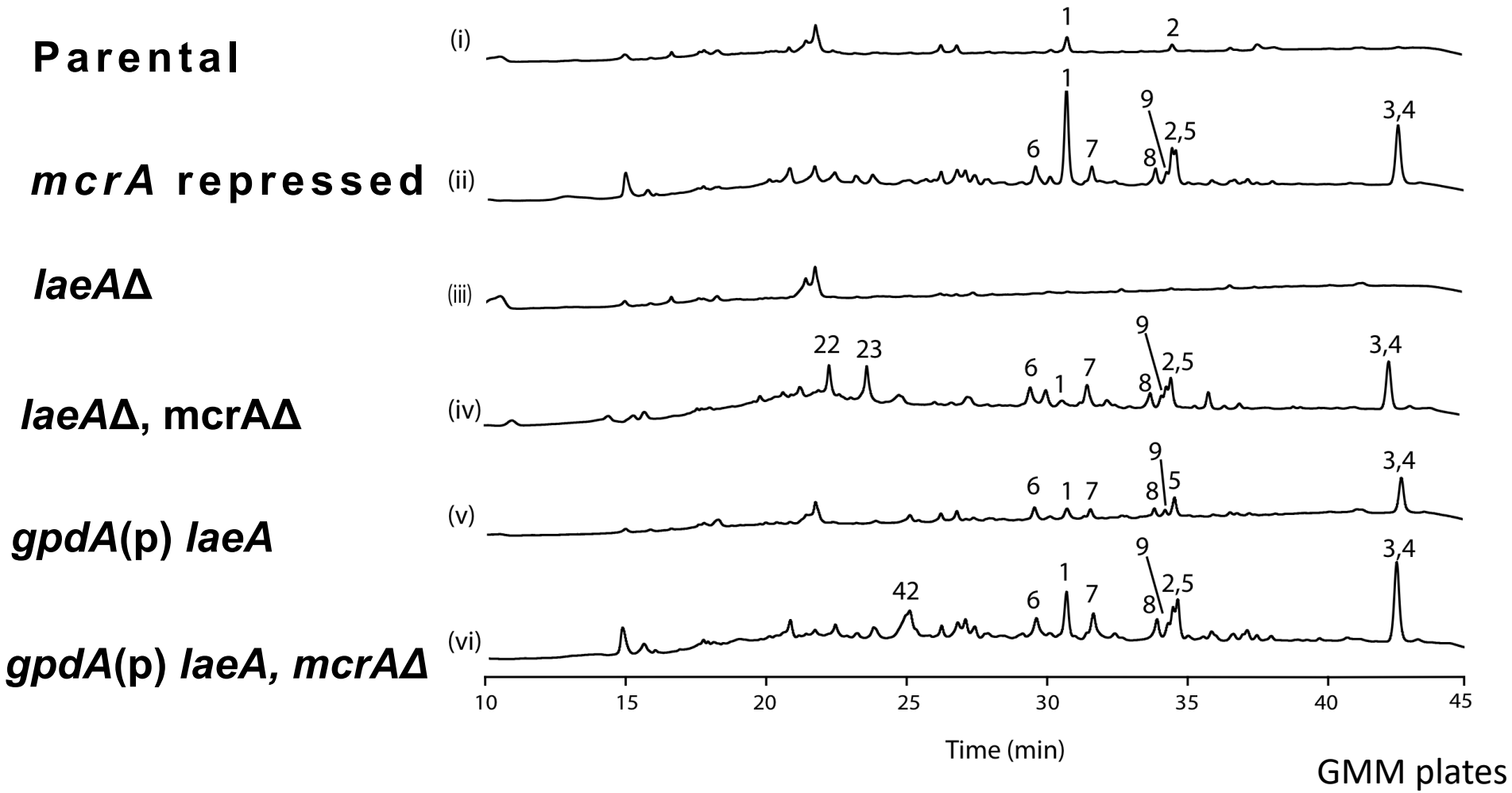


Parental

***mcrA*OE**

***mcrA*Δ**

How McrA regulates secondary metabolism



GMM plates

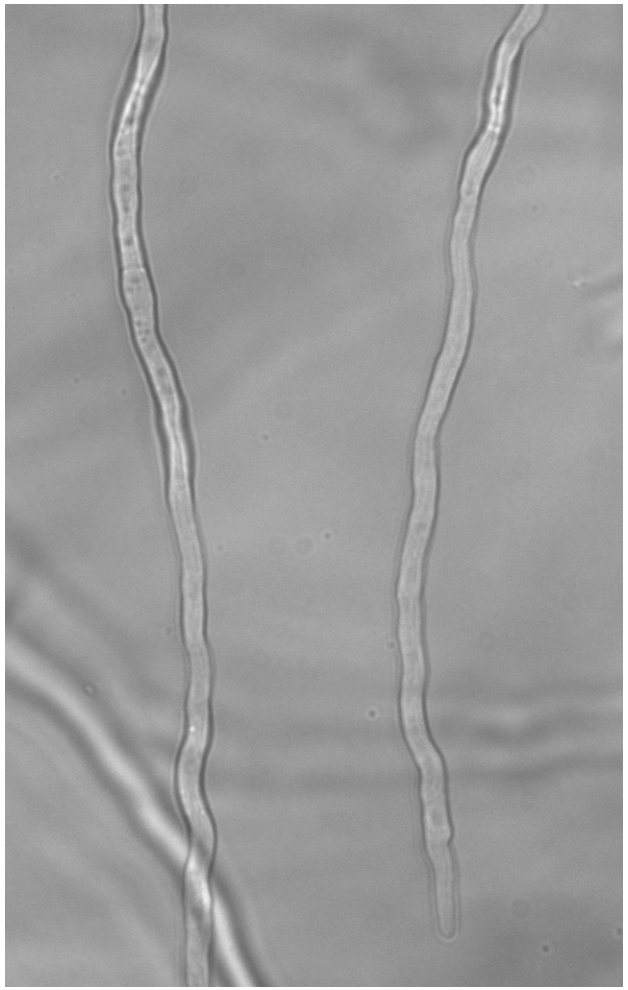
- **McrA plays a role in the regulation of at least 10 secondary metabolite gene clusters.**
- **Most are upregulated in the *mcrA* deletion.**
- **Deletions of *mcrA* homologs in *Aspergillus terreus* and *Penicillium canescens* also upregulate secondary metabolite production.**
- **Production of compounds in *mcrAΔ* strains is influenced by media (carbon source) and growth conditions.**

Effects of *mcrA* Deletion on transcription

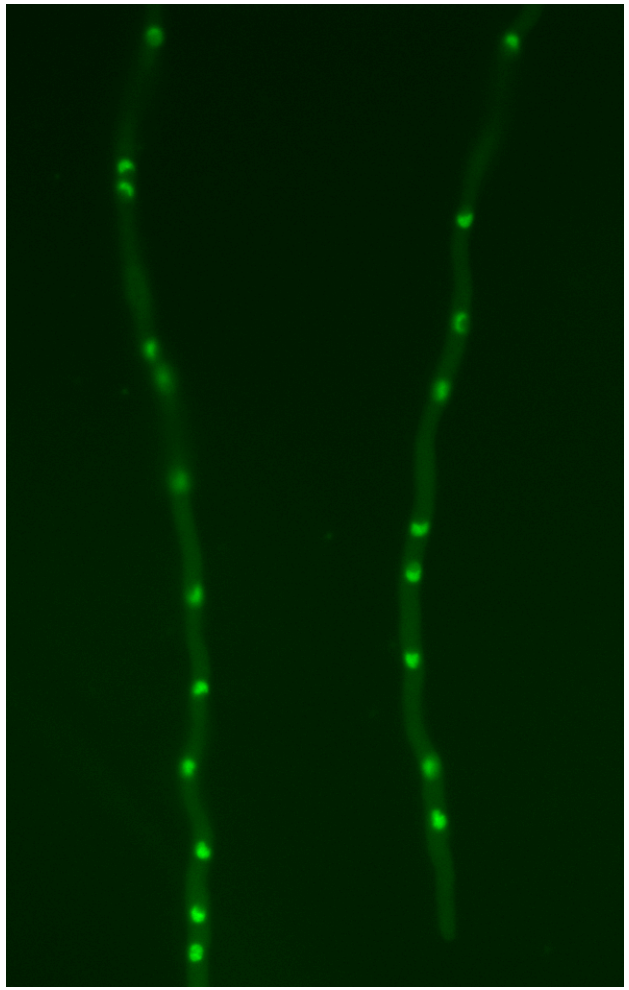
- Deletion of *mcrA* significantly alters the transcription of 1352 genes ($p<0.05$).
- Genes related to primary and secondary metabolism are enriched among transcriptionally altered strains.
- The majority of transcriptionally altered genes are upregulated.

McrA-GFP localizes to the nucleoplasm.

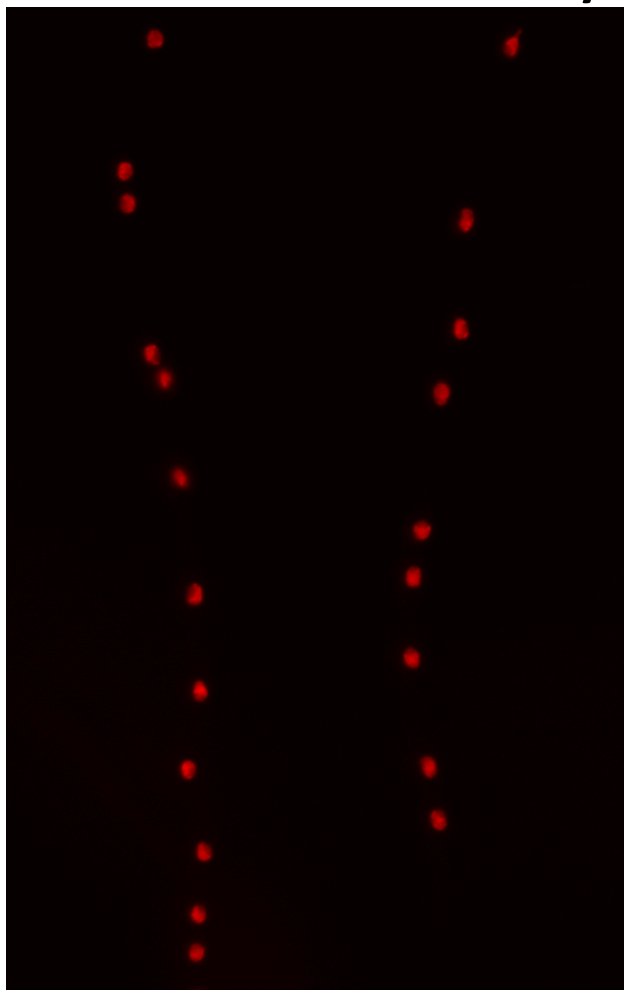
Brightfield



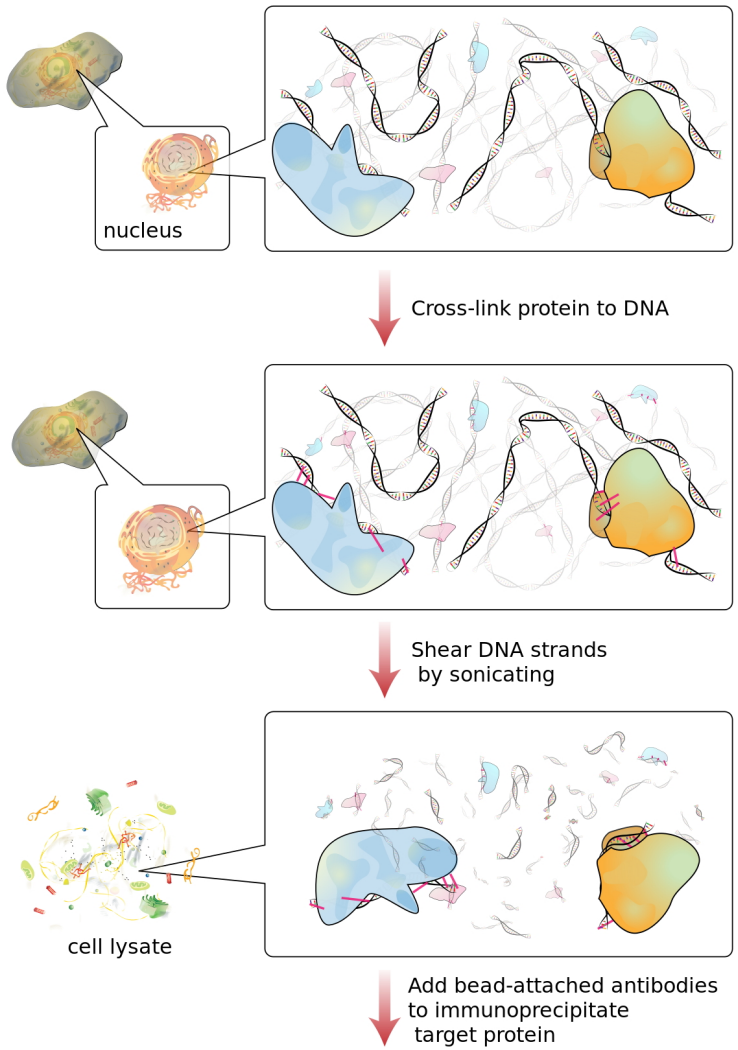
McrA-GFP

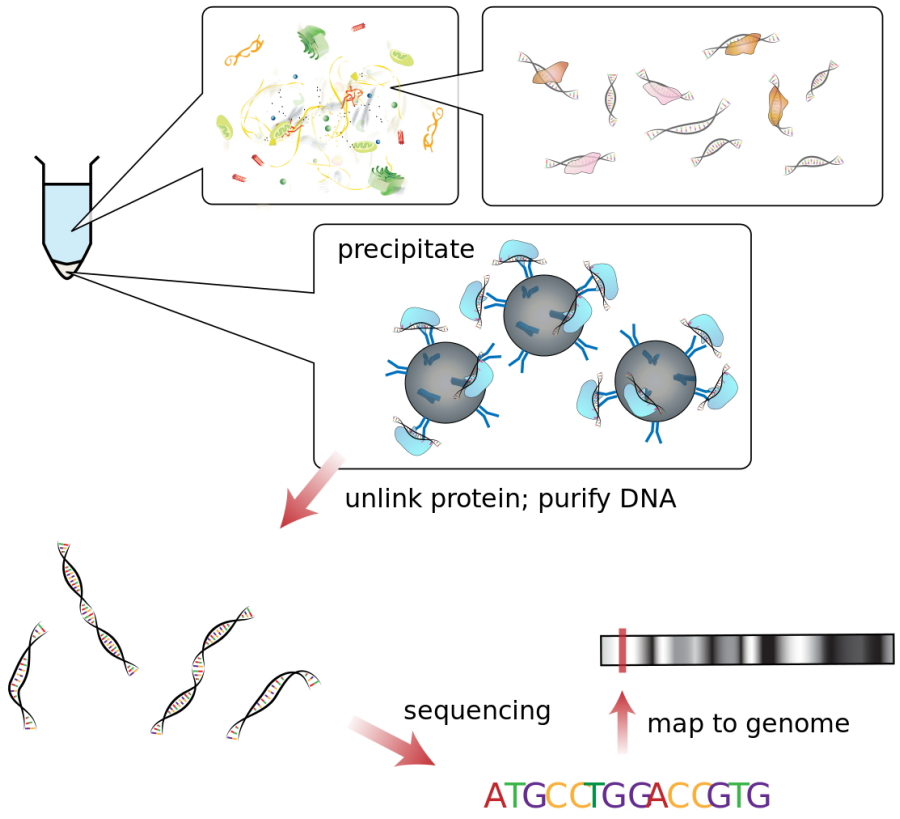


Histone H1 mCherry



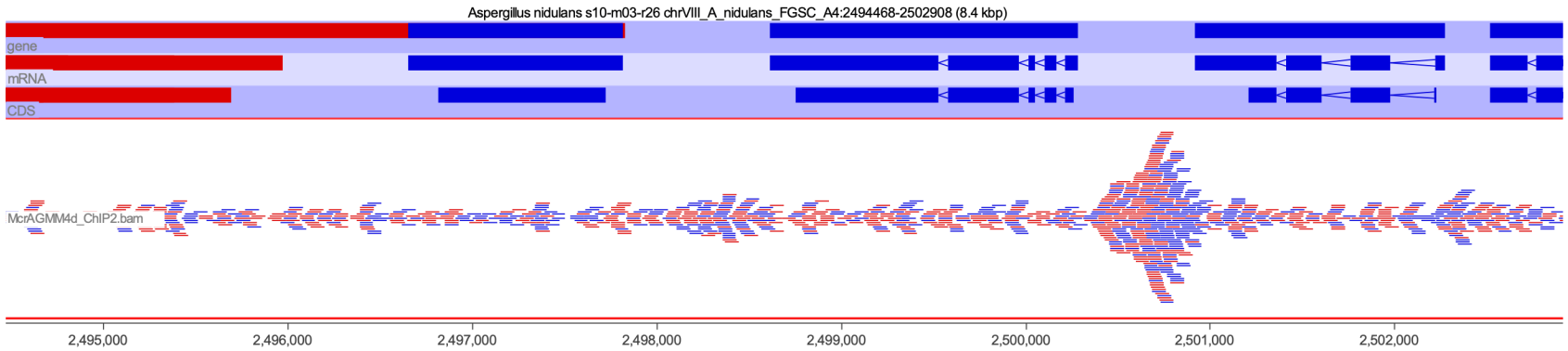
ChIP-seq with HA-tagged McrA.



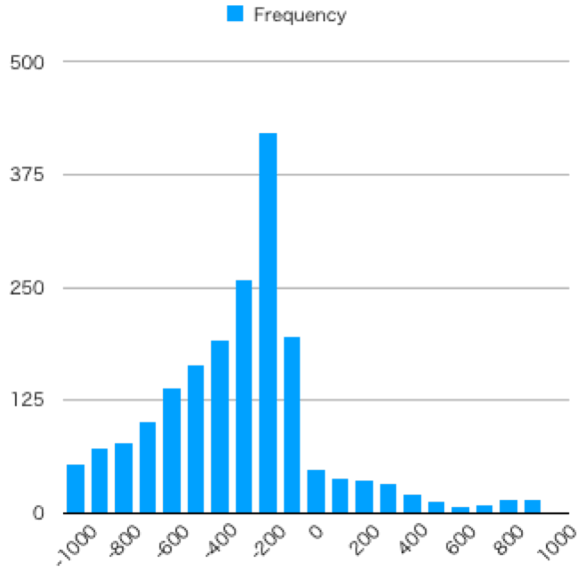


J.K.W. Chui-Wikipedia

How McrA regulates secondary metabolism



1899 statistically significant McrA ChIP-seq sites.



Distribution relative to transcription start sites. Most sites are in promoter regions of genes.

Does McrA regulate secondary metabolism biosynthetic genes directly?

*McrA*Δ dramatically up-regulates expression of two nidulanin biosynthesis genes.

Gene	WT	*mcrA*Δ	P<0.01?
nlsA (AN1242)	47.83 ± 35.08	447.26 ± 149.63	Yes
AN11934	38.86 ± 25.10	340.97 ± 67.61	Yes

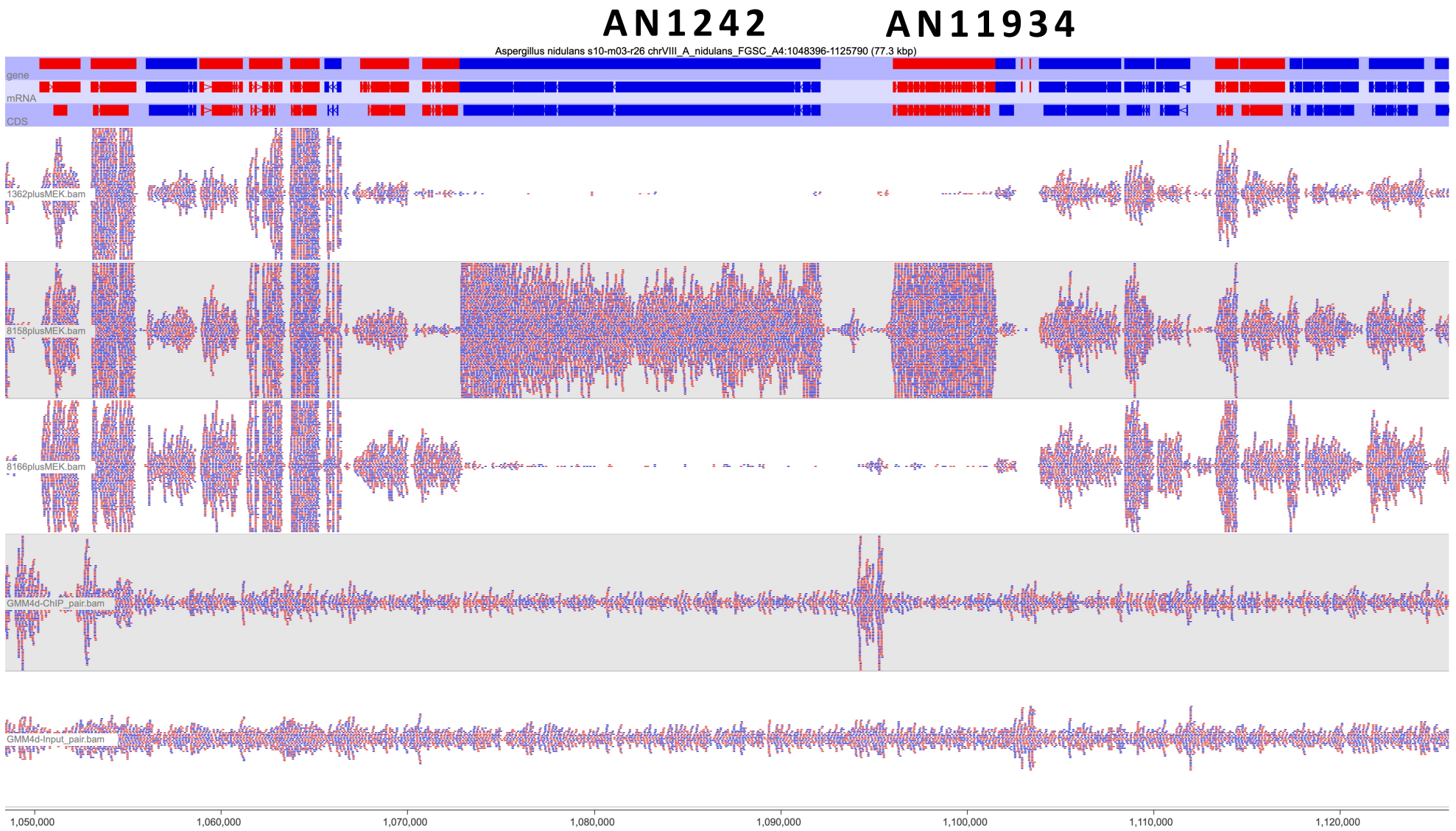
Glucose minimal medium 4 days incubation. All values are reads per million, means +/- standard deviations, three biological replicates. Incubation at 37ºC. P value from EdgeR analysis.

Over-expression of McrA dramatically down-regulates expression of nidulanin biosynthesis genes.

	Wild-type				***alcA*(p)-*mcrA***		
Gene	Uninduced	Induced	P<0.01?		Uninduced	Induced	P<0.01?
nlsA(AN1242)	72.81 ± 38.07	35.94 ± 14.43	No		142.38 ± 21.13	7.13 ± 1.92	Yes
AN11934	93.75 ± 14.23	102.75 ± 28.12	No		104.03 ± 13.51	13.57 ± 4.87	Yes

Lactose minimal medium, 3.5 days ± 12 h 50 mM methyl-ethyl ketone. All values reads per million, means ± standard deviation, three biological replicates. Incubation at 37ºC.

How McrA regulates secondary metabolism



Parental control

***mcrA*Δ**

***mcrA*OE**

***mcrA* ChIP-seq**

ChIP-seq control

Five secondary metabolism gene clusters have McrA ChIP-seq sites upstream of every gene and are regulated by McrA:

- **Nidulanins**
- **F9775A,B/Orsellinic Acid**
- **Emericellamides (Production of the compounds is downregulated by McrAOE. McrA deletion does not substantially up-regulate.)**
- **AN10886-AN12290 (de-repressed, transcript levels moderate)**
- **AN9005-AN9006 (de-repressed, transcript levels moderate)**

Many secondary metabolism gene clusters contain transcription factors that can, in principle, drive expression of the other genes of the cluster.

Does McrA regulate secondary metabolism via regulation of secondary-metabolism-specific transcription factors?

Monodictyphenone biosynthetic gene cluster. 4 days incubation on glucose minimal medium

Gene	P<0.01?	Fold increase in *mcrA*Δ	Mean RPM in *mcrA*Δ	McrA-ChIP?	Predicted function of protein
AN10023/mdpL	Yes	165.03	1203.11		Member of the monodictyphenone (mdp) secondary metabolite biosynthesis gene cluster
AN10044/mdpK	Yes	183.24	1011.36		Putative oxidoreductase
AN10038/mdpJ	Yes	193.85	1275.62		Putative glutathione S transferase
AN10035/mdpI	Yes	186.96	165.54		Putative AMP-binding CoA ligase
AN10022/mdpH	Yes	148.73	1217.80		Protein with homology to the DUF 1772 superfamily
AN0150/mdpG	Yes	107.03	1447.14		Polyketide synthase
AN0149/mdpF	Yes	282.69	1415.20		Putative zinc-dependent hydrolase
AN0148/mdpE	Yes	188.68	232.86	Yes	C6 zinc finger transcription factor similar to AflR
AN0147/mdpD	Yes	146.15	308.30		Flavin-containing monooxygenase
AN0146/mdpC	Yes	171.56	2605.77		Protein with homology to versicolorin ketoreductase
AN10049/mdpB	Yes	107.13	980.66		Protein with homology to scytalone dehydratase
AN10021/mdpA	Yes	93.26	153.55		Regulatory protein

How McrA regulates secondary metabolism

- **14 secondary metabolism gene clusters have McrA ChIP-seq sites upstream of a transcription factor.**
- **At least 6 are strongly upregulated by *mcrA*Δ under some conditions.**
- **Transcription of others may depend on the availability of an activating transcription factor, chromatin access, etc.**

Does McrA regulate regulators?

Yes

Grau, M. F., R. Entwistle, C. E. Oakley, C. C. C. Wang, and B. R. Oakley. 2019. Overexpression of an LaeA-like Methyltransferase Upregulates Secondary Metabolite Production in Aspergillus nidulans. ACS Chem Biol **14**:1643-1651.

How McrA regulates secondary metabolism

Gene	wt	mcrAΔ	p<0.01
laeA (AN0807)	20.77 +/- 4.05	37.84 +/- 11.18	no
veA (AN1052)	260.43 +/- 20.01	460.94 +/- 150.08	no
velB (AN0360)	25.98 +/- 6.44	51.93 +/- 1.52	no

GMM (4days of growth)

How McrA regulates secondary metabolism

	LMM *alcA*(p)-*mcrA* (3.5 days at 37ºC 12 h +/- 50 mM MEK)		
Gene	Uninduced	Induced	P<0.01?
laeA (AN0807)	27.64 +/- 24.87	0.94 +/- 0.92	Yes
veA (AN1052)	816.54 +/- 108.12	28.18 +/- 4.93	Yes
velB (AN0360)	65.58 +/- 8.08	61.00 +/- 4.00	No

How McrA regulates secondary metabolism

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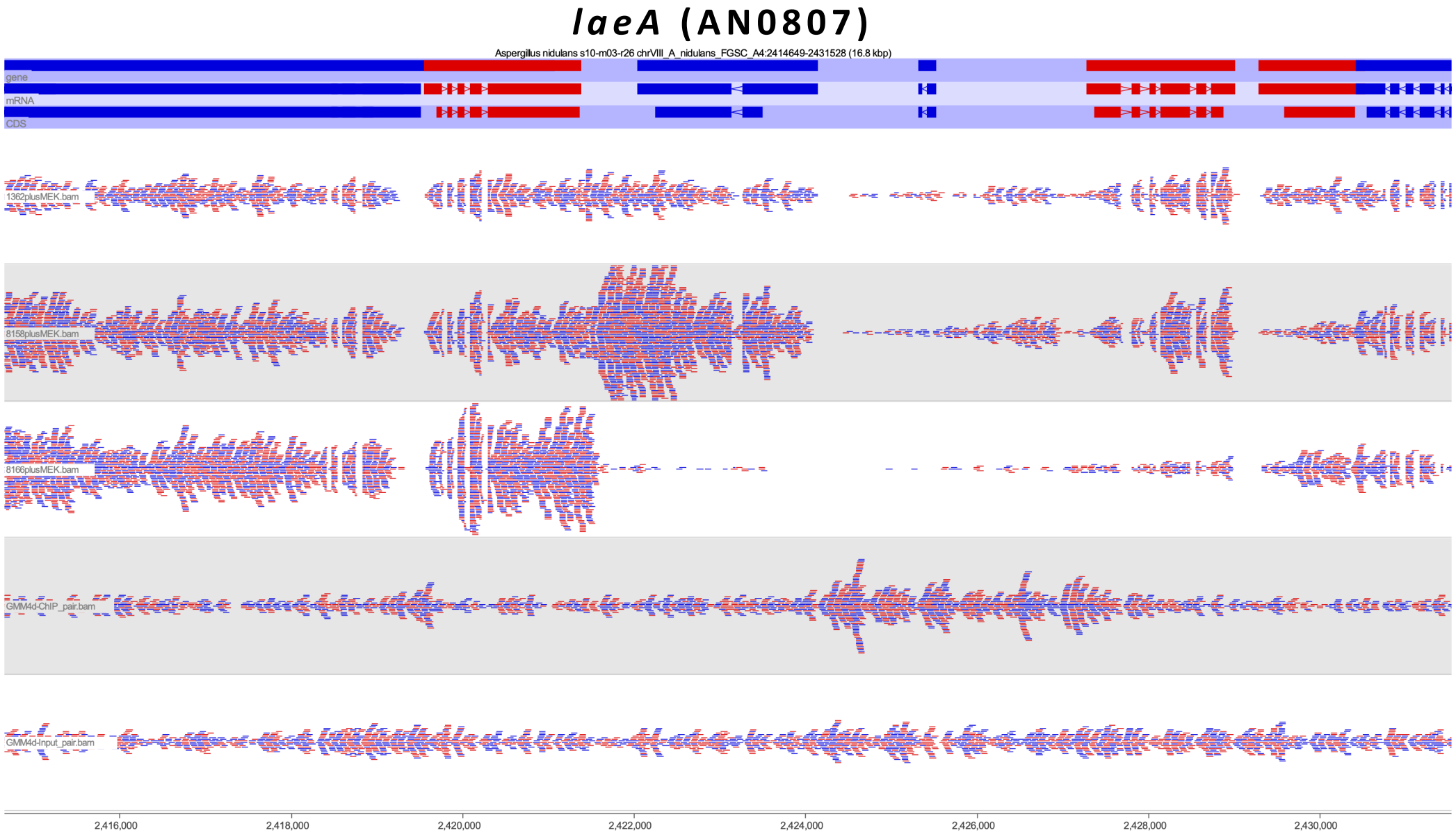

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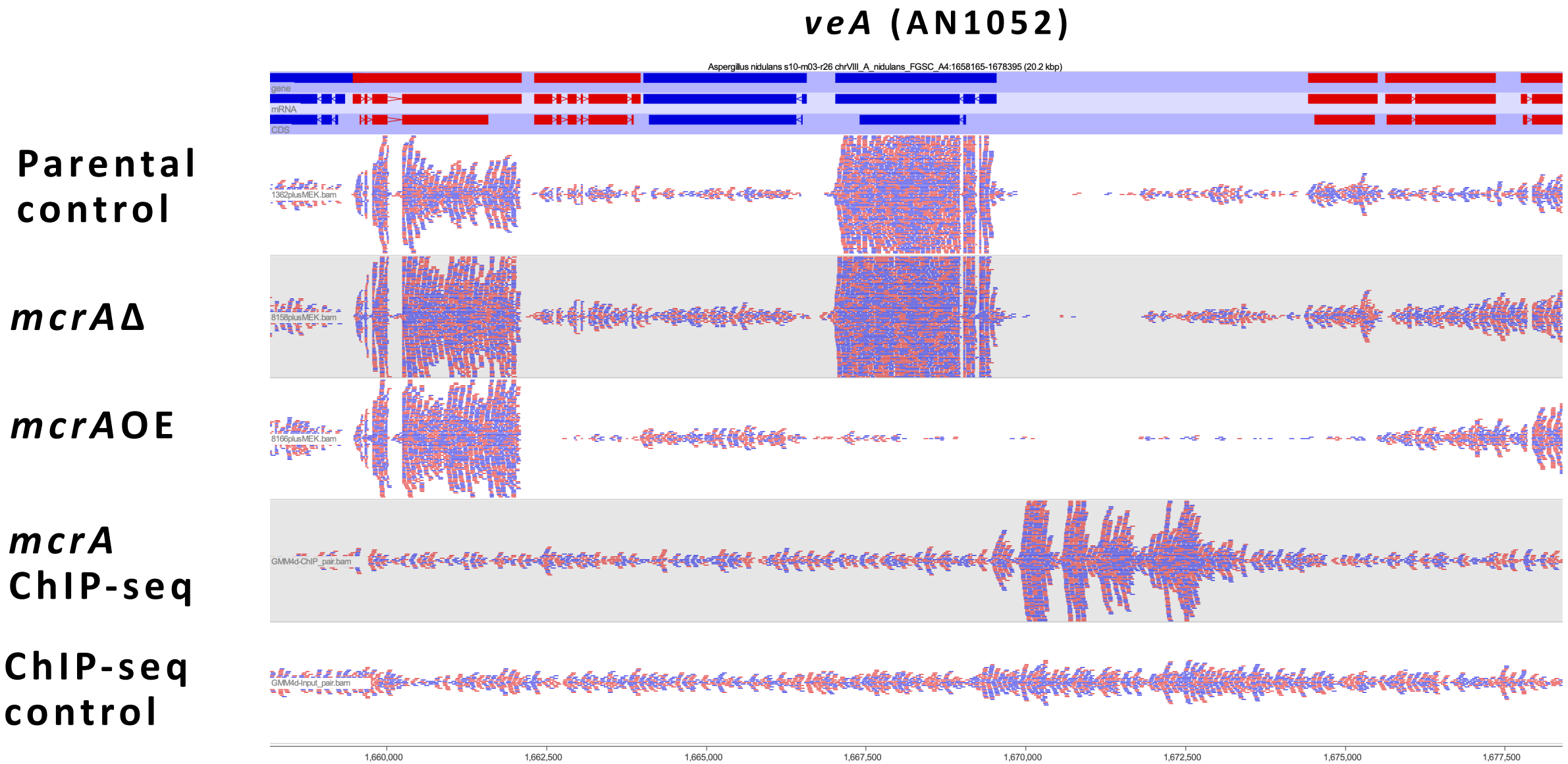
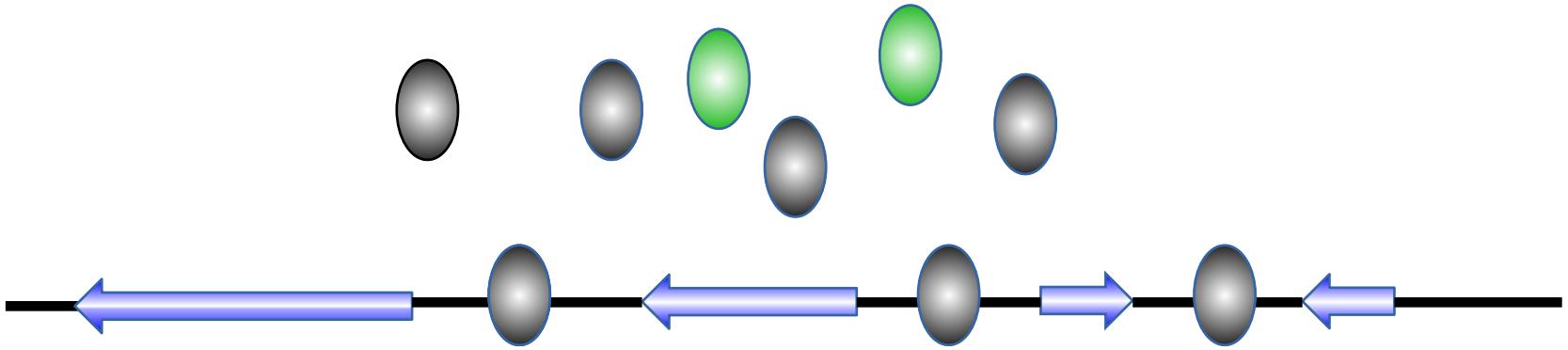


Mechanism?

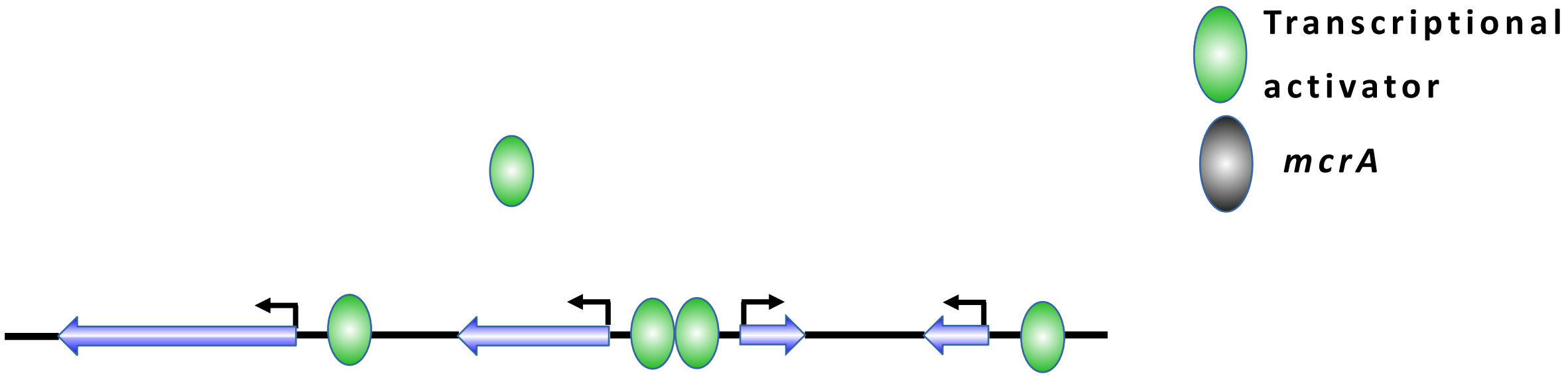
Transcriptional activator

mcrA



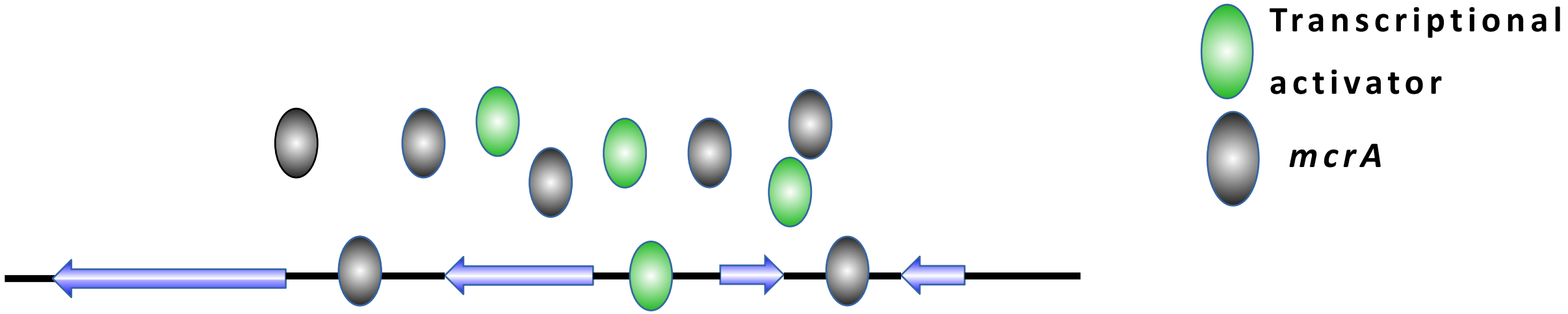
McrA binds to the promoter region of each gene and represses transcription.

How McrA regulates secondary metabolism



Deletion of *mcrA* derepresses, but levels of transcription depend on the amount and activity of the transcriptional activator(s).

How McrA regulates secondary metabolism



In the normal (*mcrA*+) situation, McrA and transcriptional activators are in equilibrium. Transcription depends on the relative abundances and activities of McrA and transcriptional activators (including affinities for binding sites).

How McrA regulates secondary metabolism

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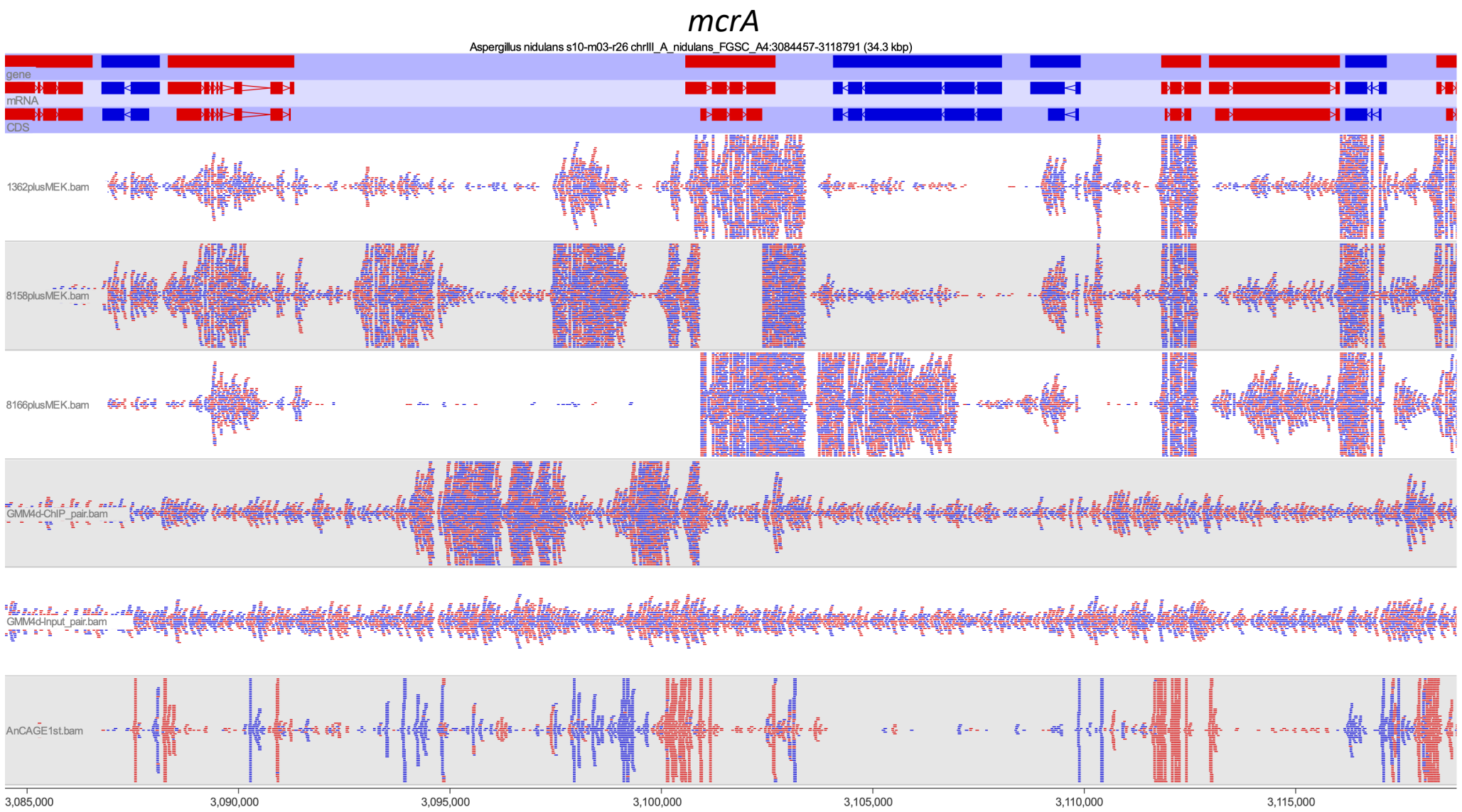

Parental

*mcrA*Δ

alcA(p)-*mcrA* (OE)

McrA ChIP

ChIP control

CAGE RNA-seq

How McrA regulates secondary metabolism

- **McrA binds upstream of many genes involved in secondary metabolism, likely functioning as a competitive inhibitor of transcription.**
- **It can regulate secondary metabolism genes directly and through cluster-specific transcription factors.**
- **It plays a role in regulating other secondary metabolism regulators.**
- **The *mcrA* region is transcriptionally complex and *mcrA* may autoregulate through a negative feedback loop.**

- Supported by the Irving S. Johnson Fund of the University of Kansas Endowment
- The Kansas City Area Life Sciences Institute and Hall Family Foundation
- NIGMS
- The H. L. Snyder Medical Foundation
- The Japan Society for the Promotion of Science.