Caulobacter crescentus as a model for the study of bacterial cell cycle regulation.

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Note:This is a modified version of the slides used as support for the talk given by Leticia Britos Cavagnaro at the Pathway Tools Workshop, at SRI, on August 24th 2009.

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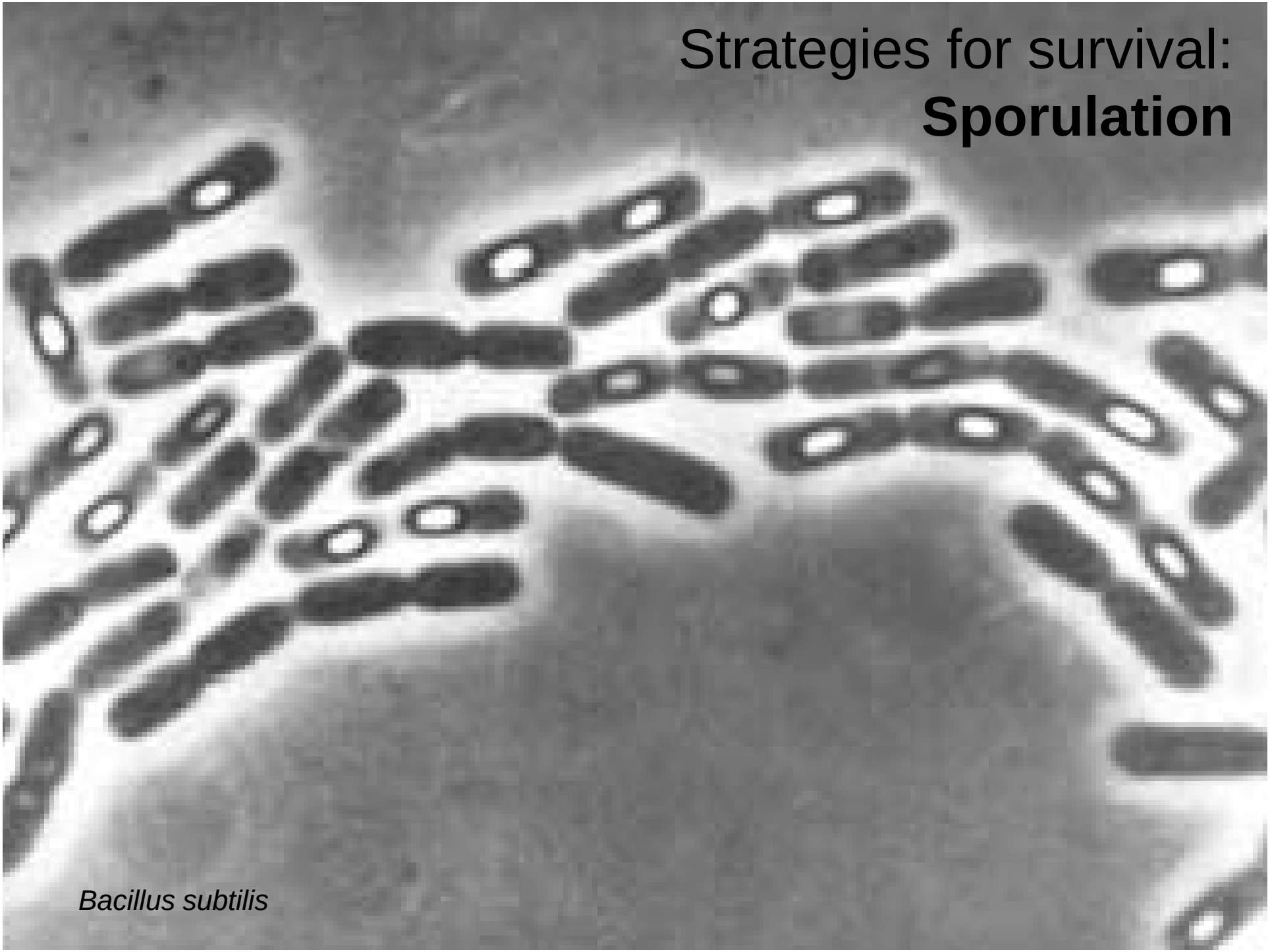
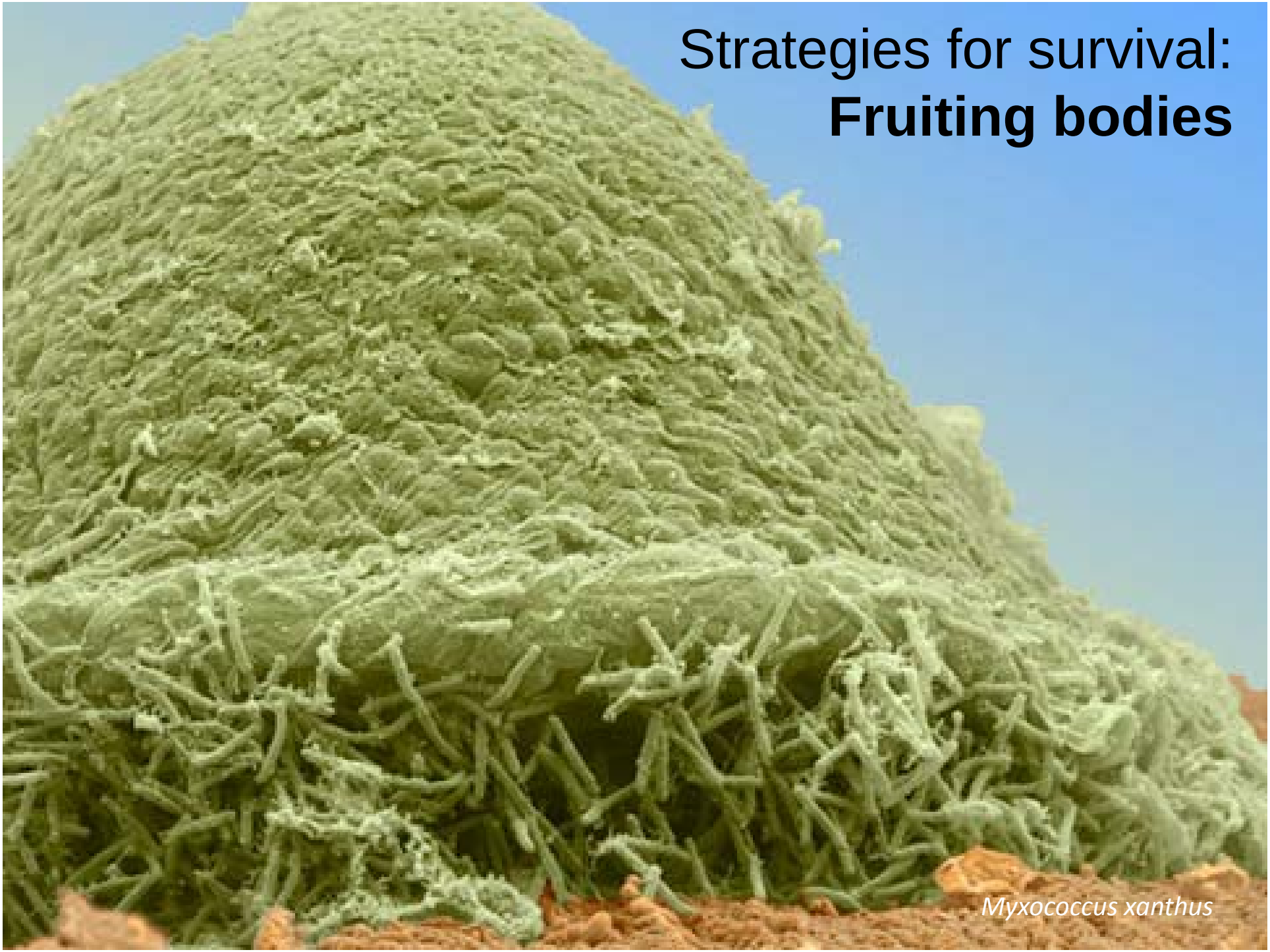
Act I

It was the best of times, it was the worst of times…

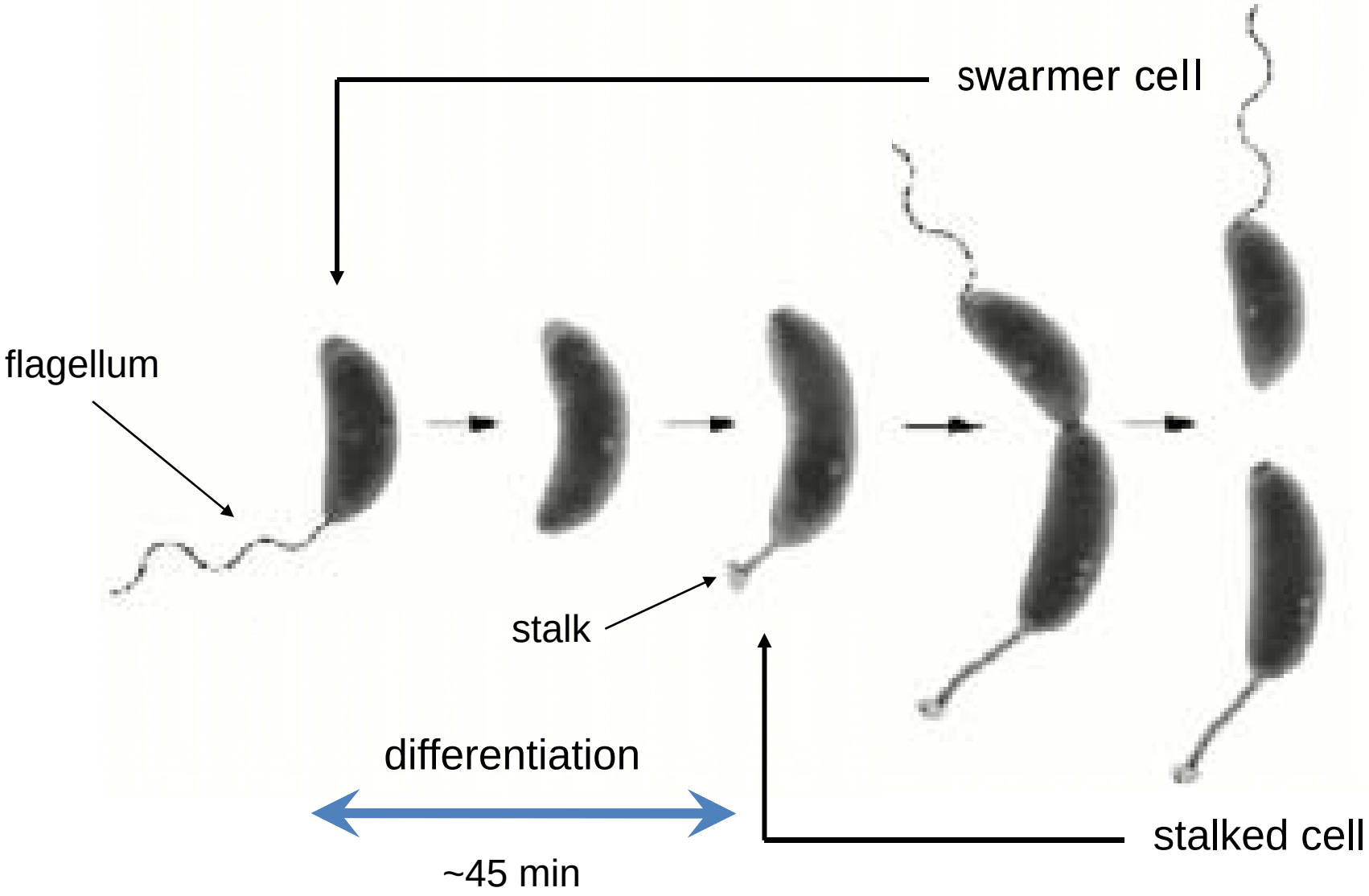
Strategies for survival: **Sporulation**



Bacillus subtilis

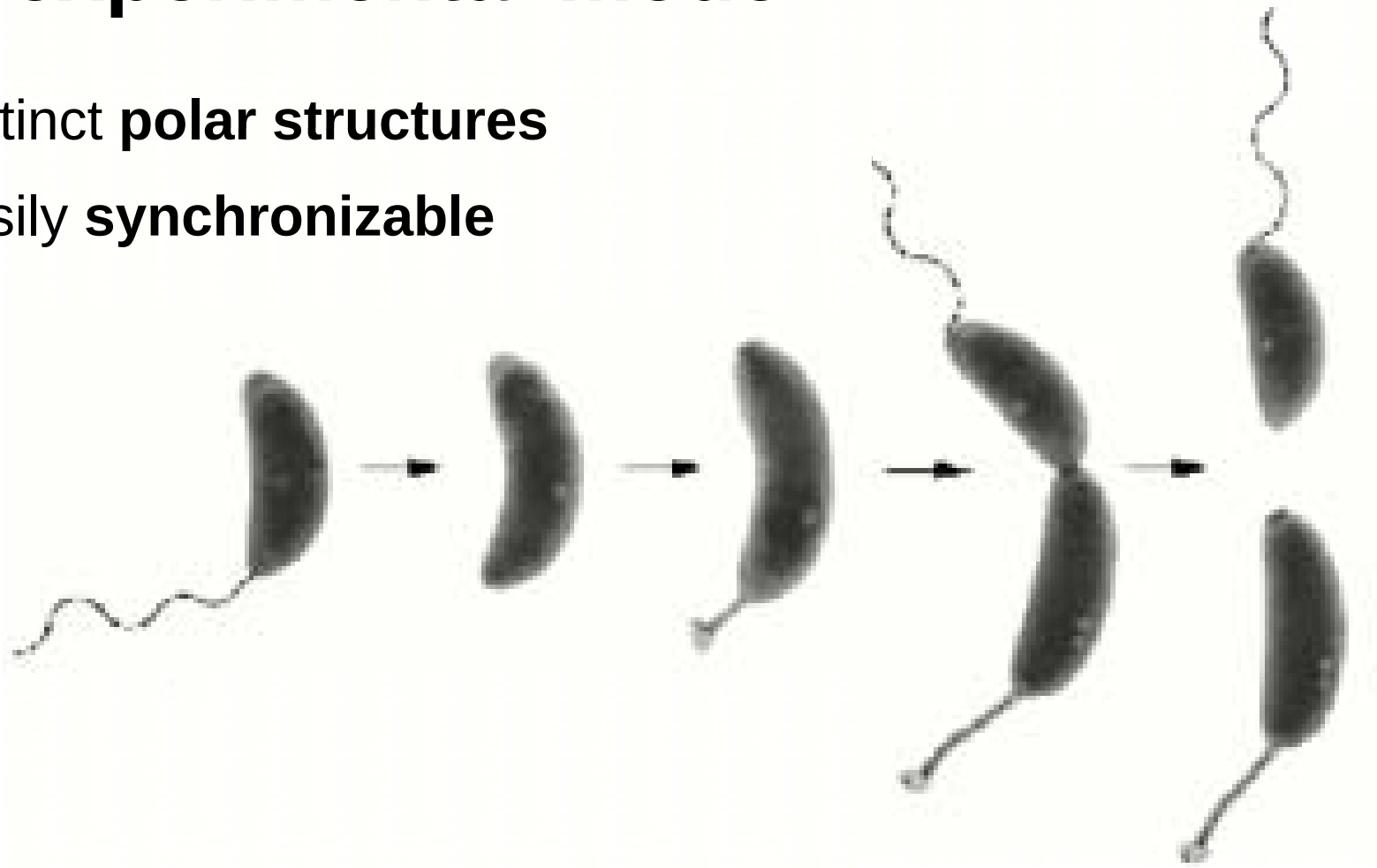

Strategies for survival:
Fruiting bodies
Myxococcus xanthus

Caulobacter's strategy

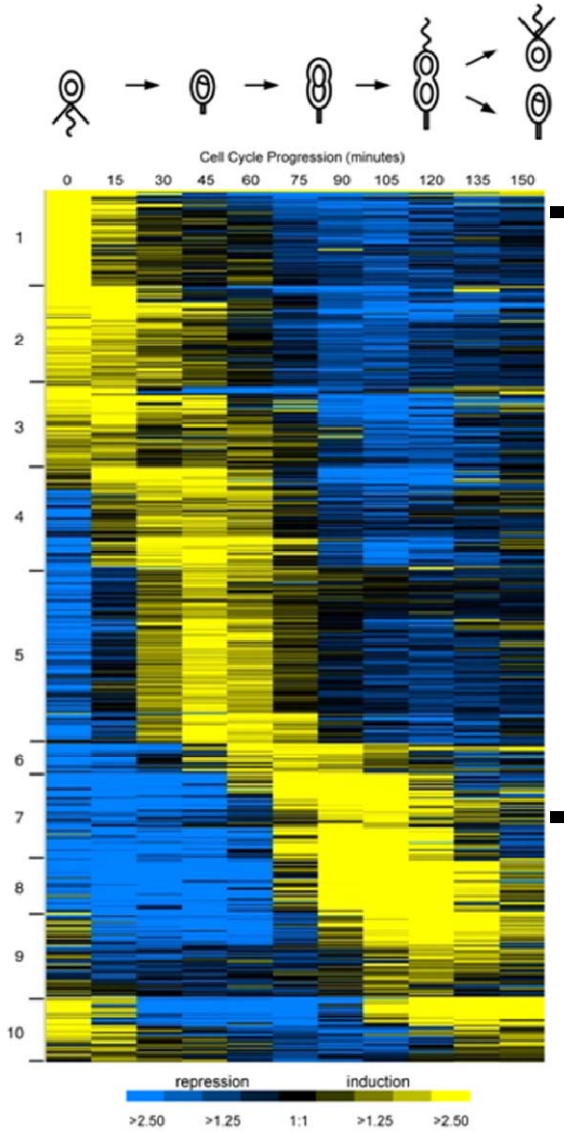


Advantages of *Caulobacter* as **experimental model**

- Distinct **polar structures**
- Easily **synchronizable**



mRNA levels of 14% of *Caulobacter* genes vary **as a function of the cell cycle**



Temporal variations in mRNA levels

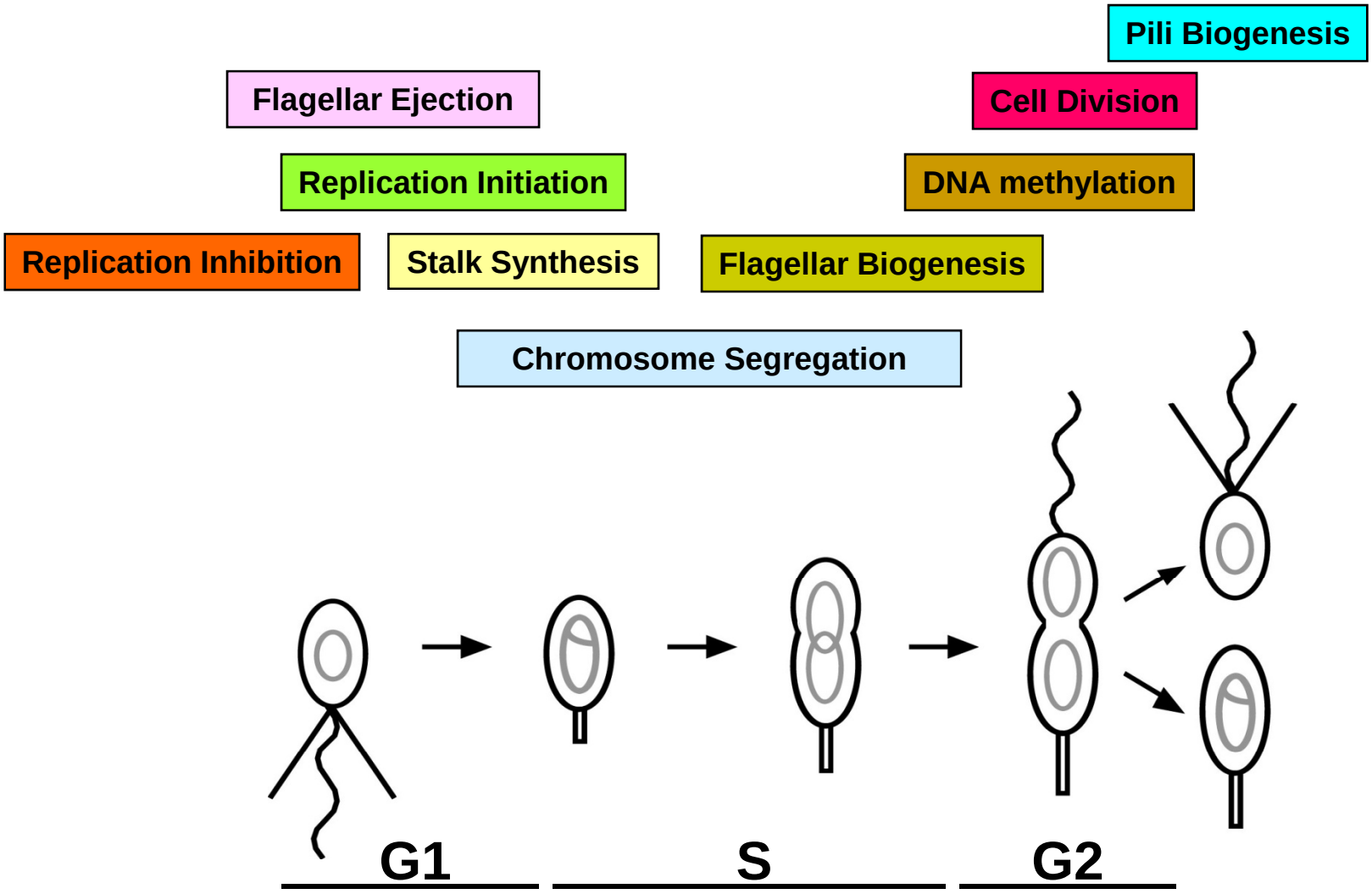
Temporal regulation of functions

dnaA → Replication Initiation

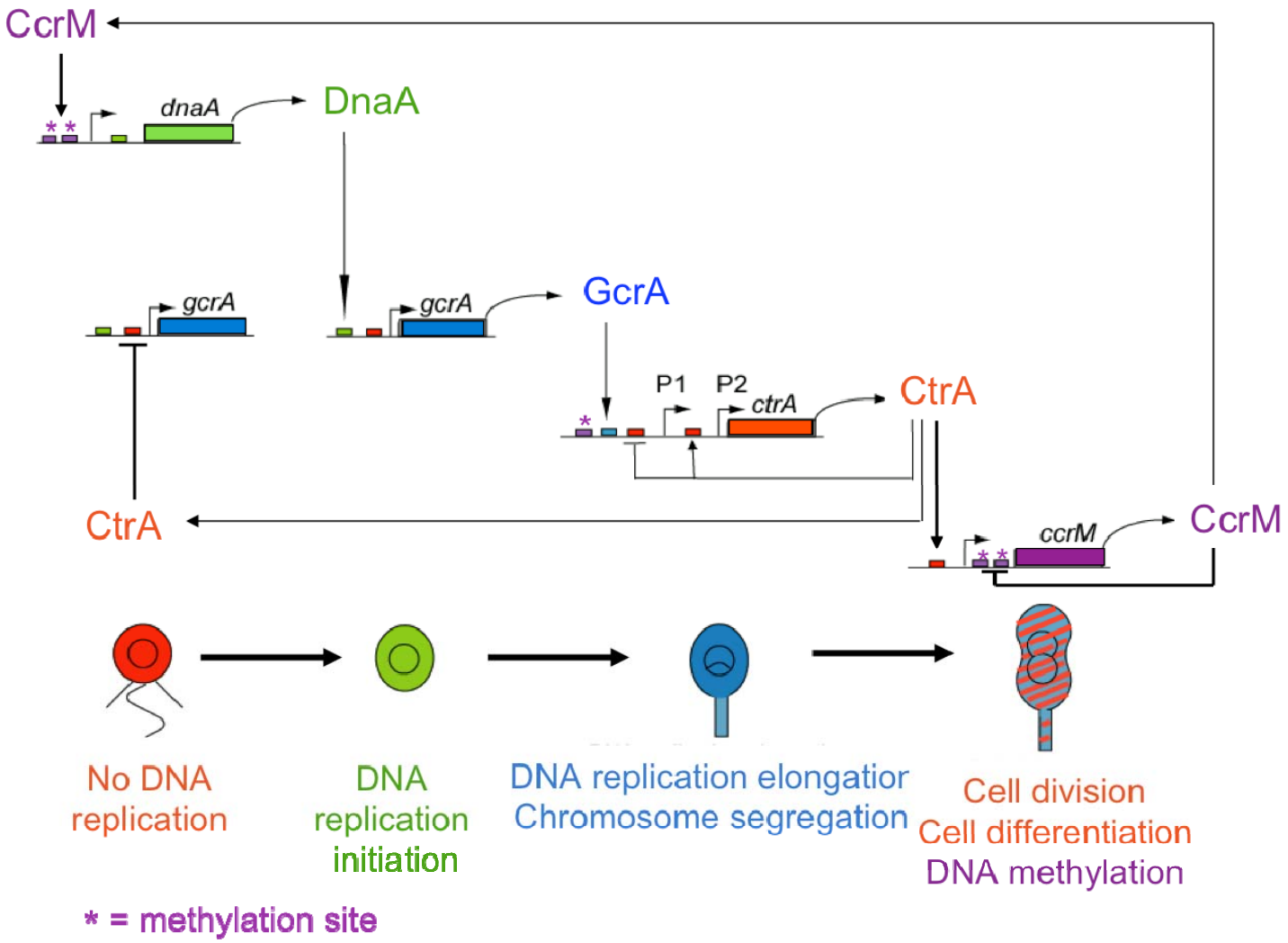
→ Flagellar Biogenesis

Laub *et al.* (2000)

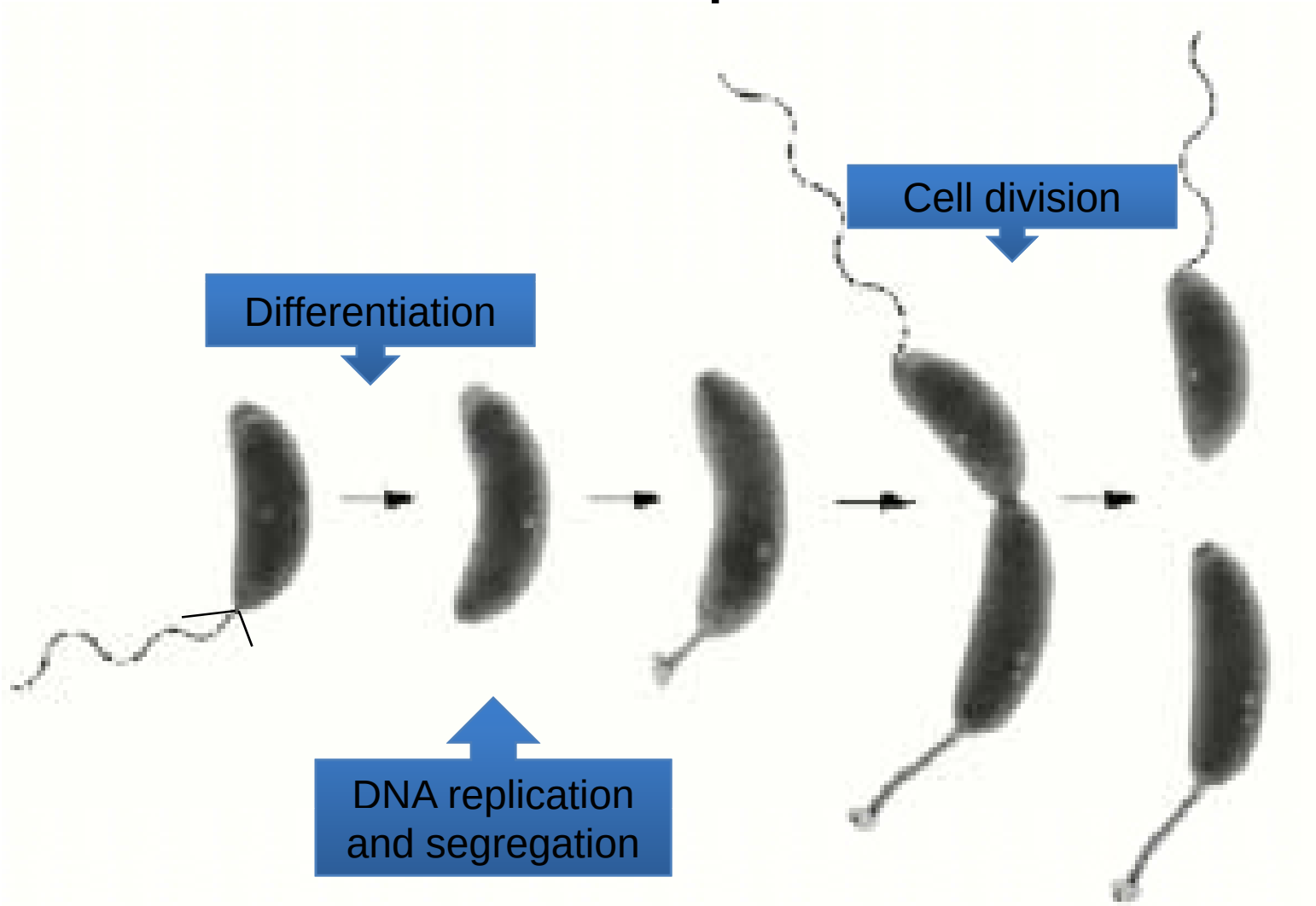
Cell cycle-regulated genes can be grouped in **functional modules**



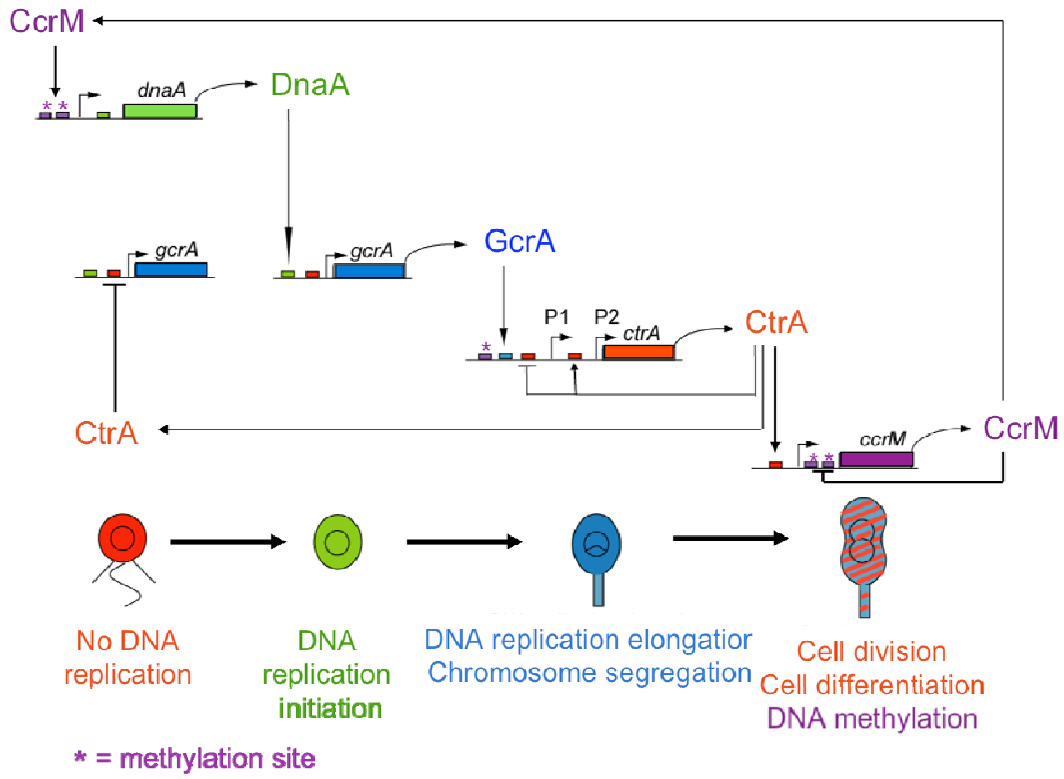
Caulobacter's cell cycle is driven by a **circuit of master regulators**



Possible arrest points upon stress or nutrient deprivation



How do cells **rely information about the environment** to the regulatory circuit that drives the cell cycle?



Visualization and analysis of microarrays and proteomics experiments

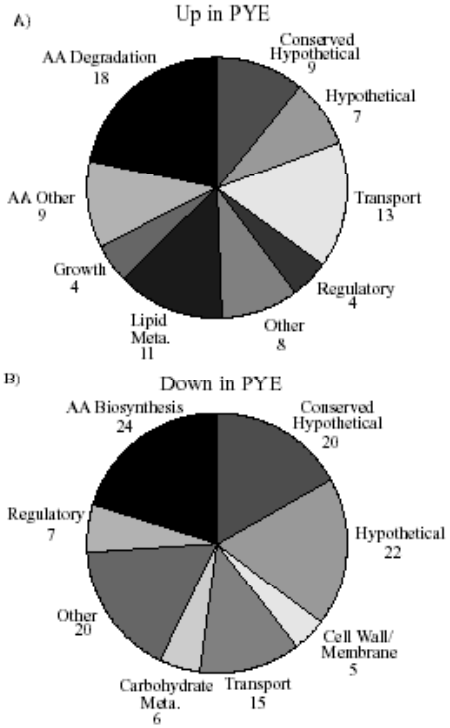
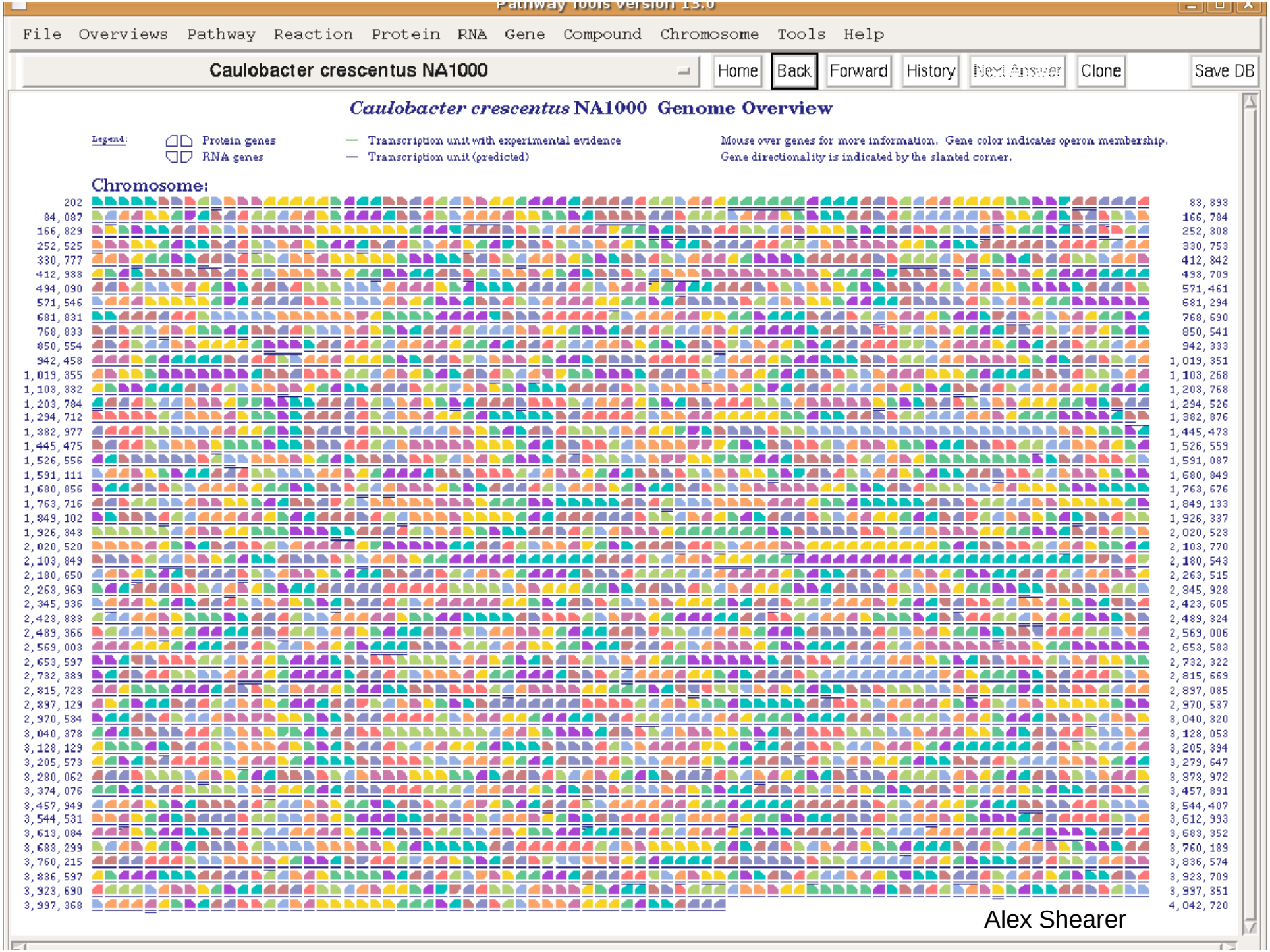


TABLE 1. Genes involved in amino acid biosynthesis or degradation that show differential expression between PYE and M2 minimal media

Pathway or gene	Relative mRNA ratio	
	PYE/M2G	PYE/M2X
Alanine degradation		
CC3574, alanine dehydrogenase, *ald*	8.64	5.32
Arginine degradation		
CC0581, arginine *N*-succinyltransferase, *astA*	3.11	3.41
CC1606, arginine *N*-succinyltransferase, *astA*	2.96	3.26
CC1607, succinylglutamic semialdehyde dehydrogenase, *astD*	2.56	2.63
CC1608, succinylarginine dihydrolase, *astB*	2.80	3.03
Arginine and glutamate degradation		
CC0584, succinylornithine transaminase, putative	2.89	3.50
Aromatic amino acid and histidine synthesis		
CC2300, phospho-2-dehydro-3-deoxyheptonate aldolase, *aroG*	0.34	0.33
CC2534, histidinol-phosphate aminotransferase, *hisC*	3.99	3.02
Aromatic amino acid synthesis		
CC1116, chorismate mutase, putative	1.55	1.78
CC2222, chorismate mutase, putative	0.52	0.52
CC2223, histidinol-phosphate aminotransferase, *hisC*	0.67	0.69
Glutamate degradation		
CC0088, NAD-specific glutamate dehydrogenase[a]	3.35	3.25
Glycine degradation		
CC3352, glycine cleavage system P protein, subunit 2, *gcvP*	3.56	4.10
CC3353, glycine cleavage system P protein, subunit 1, *gcvP*	4.34	5.27
CC3354, glycine cleavage system H protein, *gcvH*	4.76	5.32
CC3355, glycine cleavage system T protein, *gcvT*	4.36	5.09
Histidine degradation		
CC0957, urocanate hydratase, *hutU*	3.67	3.56
CC0958, formiminoglutamase, *hutG*	3.90	4.22
CC0959, histidine ammonia-lyase, *hutH*	3.54	4.80
CC0960, imidazolonepropionase, *hutI*	2.76	2.49
Incorporation of ammonia into glutamate		
CC1969, glutamine synthetase, class I, *glnA*	0.36	0.52
CC3606, glutamate synthase, small subunit, *gltD*	0.39	0.33
CC3607, glutamate synthase, large subunit, *gltB*	0.25	0.21
Isoleucine and valine synthesis		
CC2100, acetolactate synthase, large subunit, *ilvB*	0.21	0.20
CC2120, ketol-acid reductoisomerase, *ilvC*	0.39	0.40
Leucine synthesis		
CC0193, 3-isopropylmalate dehydrogenase, *leuB*	0.31	0.32
CC0195, 3-isopropylmalate dehydratase, small subunit, *leuD*	0.28	0.32
CC0196, 3-isopropylmalate dehydratase, large subunit, *leuC*	0.28	0.28
CC1541, 2-isopropylmalate synthase, *leuA*	0.23	0.21
Methionine synthesis		
CC0050, *S*-adenosylmethionine synthetase	0.56	0.58
CC0482, 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, *metF*	0.23	0.24
CC2138, 5-methyltetrahydrofolate-homocysteine methyltransferase	0.29	0.29
Phenylalanine degradation		
CC2533, 4-hydroxyphenylpyruvate dioxygenase, *hpd*	3.82	3.22
Proline degradation		
CC0804 proline dehydrogenase/delta-1-pyrroline-5-carboxylate dehydrogenase, *putA*	3.74	3.78
Serine synthesis		
CC3215, D-3-phosphoglycerate dehydrogenase, *serA*	0.23	0.22
CC3216, phosphoserine aminotransferase, *serC*	0.27	0.24
Sulfate acquisition and cysteine synthesis		
CC1119, sulfite reductase (NADPH) hemoprotein, *cysI*	0.21	0.21
CC1121, phosphoadenylylsulfate reductase, *cysH*	0.27	0.26
CC1426, cysteine synthase, *cysB*	0.69	0.63
CC1482, sulfate adenylate transferase, subunit 1/ adenylylsulfate kinase, *cysN/C*	0.20	0.19
CC1483, sulfate adenylate transferase, subunit 2, *cysD*	0.27	0.25
CC1596, sulfate ABC transporter, permease protein, *cysT*	0.33	0.34
CC1597, sulfate ABC transporter, permease protein, *cysW*	0.39	0.49
CC1598, sulfate ABC transporter, ATP-binding protein, *cysA*	0.34	0.40
CC3625, cysteine synthase, *cysK*	0.39	0.46
Threonine synthesis		
CC3399, threonine synthase, *thrC*	0.41	0.39
Valine degradation		
CC2274, methylmalonate-semialdehyde dehydrogenase, putative	3.08	2.80

[a] Annotation from COG annotations (57, 58). All other annotations came from GenBank (45).


Pathway Tools version 13.0
File Overviews Pathway Reaction Protein RNA Gene Compound Chromosome Tools Help
Caulobacter crescentus NA1000
Home
Back
Forward
History
Next Answer
Clone
Save DB
Caulobacter crescentus NA1000 Genome Overview
Legend:
Protein genes
RNA genes
Transcription unit with experimental evidence
Transcription unit (predicted)
Mouse over genes for more information. Gene color indicates operon membership.
Gene directionality is indicated by the slanted corner.
Chromosome:
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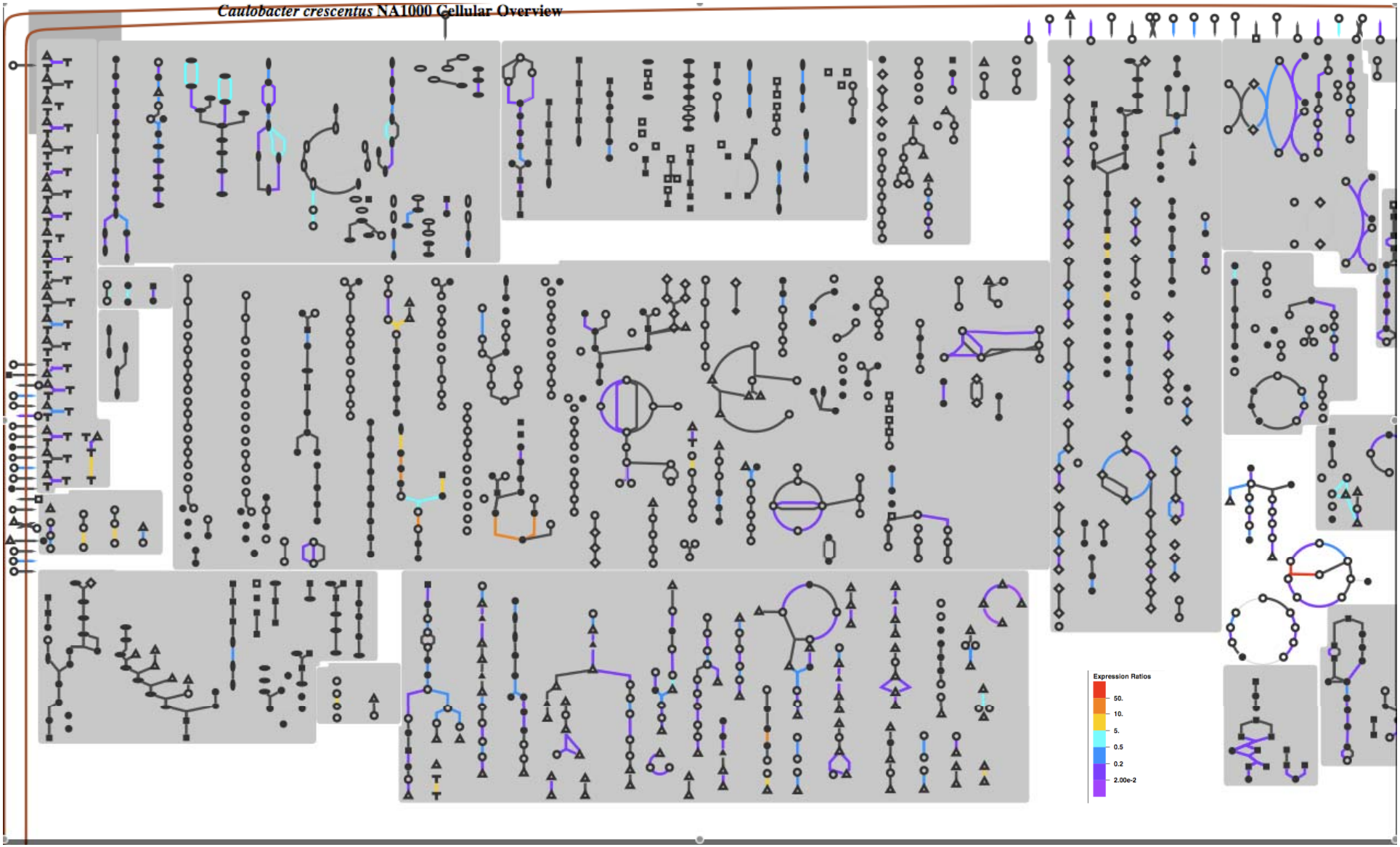
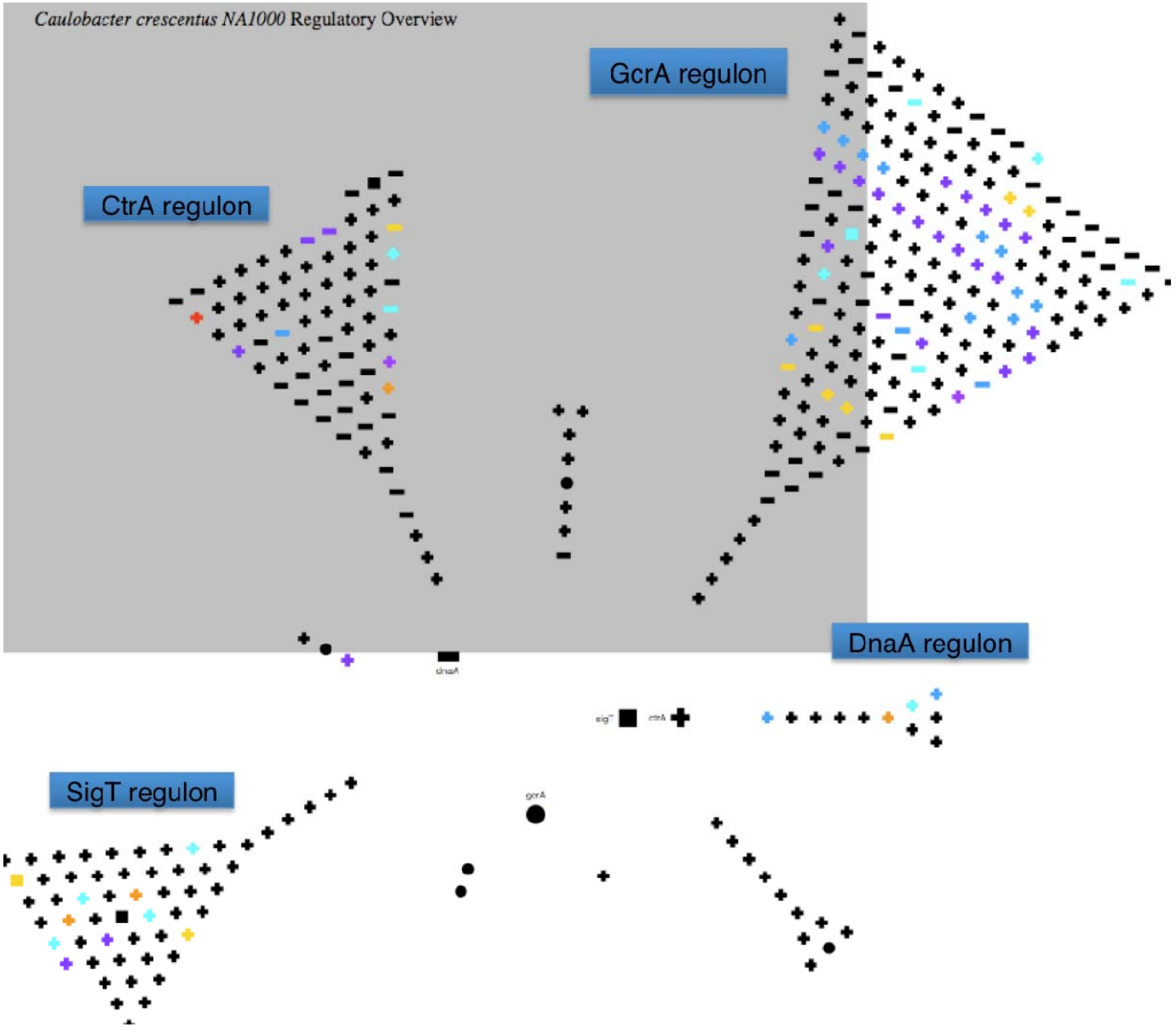
Alex Shearer

Cellular Overview

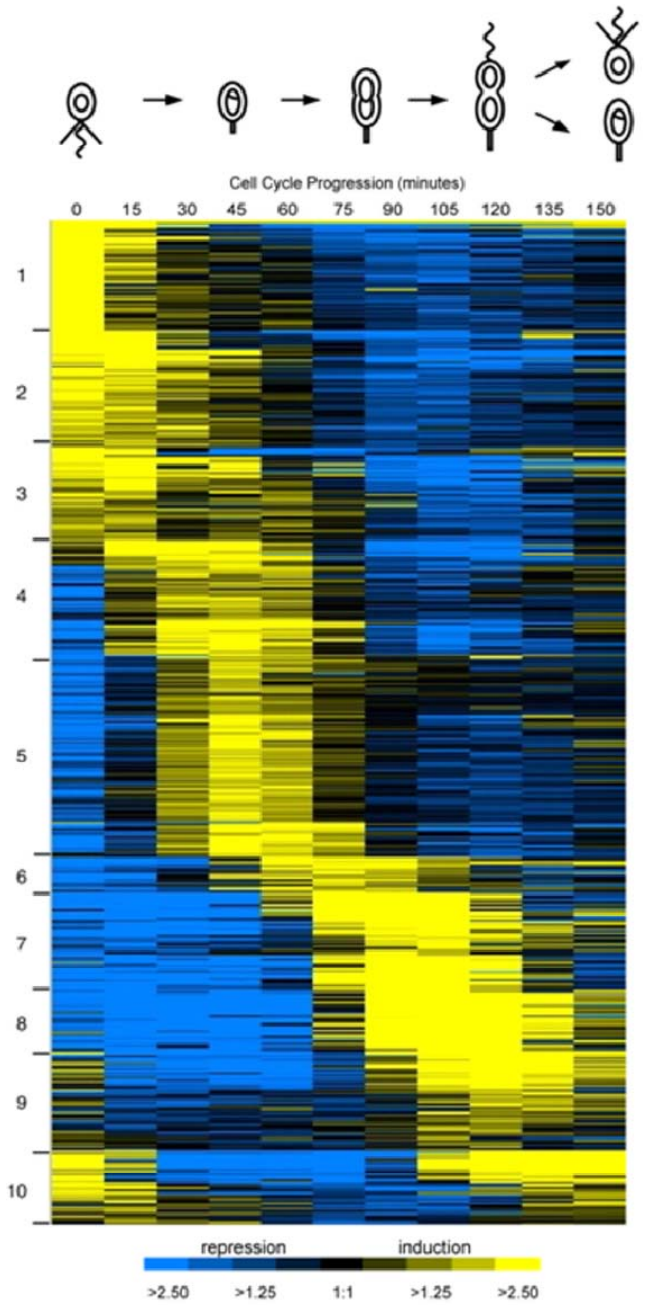




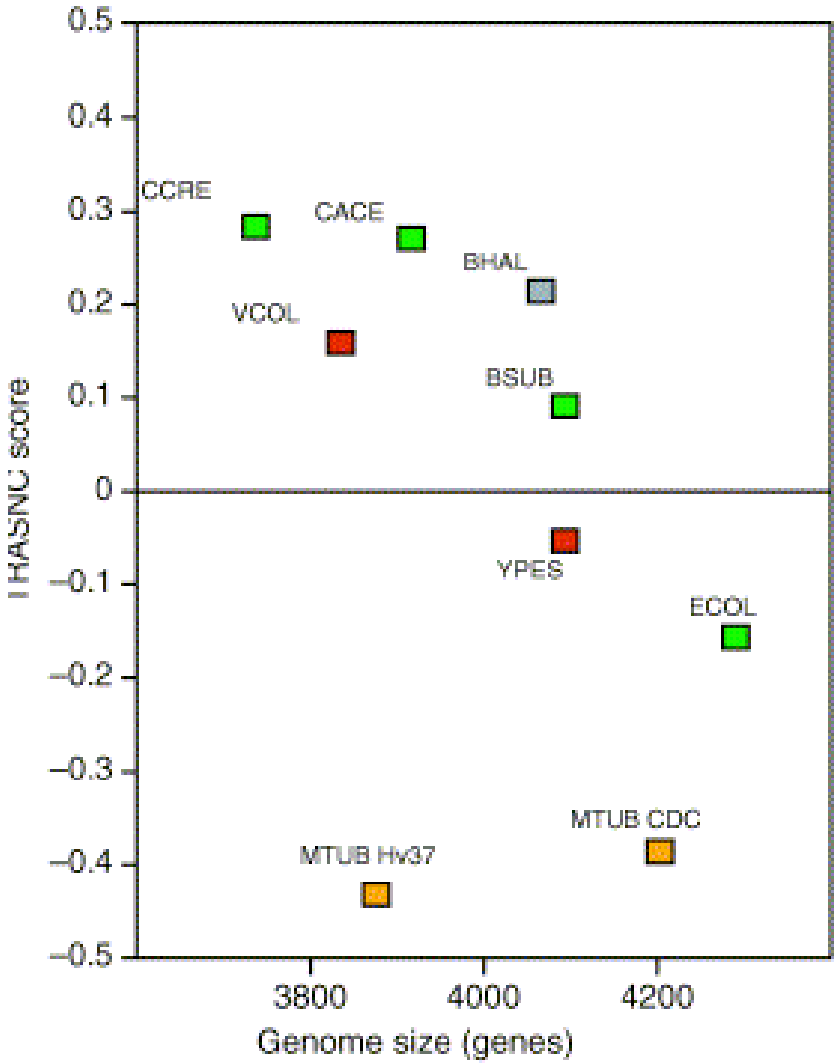
Regulatory Overview

Act II

Exploring *Caulobacter*'s transcriptional landscape



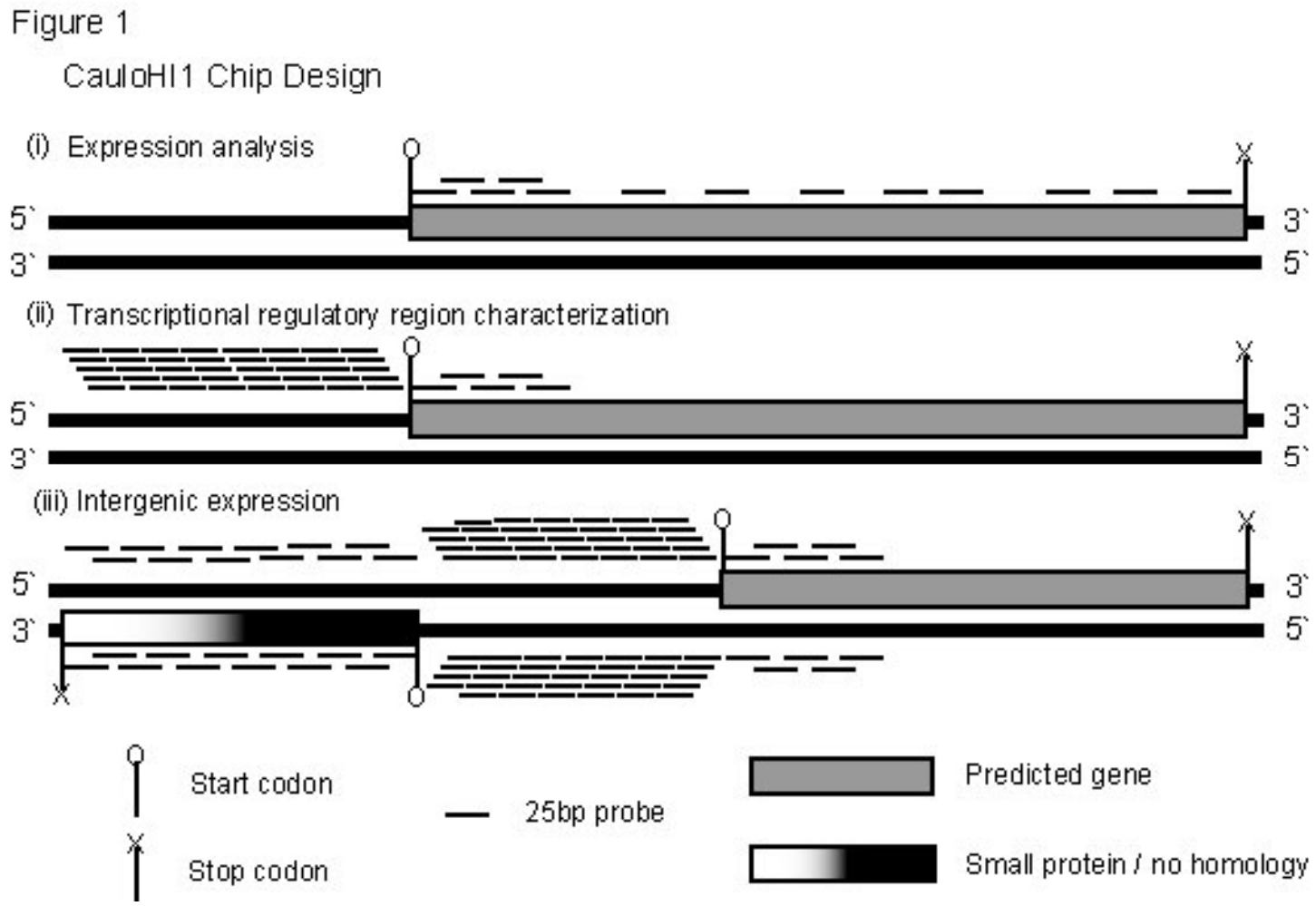
Caulobacter relies heavily on **transcriptional regulation**



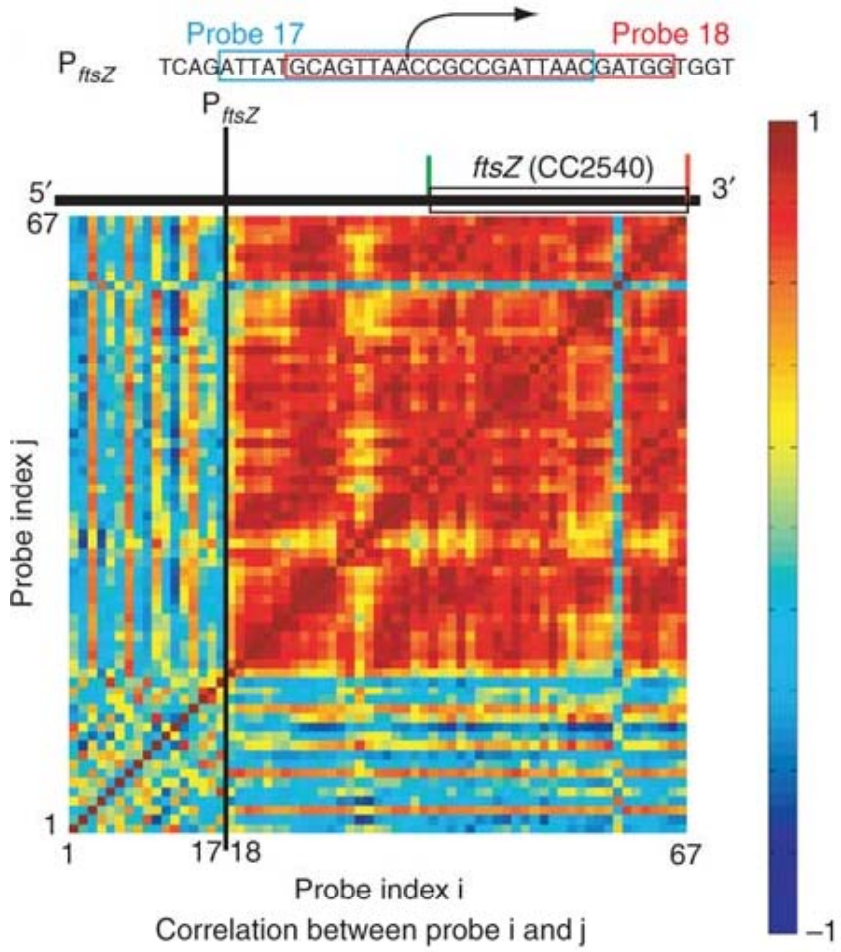
Caulobacter relies heavily on **transcriptional regulation**

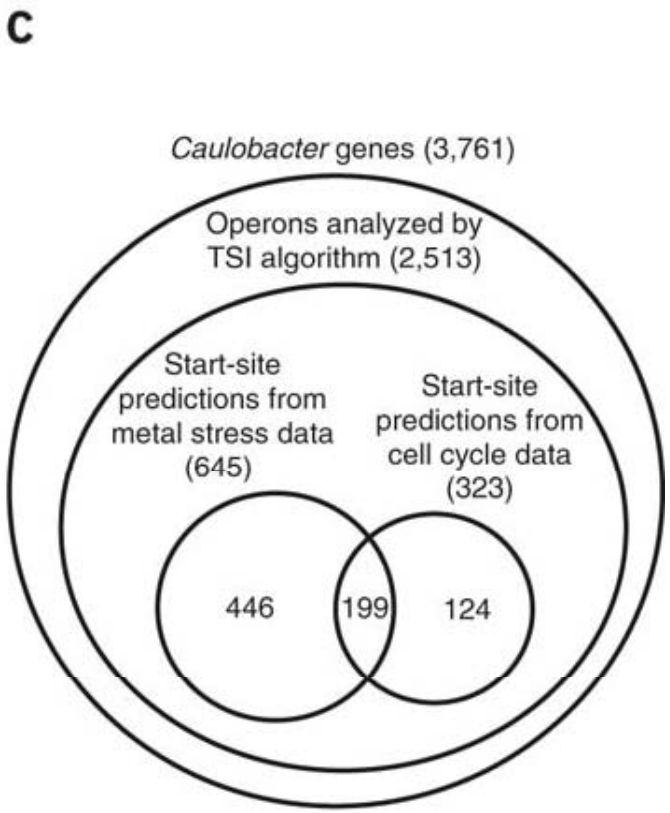
Cases *et al.* (2003)

High density **transcriptional mapping** of *Caulobacter*'s genome



Application: Identification of **transcriptional start sites**



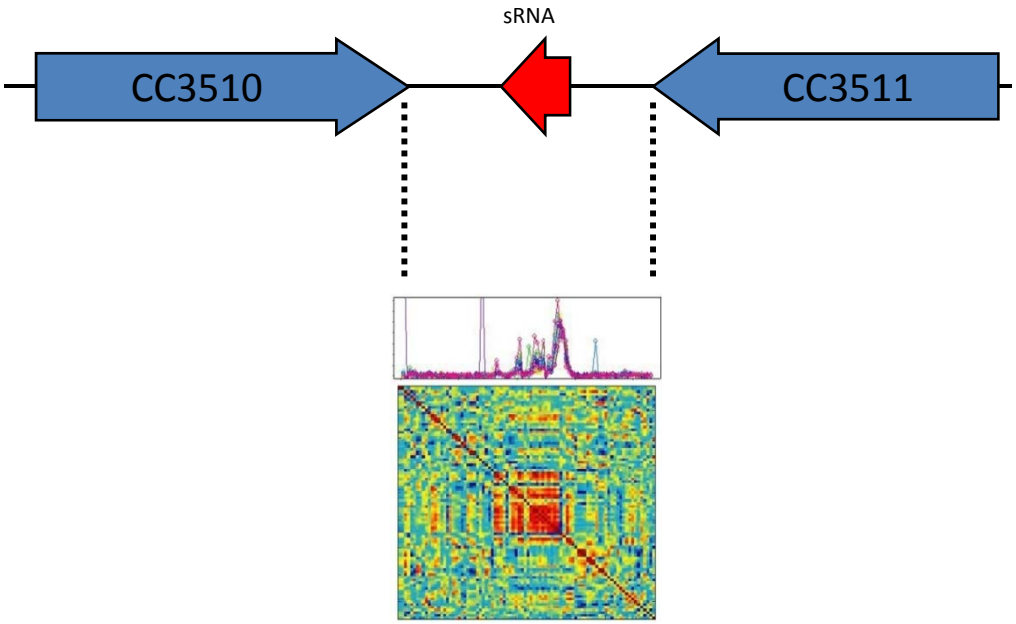


McGrath *et al.* (2007)

Application: Identification of **small RNAs**

Found and verified 27 novel small RNAs

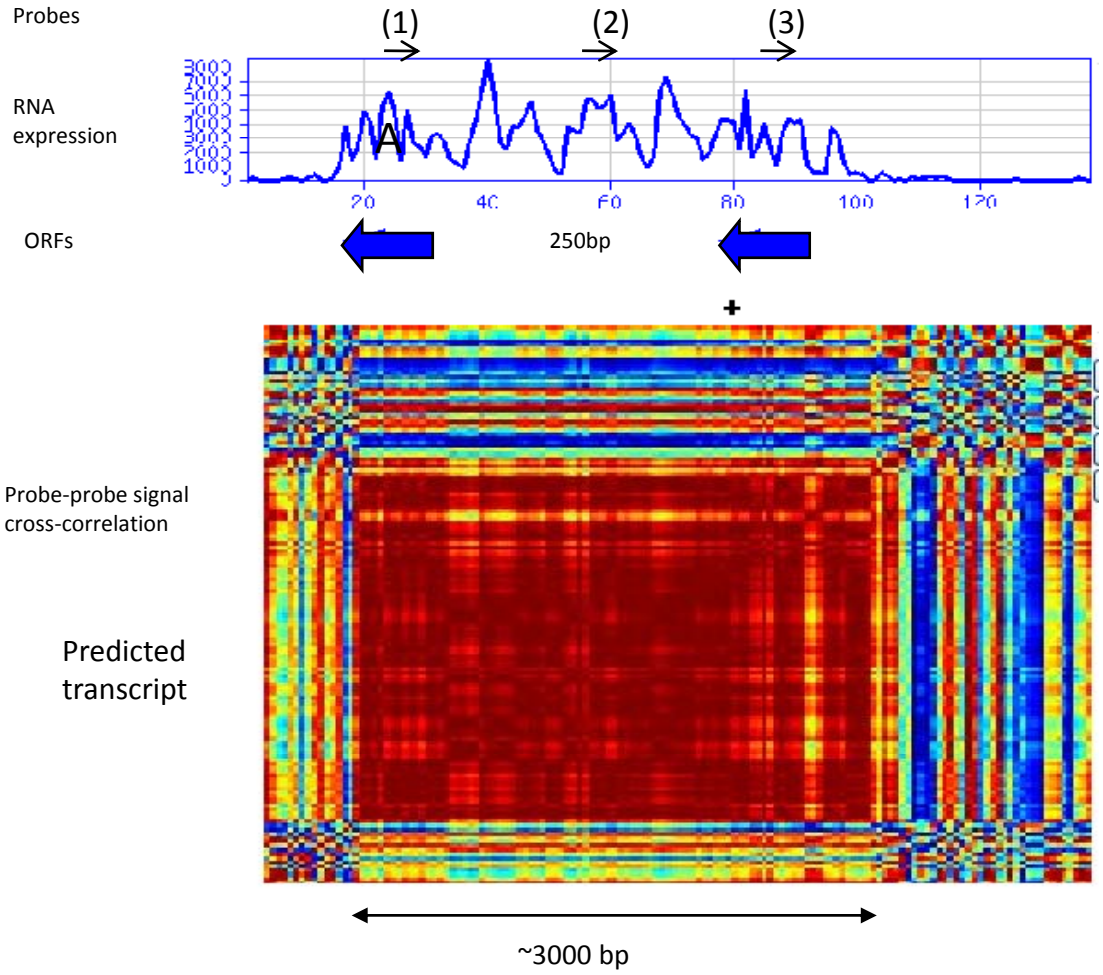
One example

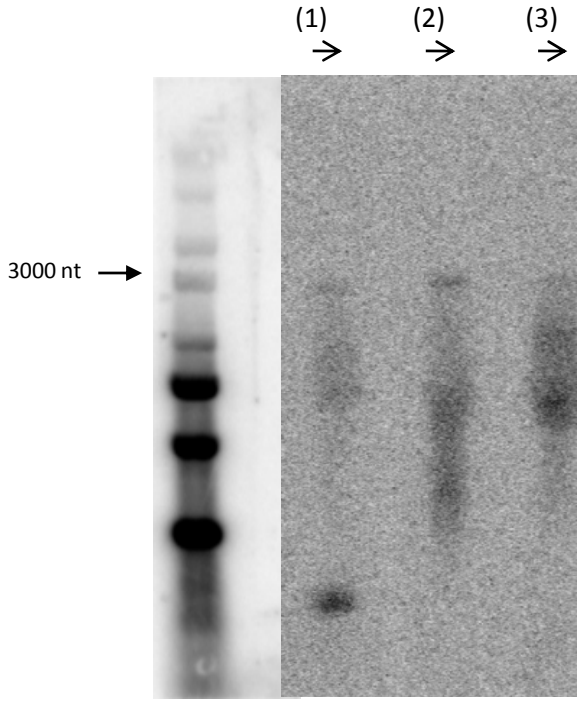


- **Activated by starvation**
- **Stops the cell-cycle**

Landt *et al.* (2008)

Application: **operon** mapping





Eduardo Abeliuk
(unpublished)

Genome Expression Browser

•Web-based browser that shows the probe expression correlations, multiple ORF annotations, mRNA cell cycle expression profiles, and other genomic features together on one display.

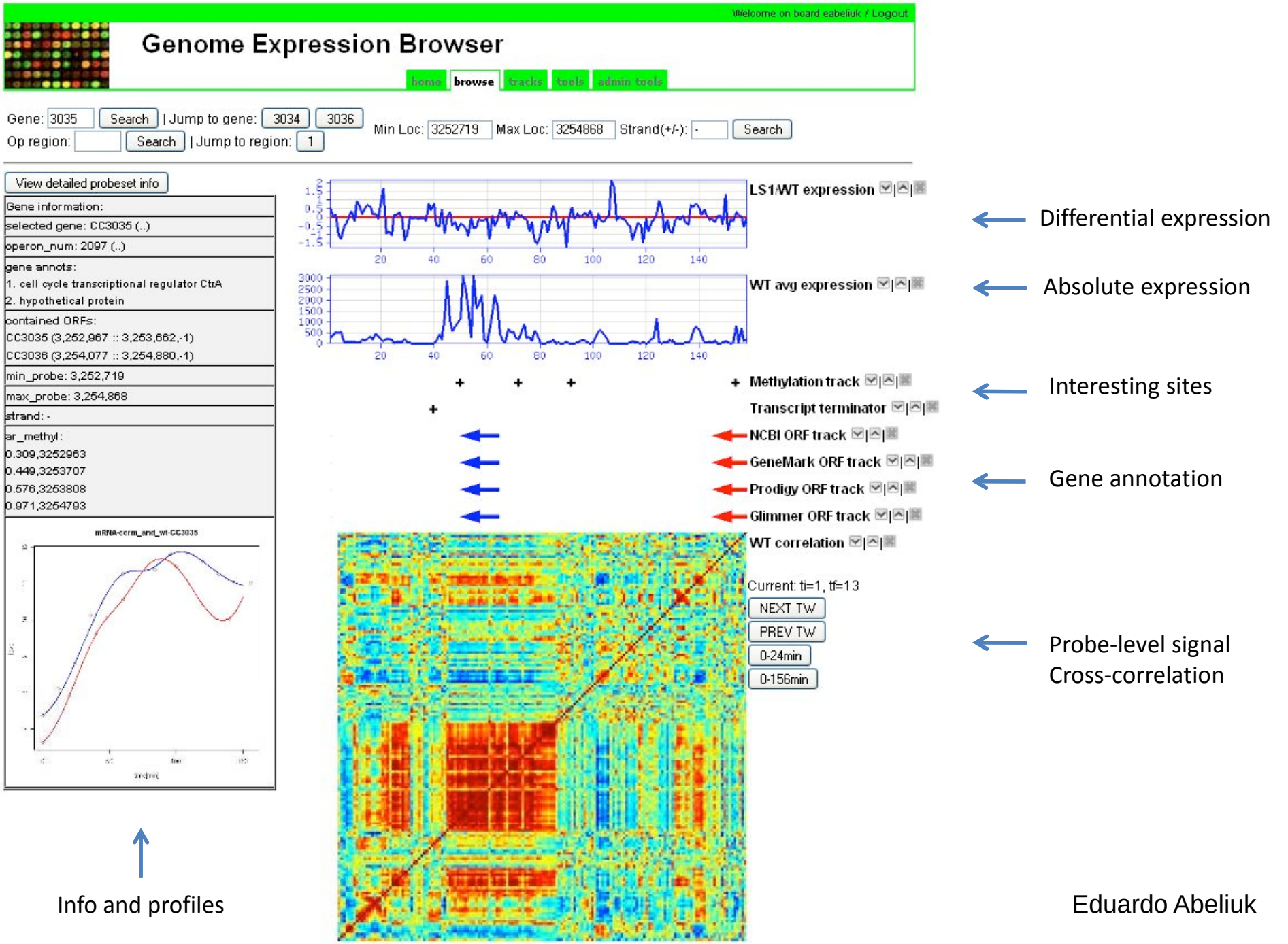
•The Genome Expression Browser can be used to visually scan an arbitrary region of the genome, and inspect interesting correlations present among different microarray experiments or genomic features.

•Well suited for integrating data from Affy high-density tiling arrays in the backend

•Currently contains Caulohi1 (*Caulobacter*) affy chip data. Other species coming soon.

•The Genome Expression Browser is in closed beta.

•Contact: Eduardo Abeliuk (eabeliuk@stanford.edu). McAdams/Shapiro Lab.



Differential expression

Absolute expression

Interesting sites

Gene annotation

Probe-level signal
Cross-correlation

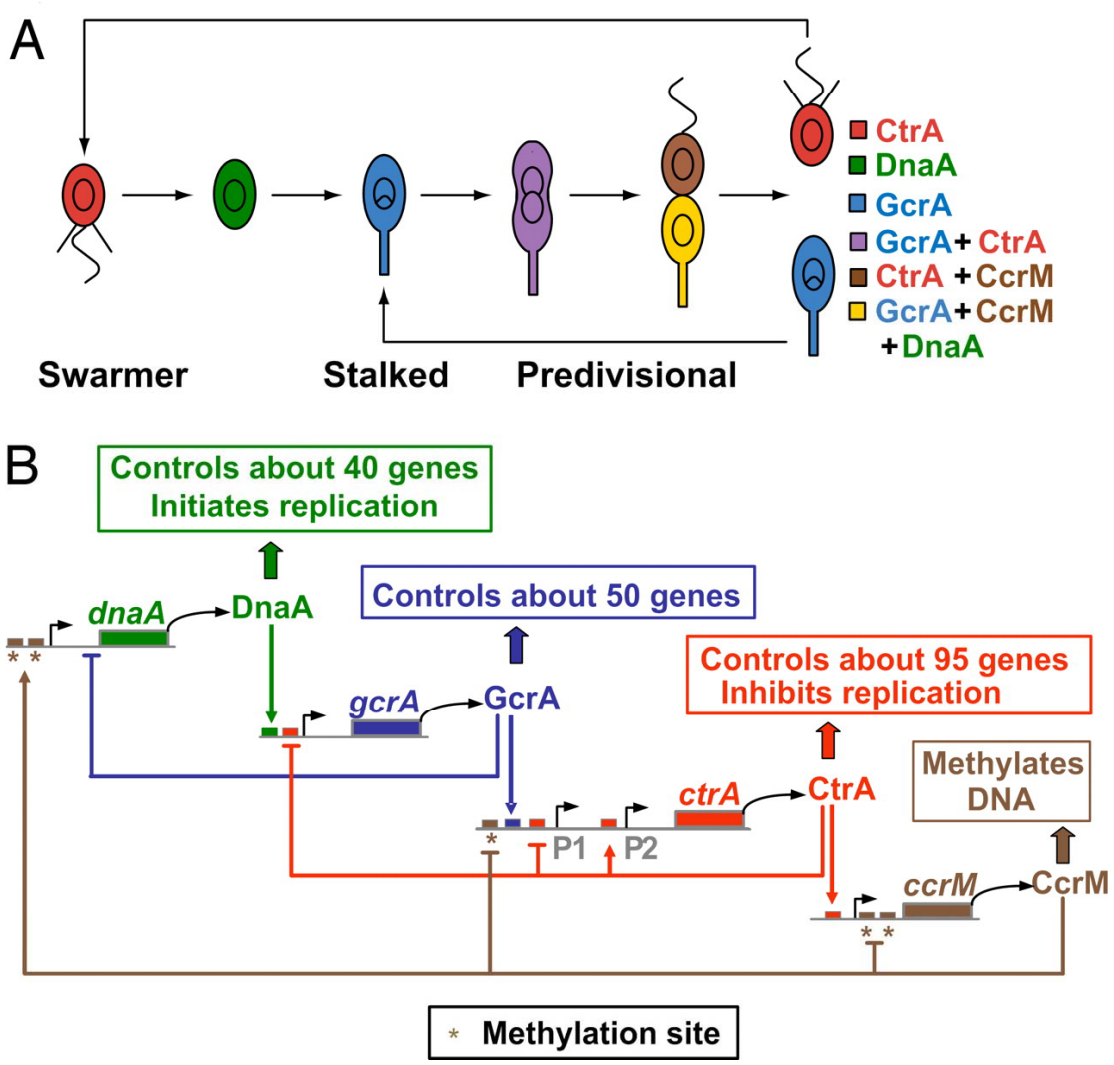
Info and profiles

Eduardo Abeliuk

Act III

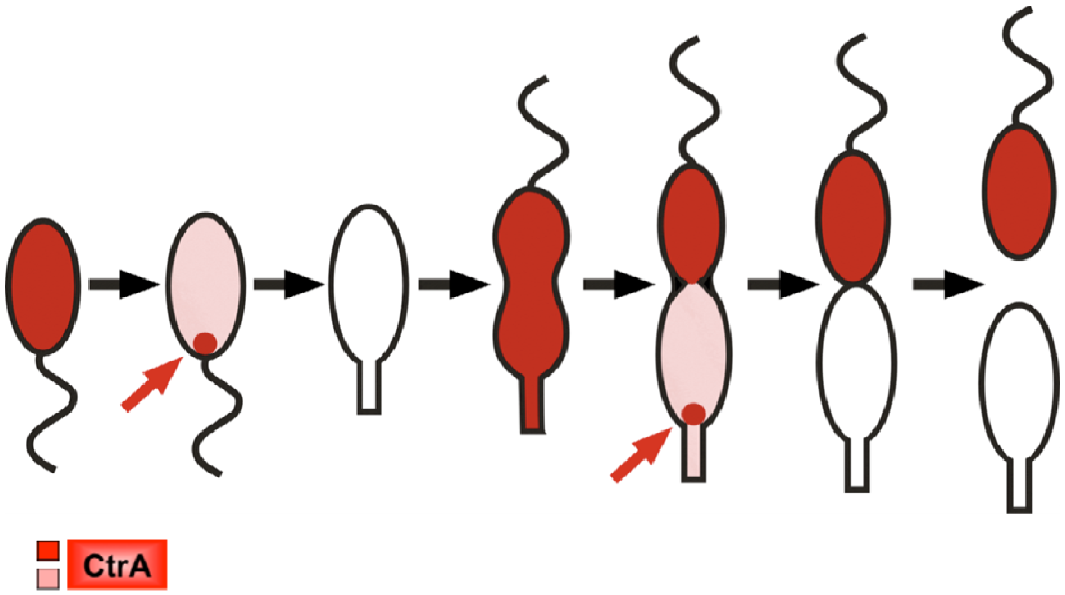
Location, location, location.

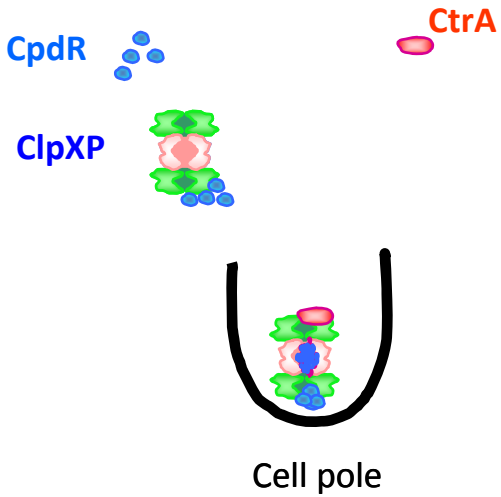
Caulobacter's cell cycle is driven by a **circuit of master regulators**



Collier *et al. (2007)*

The activity of the CtrA master regulator is controlled by **proteolysis**





Ryan *et al. (2004)*
Iniesta et al. (2006)
Iniesta et al. (2008)

Quantitative genome-scale analysis of protein localization in an asymmetric bacterium

John N. Werner, Eric Y. Chen, Jonathan M. Guberman, Angela R. Zippilli, Joseph J. Irgon, and Zemer Gitai[1]

Department of Molecular Biology, Princeton University, Princeton, NJ 08540

Edited by Lucy Shapiro, Stanford University School of Medicine, Stanford, CA, and approved March 13, 2009 (received for review February 18, 2009)

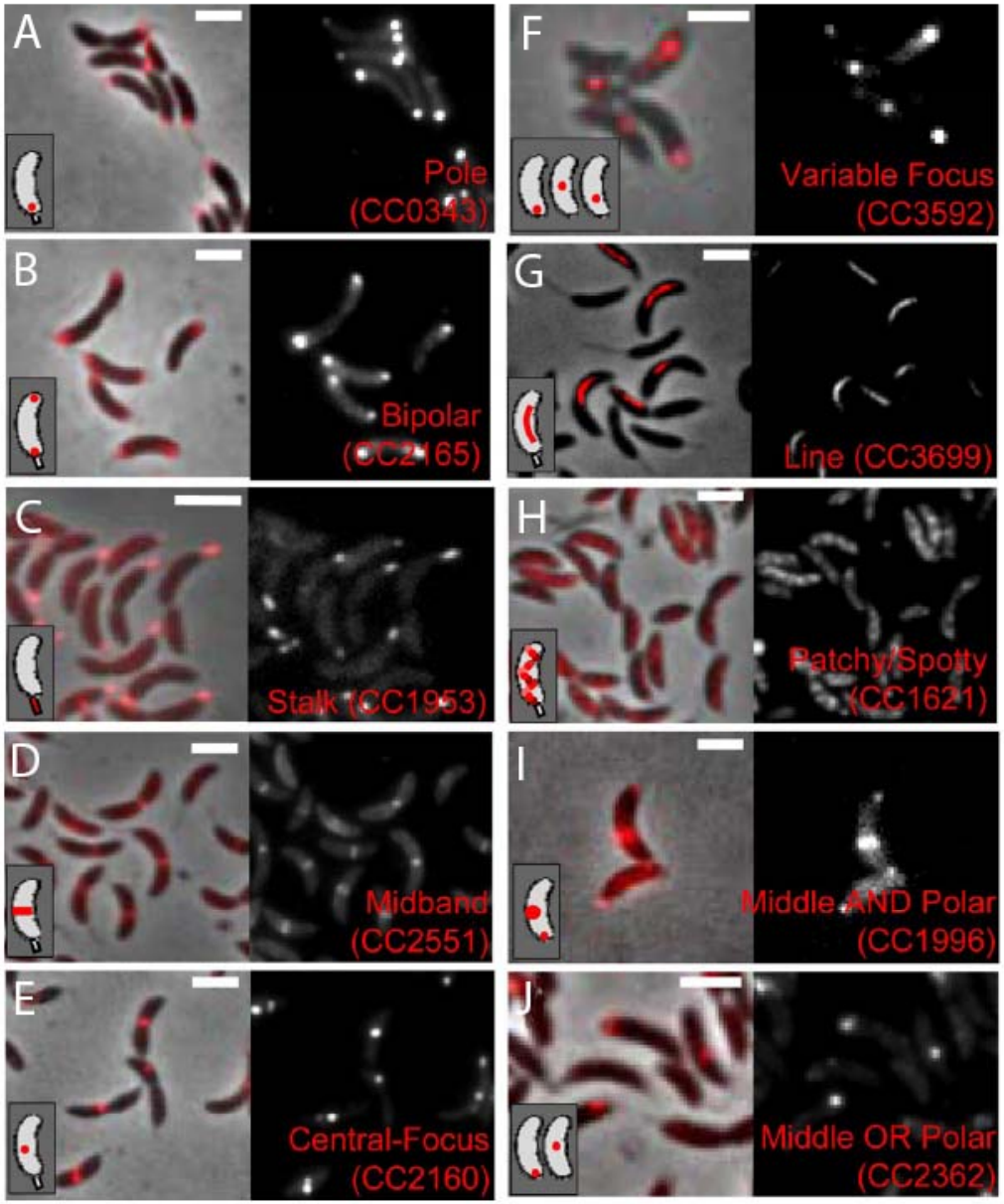
Despite the importance of subcellular localization for cellular activities, the lack of high-throughput, high-resolution imaging and quantitation methodologies has limited genomic localization analysis to a small number of archival studies focused on C-terminal fluorescent protein fusions. Here, we develop a high-throughput pipeline for generating, imaging, and quantitating fluorescent protein fusions that we use for the quantitative genomic assessment of the distributions of both N- and C-terminal fluorescent protein fusions. We identify nearly 300 localized *Caulobacter crescentus* proteins, up to 10-fold more than were previously characterized. The localized proteins tend to be involved in spatially or temporally dynamic processes and proteins that function together and often localize together as well. The distributions of the localized proteins were quantitated by using our recently described projected system of internal coordinates from interpolated contours (PSICIC) image analysis toolkit, leading to the identification of cellular regions that are over- or under-enriched in localized proteins and of potential differences in the mechanisms that target proteins to different subcellular destinations. The *Caulobacter* localizome data thus represent a resource for studying both global properties of protein localization and specific protein functions, whereas the localization analysis pipeline is a methodological resource that can be readily applied to other systems.

bacteria | *Caulobacter* | genomics | quantitative image analysis | high-throughput imaging

guished by the presence of a stalk that protrudes from only 1 pole. In addition, a number of important *Caulobacter* proteins have been shown to assume specific subcellular localizations (5). These proteins serve as positive controls for genomic studies and establish proof-of-principle examples that protein localization plays an important role in the regulation of this organism's biological activities. A recent transposon-mediated forward-genetic screen identified 11 additional localized proteins (6), but *Caulobacter* protein localization has yet to be systematically studied at a genomic scale.

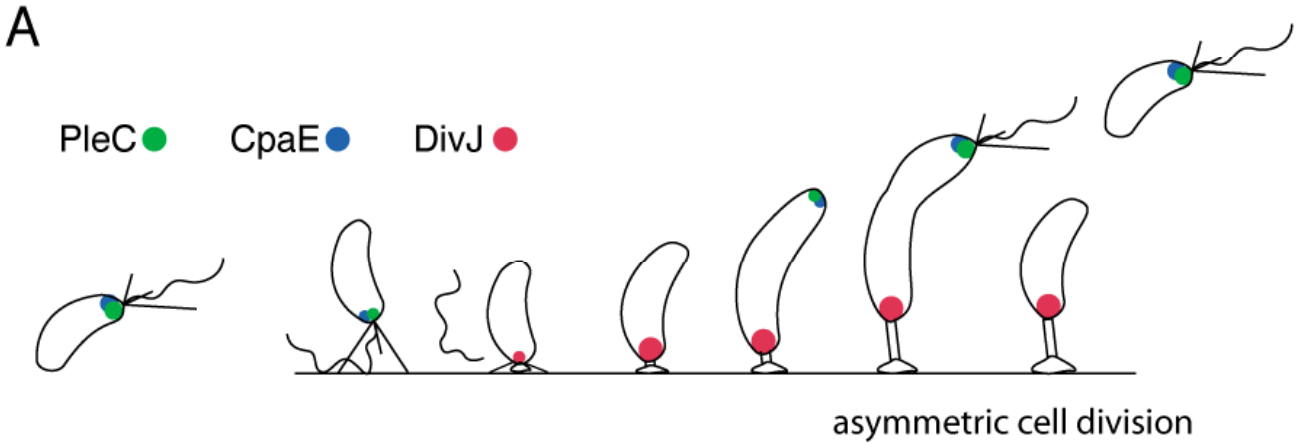
Here, we have begun to address the classical limitations of genomic localization analysis by developing a pipeline of high-throughput, high-resolution methods for generating, imaging, and analyzing fluorescent protein fusions. This approach enables the rapid, efficient, and repeated study of spatial processes on the scale of an entire genome and allowed us to reimage the localization of both N- and C- terminal mCherry fusions. The identification of 289 localized proteins represents a nearly 10-fold increase in the number of localized proteins in *Caulobacter*. By using a projected system of internal coordinates from interpolated contours (PSICIC), a recently developed software suite for automated image analysis (7), we quantitatively analyzed the accuracy and distributions of these localizations, leading to the appreciation of new aspects of *Caulobacter* proteome localization. These data thus enable the cell biological analysis of both individual proteins of interest and the general properties of the *Caulobacter* proteome.

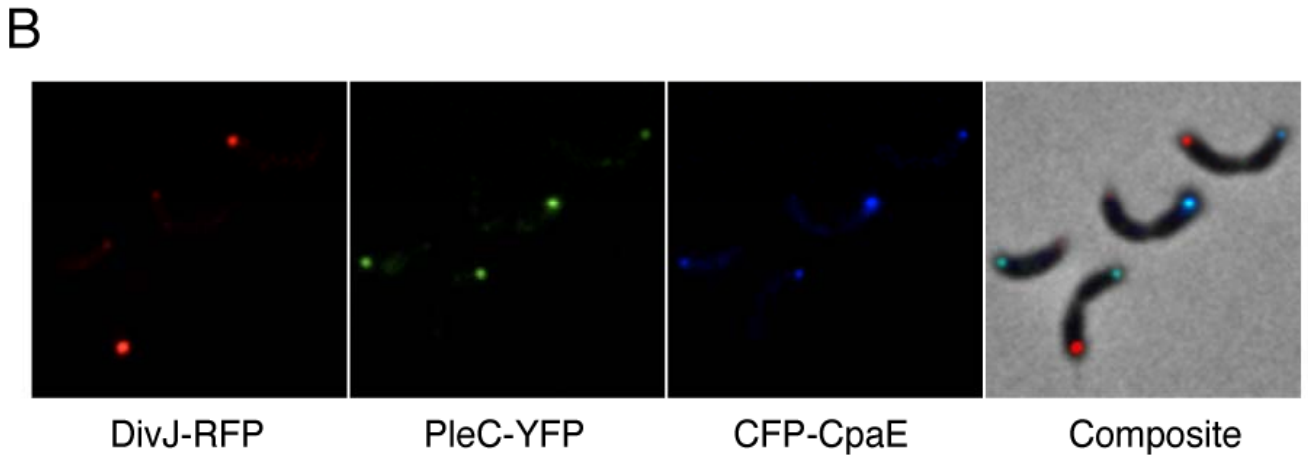
Werner *et al* (2009)



Werner *et al* (2009)

High-throughput screen for **protein localization determinants**





Beat Christen & Mike Fero
(in preparation)

High-throughput screen for **protein localization determinants**

Cell Finding

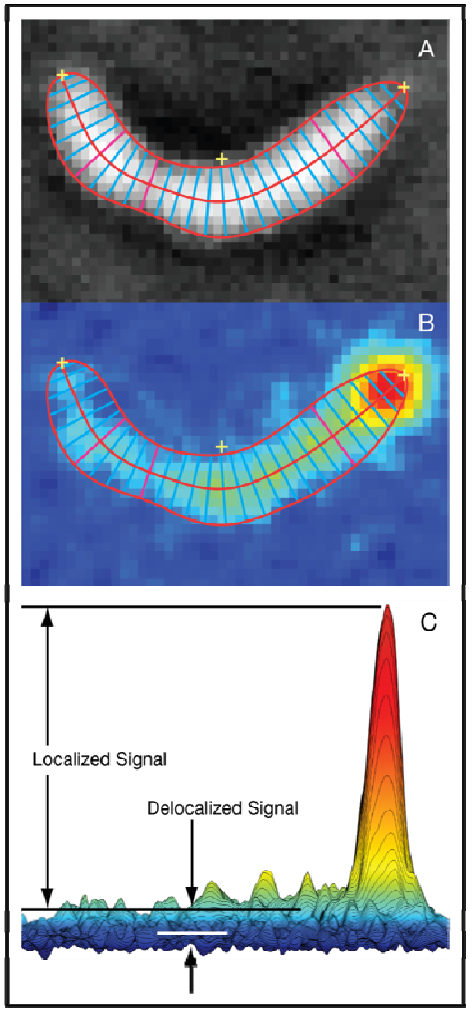
Cell Shape Parameters

Localized Fluor Signal Locations

Localized Fluor Signal Amplitudes

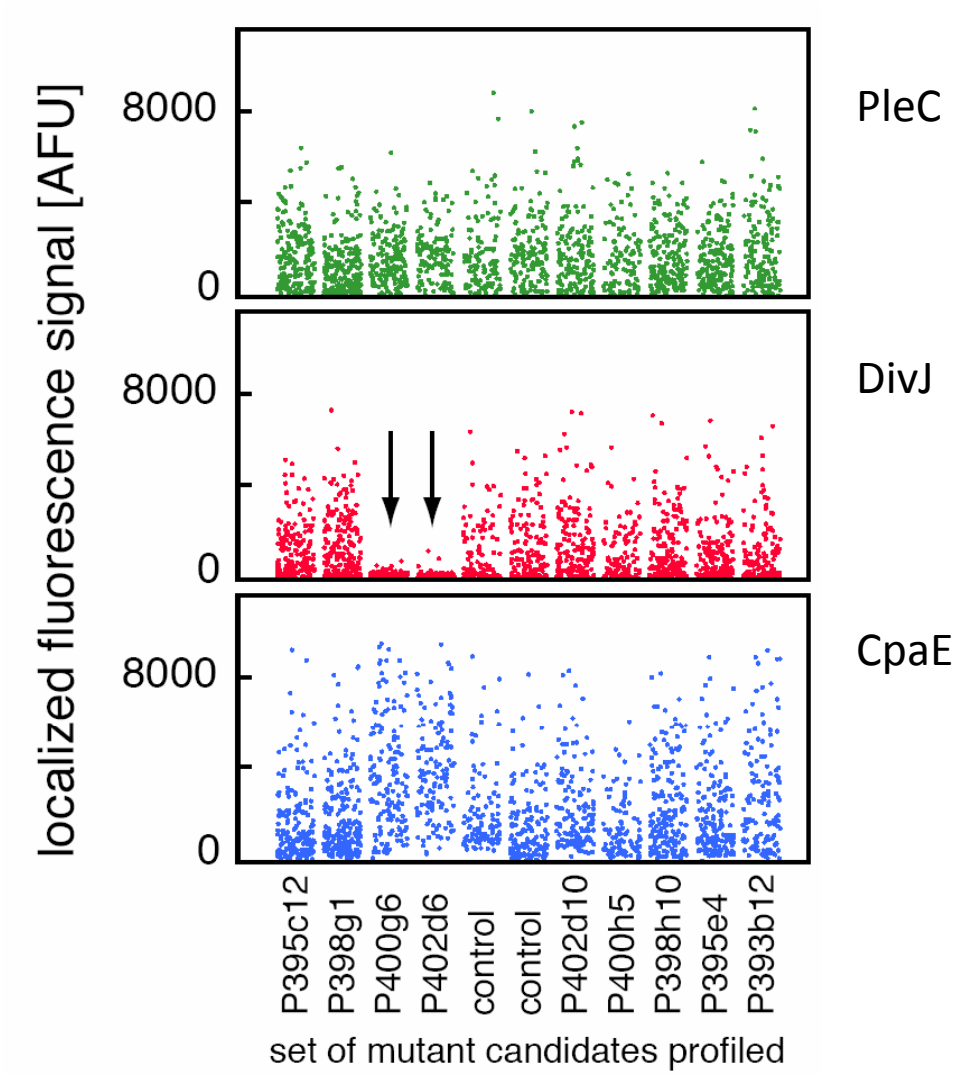
Delocalized Fluor Signal amplitude

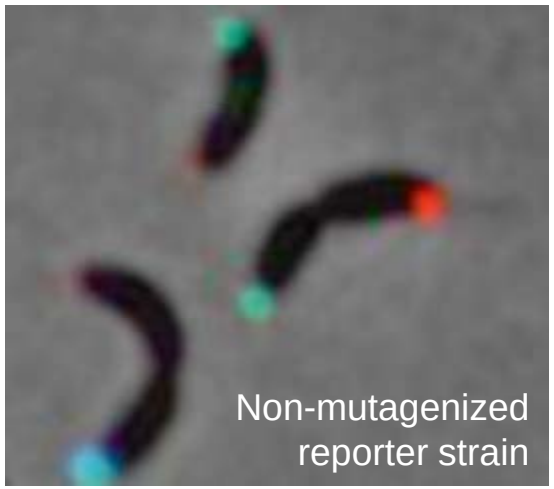
→ Summary Data Structure (no images)

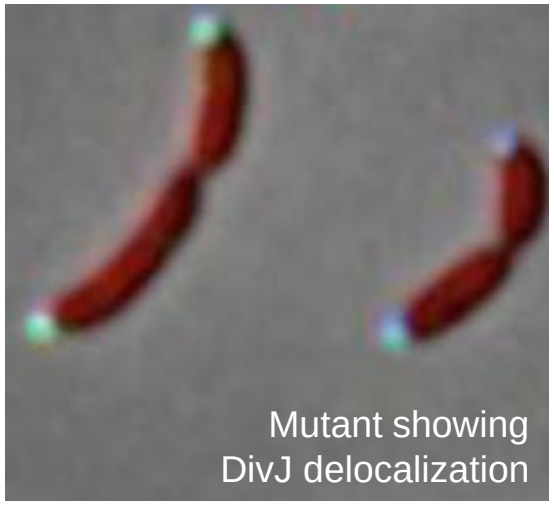


Beat Christen & Mike Fero
(in preparation)

High-throughput screen for **protein localization determinants**



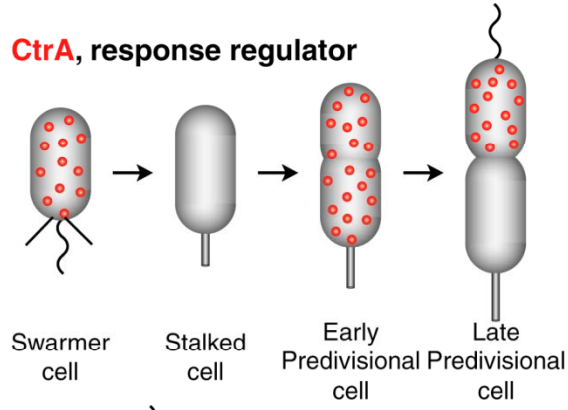




Beat Christen & Mike Fero
(in preparation)

Dynamic sub-cellular localization of prokaryotic signaling proteins

Compartmental



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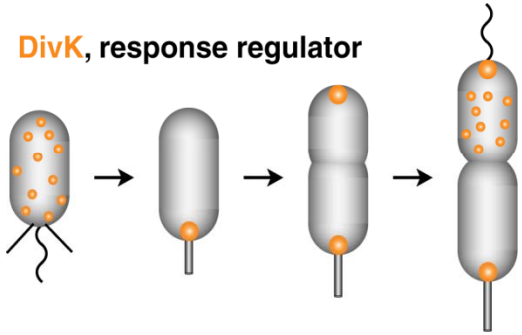

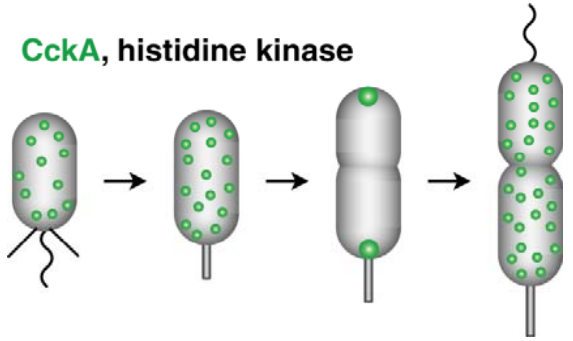

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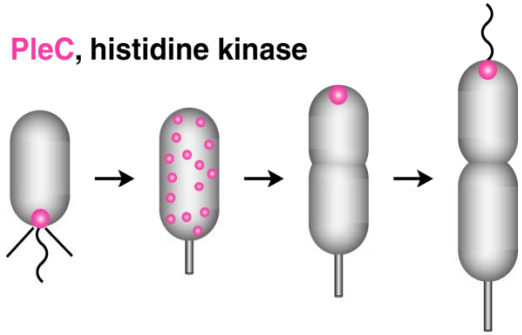
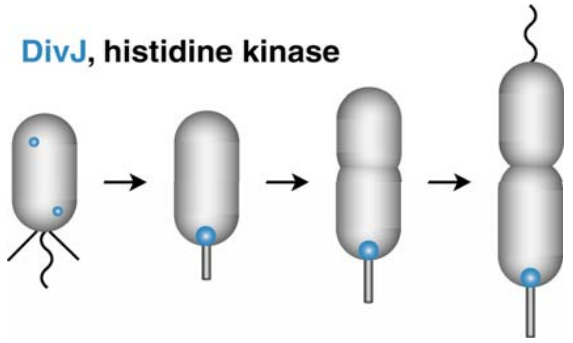
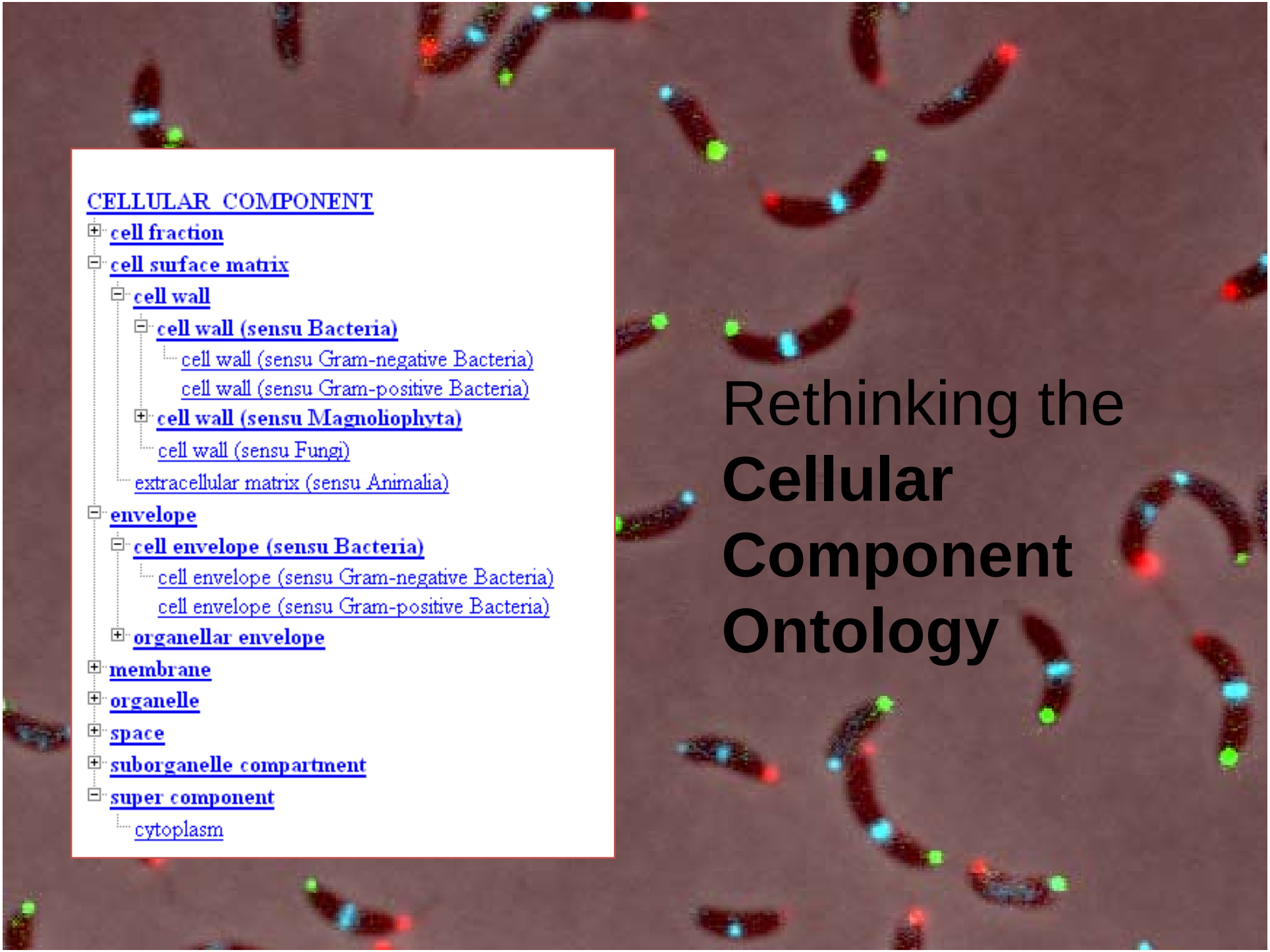
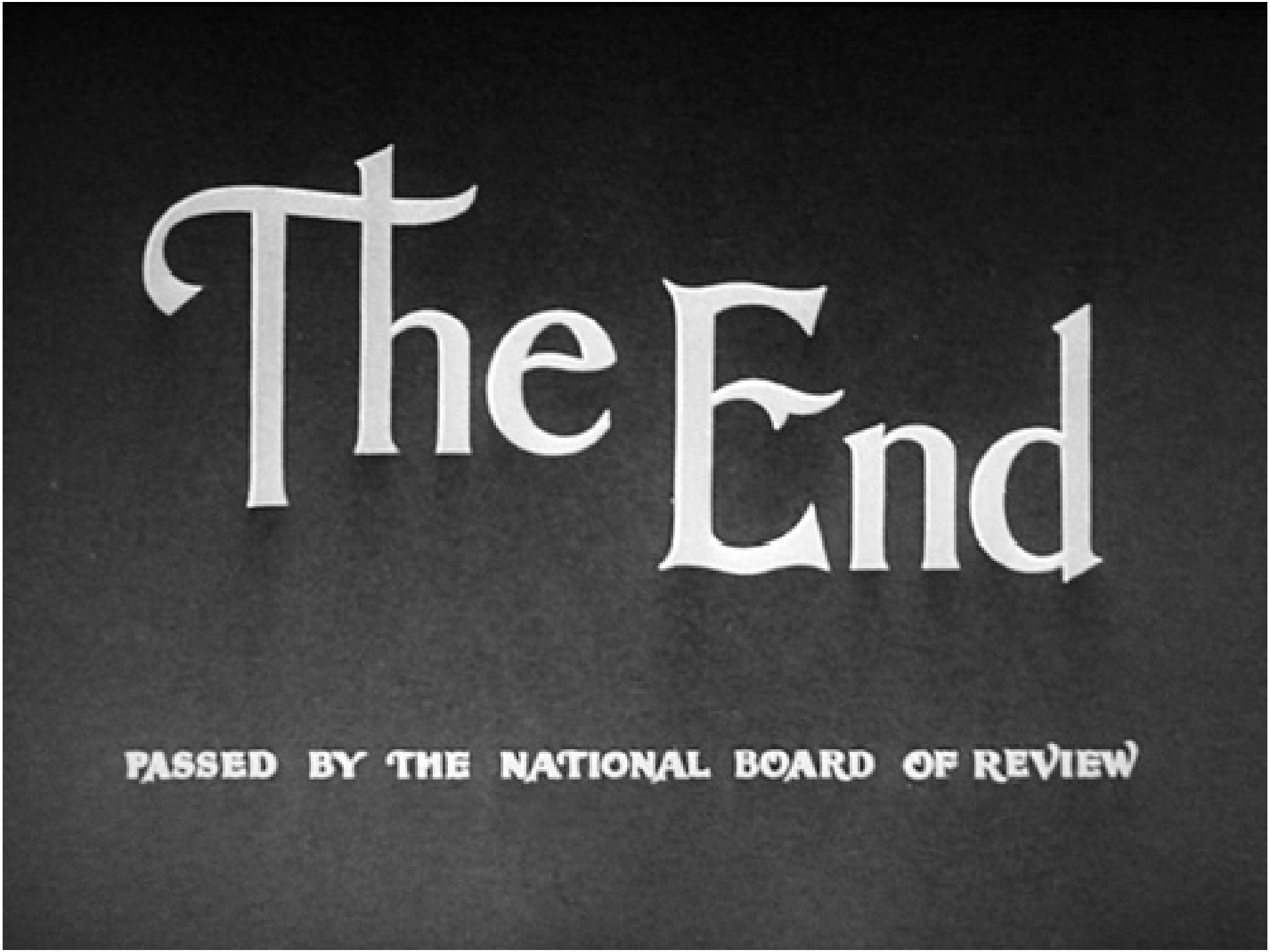





CELLULAR COMPONENT
cell fraction
cell suface matrix
cell wall
cell wall (sensu Bacteria)
cell wall (sensu Gram-negative Bacteria)
cell wall (sensu Gram-positive Bacteria)
cell wall (sensu Magnoliophyta)
cell wall (sensu Fungi)
extracellular matrix (sensu Animalia)
envelope
cell envelope (sensu Bacteria)
cell envelope (sensu Gram-negative Bacteria)
cell envelope (sensu Gram-positive Bacteria)
organellar envelope
membrane
organelle
space
suborganelle compartment
super component
cytoplasm
Rethinking the
Cellular
Component
Ontology


The End
PASSED BY THE NATIONAL BOARD OF REVIEW

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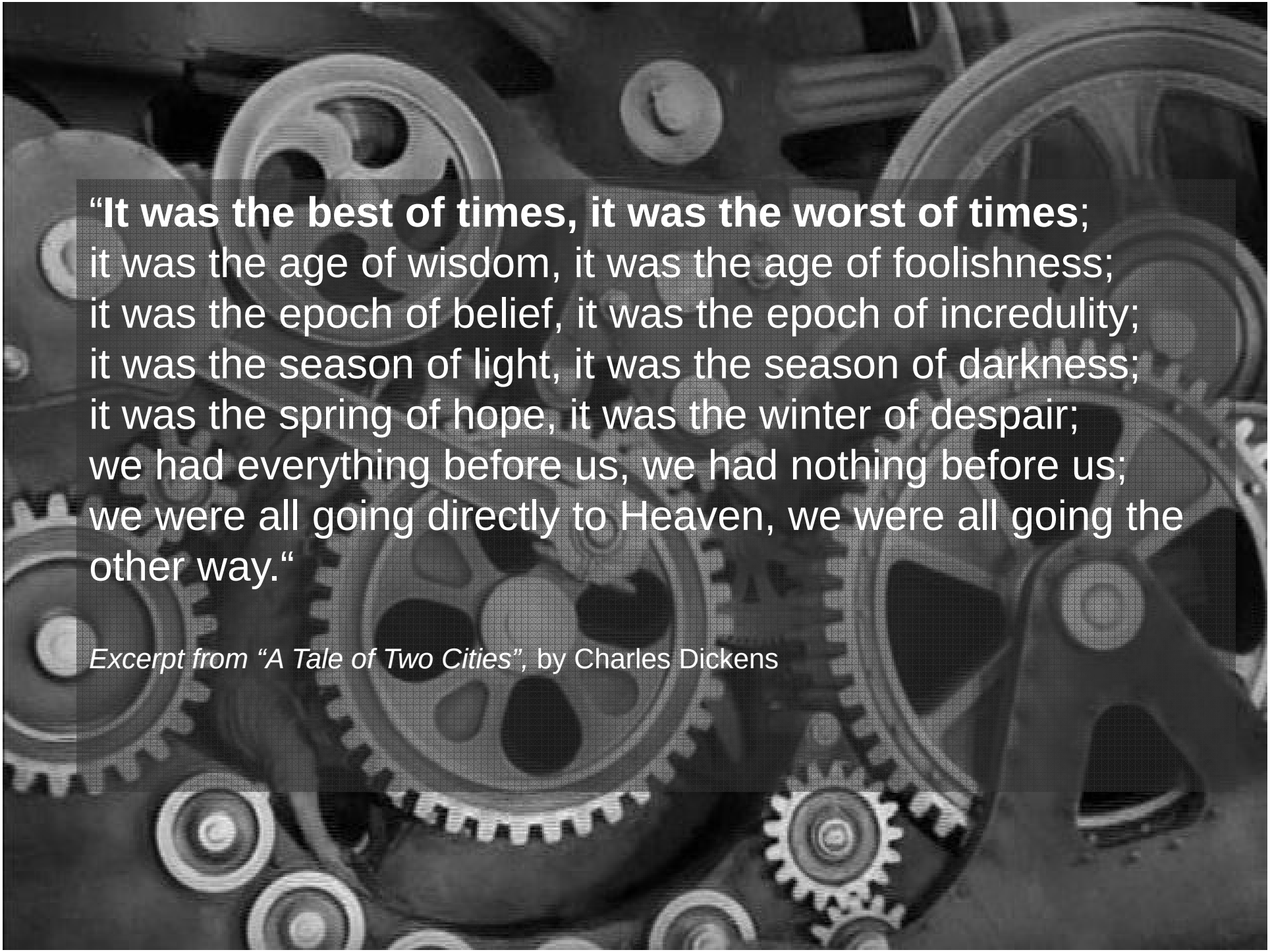
“**It was the best of times, it was the worst of times**;
it was the age of wisdom, it was the age of foolishness;
it was the epoch of belief, it was the epoch of incredulity;
it was the season of light, it was the season of darkness;
it was the spring of hope, it was the winter of despair;
we had everything before us, we had nothing before us;
we were all going directly to Heaven, we were all going the other way.“
Excerpt from “A Tale of Two Cities”, by Charles Dickens