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.
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

- swarmer cell
- flagellum
- stalk
- differentiation (~45 min)
- stalked cell
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

Laub *et al.* (2000)
Cell cycle-regulated genes can be grouped in **functional modules**

- Flagellar Ejection
- Replication Initiation
- Replication Inhibition
- Stalk Synthesis
- Flagellar Biogenesis
- Pili Biogenesis
- Cell Division
- DNA methylation
- Chromosome Segregation

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**G1** → **S** → **G2**
Caulobacter’s cell cycle is driven by a circuit of master regulators.
Possible arrest points upon stress or nutrient deprivation

- Cell division
- Differentiation
- DNA replication and segregation
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
Cellular 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

Figure 1
CauloHI1 Chip Design

(i) Expression analysis

(ii) Transcriptional regulatory region characterization

(iii) Intergenic expression

- Start codon
- Stop codon
- 25bp probe
- Predicted gene
- Small protein / no homology
Application: Identification of transcriptional start sites

McGrath et al. (2007)
Application: Identification of small RNAs

One example

- Activated by starvation
- Stops the cell-cycle

Landt et al. (2008)
Application: operon mapping

Probes

RNA expression

ORFs

Probe-probe signal cross-correlation

Predicted transcript

~3000 bp

3000 nt

Eduardo Abeliuk (unpublished)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

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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-throughout imaging

Werner et al (2009)
Werner et al (2009)
High-throughput screen for protein localization determinants

A

PleC  CpaE  DivJ

asymmetric cell division

B

DivJ-RFP  PleC-YFP  CFP-CpaE  Composite

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

- PleC
- DivJ
- CpaE

Beat Christen & Mike Fero (in preparation)
Dynamic sub-cellular localization of prokaryotic signaling proteins

Compartmental

- **CtrA**, response regulator
  - Swarmer cell
  - Stalked cell
  - Early Predivisional cell
  - Late Predivisional cell

Bipolar

- **DivK**, response regulator
- **CckA**, histidine kinase

Unipolar

- **PleC**, histidine kinase
- **DivJ**, histidine kinase
Rethinking the Cellular Component Ontology
The End

Passed by the National Board of Review
Harley McAdams
Sun-Hae Hong
Mike Fero
Eduardo Abeliuk
Mohammed AlQuraishi
Jimmy Blair
Jean Yeh
Jennifer Boyd-Kozdon
Ling Xie

Lucy Shapiro
Antonio Iniesta
Beat Christen
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Brandon Williams
Erin Goley
Esteban Toro
Grant Bowman
Jay Lesley
Jerod Ptacin
Monica Schwartz
Natalie Dye
Steve Landt
Virginia Kalogeraki

Special thanks to:
Alex Shearer, Suzanne Paley, Tomer Altman & Peter Karp (SRI)
Sam Purvine, Tom Taverner & Mary Lipton (PNNL)

Funding:
Stanford Graduate Fellowship
“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