Minireview

Anatomy of a bacterial cell cycle

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Abstract

Two recent reports describe mRNA and protein expression patterns in the bacterium *Caulobacter crescentus*. The combined use of DNA microarray and proteomic analyses provides a powerful new perspective for unraveling the global regulatory networks of this complex bacterium.

The seemingly simple process of bacterial growth and division requires an impressive orchestration of functions. Cells monitor and coordinate DNA replication with cell division to ensure that each progeny cell receives an accurate copy of the genome. Optimal growth requires coordination of the cell cycle with metabolic processes. In bacteria that undergo cellular differentiation, developmental events are coordinated with growth and with the cell cycle [1,2]. Complex regulatory networks must be in place to ensure the proper coordination of these events, not only in bacteria but in all organisms. Our understanding of these networks is still limited, even in well-studied model organisms, because most studies have analyzed expression patterns of, at best, a few genes. The advent of complete genome sequence information, coupled with post-genomic high-throughput techniques, now allows the global analysis of gene expression and protein levels throughout the cell cycle. As with so many previous revolutions in the biological sciences, microbes are providing the benchmark with which to test the new technologies [3,4]. The recent publication of two articles analyzing the cell cycle of *Caulobacter crescentus* [5,6] illustrates the advantage that the combined use of DNA microarray and proteomic methods can provide in understanding global cellular processes.

The experimental system and the methods

The alpha-purple proteobacterium *Caulobacter crescentus* has several advantages as an experimental system for studying the bacterial cell cycle. *Caulobacter* cells undergo morphological changes that allow visual tracking of cell-cycle progression. An asymmetric pre-divisional cell produces two morphologically different progeny cells (Figure 1). Many cellular events can occur only in the sessile stalked cell, including DNA replication, flagellum synthesis, and cell division. The motile swarmer cell follows its own set of cellular processes before it differentiates into a stalked cell. Developmental progression of swarmer cells through the entire life cycle can be tracked, because large populations of swarmers can be easily synchronized [7,8]. Moreover, the complete genome sequence of *Caulobacter* has recently been completed [9]. Because of its advantages as a model system, *Caulobacter* has been studied extensively, and prior to the two recent reports [5,6], cell-cycle expression data were available for 72 genes or gene products, resulting from studies of individual genes. The power of post-genomic analytical tools comes from their ability to provide a snapshot of the patterns of molecular expression levels of thousands of genes at once. Laub *et al.* [5] and Grunenfelder *et al.* [6] used two major technologies to analyze the global expression patterns in *Caulobacter* of mRNAs (with DNA microarrays) and proteins (with proteomics), respectively.

DNA microarrays depend on the hybridization of fluorescently labeled cDNAs to a compact array of up to thousands of probe cDNAs [10]. The difference in fluorescence resulting from cDNAs generated from different cell samples can be directly compared, and this gives a measure of the relative abundance of each mRNA in the different samples. Proteomic analysis involves the separation of a large number of cellular

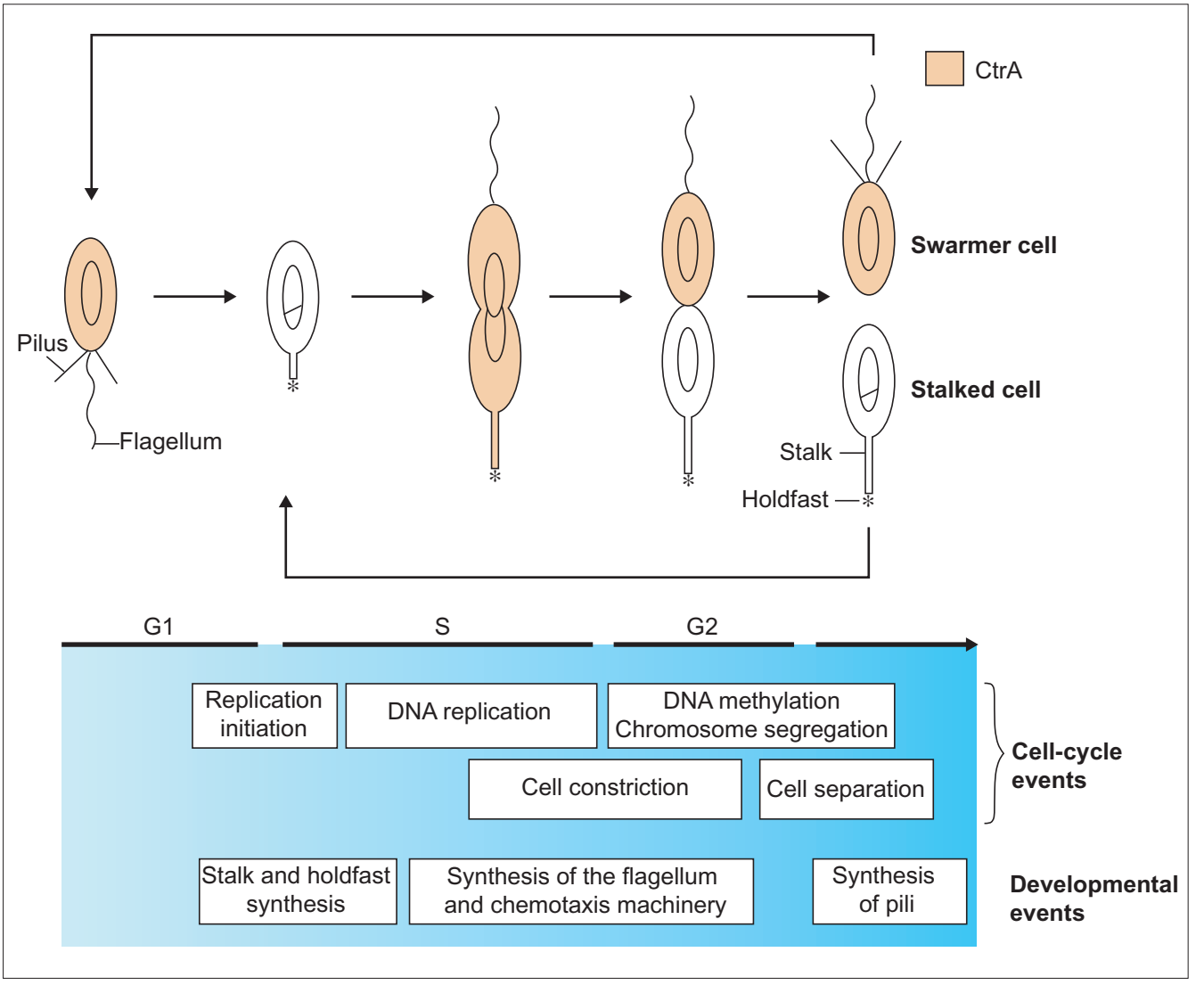

Figure 1
The life cycle of *Caulobacter crescentus*. Swarmer cells are motile, piliated, and unable to initiate DNA replication (in G1, the pre-synthetic gap phase). After an obligatory period spent as non-replicating cells, swarmer cells differentiate into stalked cells. DNA replication and cell division are initiated (in S, the synthesis phase), and the flagellum and pili are replaced by a stalk and an adhesive holdfast. Stalked cells elongate, initiate cell division, and synthesize a new flagellum at the pole opposite the stalked pole. The resulting asymmetric cell produces the two different cell types upon cell separation (at the end of G2, the post-synthetic gap phase). Swarmer cells are unable to initiate DNA replication and cell division until they differentiate, whereas stalked cells can do so immediately after the previous cell division. Immediately after cell separation, the swarmer cell synthesizes pili at the flagellar pole. The timing of various cell-cycle and developmental functions are indicated with boxes and lines, the chromosomes are represented by circles inside the cells for non-replicating chromosomes and by θ structures for replicating chromosomes. Shading indicates the presence of CtrA, and external structures are labeled. Modified from [5].

proteins in a manner that allows the measurement of protein abundance or rate of synthesis under different conditions [11]; proteins are usually separated by two-dimensional gel electrophoresis coupled to peptide-mass fingerprinting, using a matrix-assisted laser desorption time-of-flight (MALDI-TOF) spectrometer, to identify individual proteins. Each technique has both advantages and disadvantages. Microarrays allow the expression of every gene of a microorganism to be assayed simultaneously - but not all changes in mRNA level are reflected in a change in protein concentration. Proteomic analysis has the advantage of biological relevance, since proteins, not mRNAs, carry out most cellular processes. Also, two-dimensional gels can detect both protein modifications and protein degradation; these are certainly not detected by DNA microarrays. Integral membrane proteins are not resolved by two-dimensional gels,

however, so they are currently excluded from proteomic analyses. Even for soluble proteins, the current sensitivity levels for both detection and identification restrict our ability to identify low-abundance proteins. Ideally, both global mRNA and protein expression studies will be performed to provide complementary data, much as northern and western blotting analyses have been used for single gene and single protein studies.

Necessary functions for cell-cycle progression

The two recent papers [5,6] describe the cell-cycle expression patterns in *Caulobacter* of 2,966 genes (out of a total of 3,767 predicted open reading frames) [5] and 979 proteins [6], respectively. Even though these studies were done independently in two different laboratories, together they provide the first global view of the gene products required at specific stages of the bacterial cell cycle. The majority of the cell-cycle-regulated gene products (49 of 63, or 78%) analyzed in both studies had similar or identical mRNA and protein expression patterns. Microarray analysis alone would not have detected ten cell-cycle-regulated proteins that showed no variation of mRNA level, however. In addition, four RNAs and their corresponding protein products exhibited inverse expression patterns (protein high, mRNA low, or vice versa). These results support the validity of using DNA microarray data to make predictions about protein expression, but they also indicate that post-transcriptional regulation is the main determinant of the level of a substantial number of proteins, underscoring the importance of confirming microarray data with proteomic methods. A similar conclusion was reached in a recent integrated genomic and proteomic study of the yeast galactose utilization pathway [12]. Each of the studies identified clusters of mRNAs or proteins with similar patterns of induction and repression through the cell cycle. Clustering showed that genes contributing to a single function are often expressed in the same sequence in which they are utilized. The results of the cluster analysis are best discussed by relating them to stages of the cell cycle.

Swarmer cell (G1 stage)

Microarray analysis [5] of early swarmer cells revealed the induction of expression of genes known to be involved in the initiation of DNA replication, including *dnaA* and *dnaB*. Other genes expressed very early in the cell cycle included a subset of replication genes, several genes encoding ribosomal proteins, and nine cell-envelope metabolism genes. The proteomic study [6] showed that proteins whose highest levels occurred in the swarmer stage included proteins involved in cofactor metabolism, carbohydrate metabolism, lipid metabolism, and protein folding, as well as cell-envelope synthesis. A subset of enzymes required for the production of redox cofactors is also transcribed specifically at this stage [5]. Thus, the swarmer cell prepares for later cellular events, such as DNA replication and stalk formation, by inducing genes required for the biosynthesis of macromolecular building blocks.

Swarmer cell differentiation (G1 to S)

Genes involved in DNA replication, nucleotide synthesis, and DNA repair were induced at the time of swarmer-to-stalked cell differentiation, or shortly before [5]. The *ftsZ* gene, which encodes the earliest-acting cell division protein, was also induced during swarmer cell differentiation, in agreement with earlier results [13]. The expression of genes and/or proteins involved in energy metabolism, amino-acid biosynthesis and macromolecular synthesis was also increased during this time. This probably reflects the increasing energy requirement of the cell as its growth rate increases during differentiation.

Stalked cell (S phase)

Early in S phase, several genes and proteins that were highly expressed during G1 exhibited a substantial drop in expression, including proteins involved in carbohydrate metabolism and cofactor metabolism. Proteins with increased levels in early S phase included FtsZ, several energy metabolism enzymes, DNA-single-stranded binding protein (SSB), and at least five proteins of unknown function [6]. Microarray data confirmed the order of expression of three cell division genes: *ftsZ* was expressed first, with *ftsQ* and *ftsA* transcription occurring in late S phase. Late *ftsQA* transcription forms part of a checkpoint coupling DNA replication to cell division [14]. Interestingly, transcription of *ftsZ* was followed by *ftsI*, then *ftsW*, prior to *ftsQ* and *ftsA* transcription [5]. The order of gene expression often reflects the order of assembly of protein products, as was shown previously for the hierarchy of flagellar genes [15] and genes required for the synthesis of pili [16]. The microarray results suggest that the order of assembly of cell division proteins in *Caulobacter* may be different from that suggested by studies in *Escherichia coli* [17], but it is also possible that the order of action of cell division proteins in *E. coli* is in fact different from the current model.

Pre-divisional cells (G2 phase)

In total, 48 cell-cycle-regulated proteins were found to be degraded within one cell cycle [6]. Proteins critical for cell-cycle progression, such as the master response regulator CtrA [18], the essential DNA methyltransferase CcrM [19], and FtsZ [13] were part of the set of 'unstable' cell-cycle-regulated proteins, confirming previous studies. This set of proteins includes CheYI and CheD, suggesting that chemotaxis proteins are degraded at every cell cycle; this degradation might take place simultaneously with degradation of the chemoreceptor McpA [20] during swarmer cell differentiation. Approximately half of the unstable proteins were also differentially synthesized during the cell cycle, strongly suggesting that these proteins are present only during a short time and that their levels are tightly regulated in a variety of ways. Given that highly unstable proteins are often regulatory

proteins [21], the Grunenfelder *et al.* [6] study is the first to suggest that proteolysis is a global method of regulation in bacteria as well as eukaryotes.

Why are so many genes and proteins cell-cycle-regulated?

Two main advantages of cell-cycle regulation might be energy conservation and maintenance of the order of cell-cycle events. Certain cell-cycle events, such as the initiation of DNA replication, chromosome segregation, and cell constriction, occur only once per cell cycle in the cells of essentially all organisms. For these events, order is critical. A cell should not divide before DNA replication has produced two complete copies of the genome, otherwise at least one daughter cell will not be viable. Other processes specific to the developmental life cycle of *Caulobacter* also occur only once per cell cycle. These periodic events include synthesis of the stalk, holdfast, flagellum, and pili (Figure 1). Because *Caulobacter* cells typically live in nutrient-deprived environments [7], it may be advantageous to limit the energy spent on each process; and certain processes are antithetical, such as swimming and adhesion. Synthesis of the holdfast follows ejection of the flagellum during swarmer cell differentiation, ensuring that the energy required for rotation of the flagellum is not spent needlessly when the cell is cemented to a substrate. One exception to this exclusive presence of flagellum and holdfast is found in pre-divisional cells, where the flagellum and the stalk are at opposite poles. In this case, activation of flagellar rotation immediately before cell separation probably provides the force necessary to complete cell separation; use of flagellar momentum eliminates the need for a separate cell-division force-generating mechanism, so it is energetically favorable.

It is likely that many regulators are required to coordinate progression through the cell cycle. Possibly the most exciting aspect of these two studies [5,6] is the identification of 247 mRNAs and more than one dozen proteins of unknown function that have cell-cycle-regulated expression patterns. Twenty-seven of the unknown, but differentially expressed, genes are possible regulators of morphological development or cell-cycle progression. These genes encode homologs of response regulators, histidine kinases, and sigma factors and are expressed at each stage of the cell cycle, suggesting that each may be involved in regulation of specific cell cycle and/or developmental events. One or a few of these genes could be master regulatory proteins similar to the global cell-cycle response regulator CtrA (see Figure 1), which was found to regulate 26% of the *Caulobacter* cell-cycle-regulated genes either directly or indirectly [5].

Much work lies ahead before we fully understand the regulation of *Caulobacter* development and cell cycle - but these two landmark papers [5,6] have advanced both our knowledge of *Caulobacter* expression patterns and an awareness of the synergy attained by combining two post-genomic techniques.

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