

instructions, such as “report ‘T’s of any color”. Without this letter monitoring task, the rapidly flashing noise images, which conceal the invisible tool images, would presumably capture and hold the observers’ visual attention in the peripheral visual field. So what did Bahrami *et al.* [6] hope to achieve by removing both awareness and attention?

To understand, we need to recall some basic facts about attention. In our subjective visual experience, we encounter attention in the form of visual effort and voluntary control. Formally, attention selectively enhances and attenuates visual processing to meet current behavioural goals [7]. Attention is not associated with one particular brain site, but seems to result from dynamic interactions between multiple brain areas encoding visual and goal information [8,9]. When a given stimulus is selected by attention, it typically evokes stronger responses at all neural levels: in the visual thalamus, in early retinotopic areas of cortex, and in higher areas of the ventral and dorsal visual cortex [10]. Psychophysical evidence shows many qualitative and quantitative improvements in the visual awareness of an attended stimulus [11].

What Bahrami *et al.* [6] did, therefore, was to ask whether attention modulates responses evoked by an invisible image. In fact, they found that the fMRI activation by invisible tool images did indeed prove higher when attention was allowed to select the image locations — with simple letter monitoring — than when attention was assiduously drawn away — with complex letter monitoring. This result, which was obtained in all three investigated areas (V1, V2 and V3), implies that a neuronal response need not contribute to visual awareness, even though it is enhanced by visual attention. In short, attention does not guarantee awareness.

The dissociation observed by Bahrami *et al.* [6] — attention without awareness — reinforces previous reports of the opposite dissociation, namely, that observers tend to be aware of salient stimuli outside the current

focus of attention — awareness without attention [11,12]. It looks less and less likely, therefore, that a neural correlate of visual awareness, which is the ultimate goal of this line of research, will bear a close resemblance to the neural basis of attention [13]. The question remains wide open, as to what form a neural correlate of awareness may take — activity of particular cell types, activity of particular areas or connections, or particular forms distributed activity have been considered, with plenty of other possibilities offering to a fertile imagination. The contribution of studies such as those by Fang and He [4] and by Bahrami *et al.* [6] lies in the neurophysiological dissociation of psychologically defined processes — attention and awareness — that normally operate in tandem and are thus all too easy to conflate.

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Bacterial Cell Cycle: Completing the Circuit

Recent advances in understanding bacterial cell-cycle regulation suggest circuit control mechanisms that operate analogously to those in the eukaryotic cell cycle.

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The cell cycle can be thought of as a ‘circuit’ of regulatory components which, by enabling an efficient flow of information, triggers events critical for cellular reproduction. Like industrial spies in Silicon Valley, biologists are trying to peer into cells to map out their circuit components and connections. New work on the

bacterium *Caulobacter crescentus* has, for the first time, laid out connections between key regulators of all the major events in this microbe’s cell cycle [1]. The design principles of the *Caulobacter* circuit parallel those used in the yeast *Saccharomyces cerevisiae* [2], a model for studies of the eukaryotic cell cycle, suggesting that these principles may be of fundamental importance.

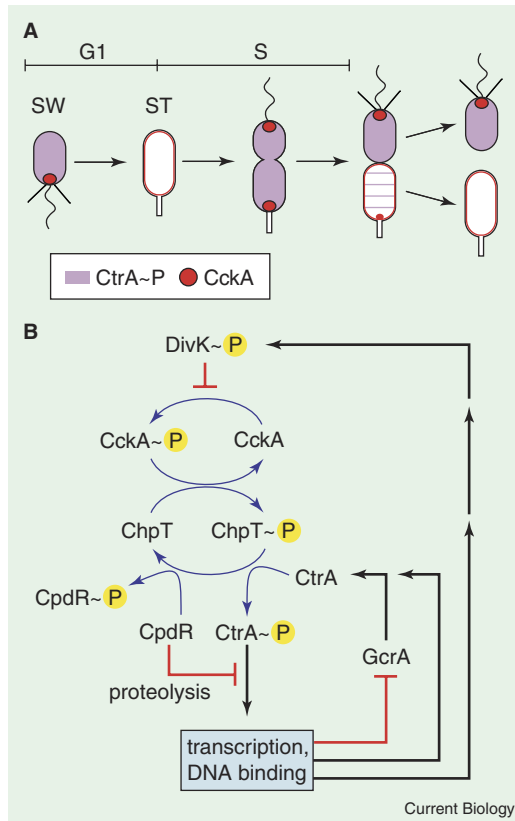


Figure 1. Regulation of the *Caulobacter* cell cycle.

(A) The activities of CtrA and CckA oscillate as *Caulobacter* progresses through the cell cycle. During G1, the swarmer (SW) cell has pili (straight lines) and a flagellum (wavy line) at one pole. Active CckA localizes to this pole, and CtrA is present and phosphorylated. The swarmer cell differentiates into a stalked (ST) cell to enter S phase, replacing pili and flagellum with a stalk (straight rod). At this point, CckA delocalizes and CtrA is degraded. As the stalked cell elongates during S phase, CtrA is synthesized and phosphorylated again, because CckA is re-localized to the poles and active. After cell division, the swarmer progeny repeats the entire cycle, while the stalked progeny re-enters S phase. (B) Feedback loops control CtrA activity and cell-cycle progression. CckA kinase phosphorylates both CtrA and CpdR via the ChpT phosphotransferase. This phosphorelay activates and

stabilizes CtrA, because only the unphosphorylated form of CpdR promotes CtrA proteolysis. Active CtrA drives *divK* expression, and accumulation of phosphorylated DivK inhibits the activity of CckA. CtrA also promotes its own expression and represses expression of GcrA. After CtrA is destroyed during the swarmer-to-stalked transition, GcrA induces a burst of CtrA production, which positively feeds back to generate more CtrA. (Adapted with permission from [1].)

Caulobacter is an appealing model for investigating the bacterial cell cycle [3] because cells can be easily synchronized in the 'swarmer' stage, which is analogous to the G1 phase of eukaryotes. The swarmer has pili and a single flagellum at one cell pole, which are replaced by an adhesive stalk when the swarmer differentiates into a stalked cell (Figure 1A). The stalked cell enters S phase, and as the cell replicates the chromosome and elongates, new swarmer structures develop at the pole opposite the stalk. Following asymmetric division, the new swarmer cell finds itself in G1 again, whereas the stalked progeny cell immediately re-enters S phase.

This series of events is coordinated by several signal transduction proteins, foremost among them CtrA, an essential transcription factor [4]. CtrA controls the expression of many

cell-cycle-regulated genes [5]; it also binds to and silences the origin of replication [6]. CtrA activity is modulated by differential expression, phosphorylation and proteolysis [4,7]. In G1, phosphorylated CtrA inhibits DNA replication until the swarmer-to-stalked transition, when CtrA is degraded (Figure 1A). CtrA is then re-synthesized and activated by phosphorylation in the elongating stalked cell. Following cell division, CtrA is removed specifically from the stalked progeny, to allow chromosome replication. How the phosphorylation and degradation of CtrA are temporally and spatially controlled has been a long-standing mystery. Biondi *et al.* [1] have now identified a missing link in the circuitry, a 'phosphotransferase' called ChpT, which is necessary for both types of regulation.

All previous evidence fingered CckA, a membrane-bound histidine kinase, as the source of phosphate for CtrA *in vivo* [8,9], but researchers have been frustrated for years by an inability to demonstrate direct phosphorylation of CtrA by CckA *in vitro*. In a canonical bacterial two-component signal transduction system, the kinase autophosphorylates on a conserved histidine residue, then transfers the phosphoryl group to a conserved aspartate in the 'receiver' domain of its partner response regulator, usually a DNA-binding protein [10]. But CckA is a 'hybrid kinase', containing its own receiver domain, and hybrid kinases do not necessarily play by the same rules. So, Biondi *et al.* [1] looked at the ability of CckA's kinase domain to phosphorylate purified versions of every response regulator and receiver domain encoded in the *Caulobacter* genome. They found that CckA phosphorylated its own receiver domain, but not CtrA. This result was reminiscent of earlier work in which Biondi *et al.* [11] demonstrated that stalk biogenesis is controlled by a 'phosphorelay' of signals from a hybrid kinase to a histidine phosphotransferase to a response regulator. Might a separate histidine phosphotransferase serve as an intermediary between CckA and CtrA?

Unlike kinase and receiver domains, which tend to be highly conserved, histidine phosphotransferases vary greatly in sequence. As Biondi *et al.* [11] pointed out in their earlier work, though, histidine phosphotransferases have certain features in common: relatively small size (<250 amino acids), predominantly α -helical structure and a histidine residue in a predicted α helix. Bioinformatic screening of the *Caulobacter* genome with these criteria yielded more than 50 candidates, but the field was narrowed further by looking for those that are present only in organisms that also contain *cckA* and *ctrA* orthologues — the

rationale being that essential components of the same regulatory pathway should be conserved across species. A single candidate gene, *chpT*, emerged after this criterion was added [1]. Genetic and biochemical analysis confirmed that purified ChpT passes a phosphoryl group from the CckA receiver domain to CtrA, and thus represents the missing link in this signal transduction pathway.

Whether ChpT is a passive transfer vehicle, or itself mediates some form of regulation, is not yet known (though one might suspect there is a reason the CckA–CtrA pair did not evolve the ability to interact directly). At any rate, CckA–ChpT does not work just with CtrA. The CpdR response regulator directs the protease responsible for CtrA degradation to the stalked pole [12], where doomed CtrA molecules are sent for destruction during entry into S phase [13,14]. Only unphosphorylated CpdR localizes the protease; CckA protects CtrA from degradation by promoting CpdR phosphorylation [12]. Biondi *et al.* [1] found that ChpT also mediates phosphoryl transfer from CckA to CpdR. ChpT thus sits at the junction of a bifurcating pathway that allows CckA to control CtrA by both phosphorylation and inhibition of proteolysis (Figure 1B).

What is upstream of CckA in the circuit? CckA activity is correlated with polar localization [8,9], which is controlled by yet another response regulator, DivK [1]. An increase in phosphorylated DivK appears to inhibit polar localization of CckA (Figure 1B). During S phase and prior to cell division, CckA localizes to both cell poles (Figure 1A). The level of phosphorylated DivK is moderated by a kinase (DivJ) at the stalked pole and a phosphatase (PleC) at the opposite pole [15]. After division, the stalked progeny inherits the DivJ kinase; so phosphorylated DivK accumulates, and CckA becomes delocalized and inactive. In contrast, the swarmer progeny inherits the PleC phosphatase,

which dephosphorylates DivK to allow active CckA to remain at the flagellated pole; phosphorylated CtrA consequently represses DNA replication. PleC is replaced by DivJ during the swarmer-to-stalked transition, inactivating CckA and leading to destruction of CtrA. After a lag, CtrA synthesis starts up again with the help of GcrA, a regulatory protein whose expression is derepressed when CtrA is degraded (Figure 1B) [16]. After this kick start, CtrA feeds back to boost its own expression, and proceeds to activate a large regulon of genes until cell division triggers the DivK pathway for its destruction in the stalked progeny.

Thanks to systems-level approaches, the molecular details of the *Caulobacter* cell cycle circuit are becoming increasingly clear. We cannot claim to understand all the information the cell is processing as it moves through its life cycle, but the work of Biondi *et al.* [1] lays out a lucid model, connecting regulatory components through interlinked sequential gene expression pathways and feedback loops dependent on spatial positioning of proteins. Cell growth and division provide critical timing functions, creating distinct compartments and subcellular structures that interact differentially with regulatory components to influence their activities. Analogous observations have been made in *S. cerevisiae*, in which two distinct oscillatory mechanisms are interlinked to control cyclin synthesis, phosphorylation, and destruction [2]. Subcellular localization of key regulators is important in *S. cerevisiae* as well; the CDC14 phosphatase, for example, is sequestered in the nucleolus during most of the cell cycle, then released to trigger mitotic exit by dephosphorylating critical proteins [17]. The players are not evolutionarily conserved between the prokaryotic *Caulobacter* and eukaryotic *Saccharomyces* cells; in fact, the *Caulobacter* regulatory proteins are for the most part only conserved within the ‘alpha’ family of Proteobacteria. Nevertheless,

the conceptual similarities in the cell cycle regulatory circuitry between these prokaryotic and eukaryotic model systems suggest that these are robust design elements, likely to be applied repeatedly.

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Vertebrate Gastrulation: The BMP Sticker Shock

BMPs are essential regulators of cell fate during early embryonic development. Molecular genetics and *in vivo* imaging of cell behaviors in zebrafish now demonstrate a role for BMPs in the control of cell adhesion. The work reveals an important new mechanism governing cell movements during gastrulation.

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Even though embryos of diverse vertebrate groups develop a similar body plan after neurulation, the most cursory inspection of early developmental stages shows enormous differences in how these animals reach this phylotypic stage. Many of the differences in early development reflect constraints of embryo nutrition, such as the need for a large yolk supply in egg-laying vertebrates versus the need to implant in the uterine wall in mammals [1]. But even with those obvious constraints, one of the shocking features of early vertebrate development is that completely different cell behaviors are used during gastrulation [2–4].

For example, the chick and the mouse show large-scale epithelial-to-mesenchymal transitions, whereby cells ingress from the epiblast to form the mesoderm. In contrast, sheets of cells remain coherent as they move into the embryo in the frog *Xenopus*. Do these differences illustrate deep divergence in the mechanism of gastrulation, or are we as yet too ignorant to see the underlying similarities? Of course any understanding of such issues will require us to know much more about the mechanisms that control cell behaviors

during gastrulation. A new paper from Hammerschmidt and colleagues in this issue of *Current Biology* makes a very welcome contribution [5].

A popular hallmark of frog and fish gastrulation is the movement of convergence and extension, during which tightly packed cells converge and intercalate to lengthen and narrow the anterior-posterior axis. However, in the bony fishes, the ventral and lateral cells initially migrate as loose cells towards the dorsal midline in a luxuriant extracellular matrix [6–8] — a behavior that is conspicuously absent from *Xenopus* [2–4] (Figure 1A,B). Previous analyses of mutant zebrafish had indicated a negative role for bone morphogenetic proteins (BMPs) in this dorsal migration of lateral mesoderm cells [9]. However, as ventral identities are also specified by BMP signaling and ventral cells do not engage in robust cell movements, it can be difficult to deconstruct how immediately BMPs affect morphogenesis (e.g. [10–12]). Are BMPs directly involved in the cell movements, or do BMPs simply specify a cell fate that then displays a certain morphogenetic property?

In the new work, von der Hardt *et al.* [5] reveal that BMPs have direct effects on cell adhesion and thereby affect lateral cell

movements during zebrafish gastrulation. Not only do the authors separate cell fate and cell movement, but they also exploit high-resolution imaging of the cells to gain insight into the underlying cellular mechanisms. Global morphogenesis is something that can be disrupted all too easily, but cellular behaviors often respond to molecular manipulations with great specificity, so this type of analysis provides additional strength to the conclusions.

To modulate BMP signals the authors used an array of mutants and morpholino oligonucleotide-mediated knockdowns — either of the genes for the ligands, the Alk8 receptor, or of the cytoplasmic transducer Smad5. BMP signaling was then restored locally by application of BMP beads. Ventral placement of such beads restored a normal dorsally directed migration of lateral mesoderm cells. Strikingly, however, dorsal placement of such beads in *bmp2* morphants was sufficient to drive cell migration ventrally, leading to a piling up of mesodermal cells on the ventral side (Figure 2A–C).

One key experiment was to dissociate the effects of BMP signaling on cell movement from its effects on cell identity. The authors found that the migratory properties of cells were radically changed between embryos that otherwise showed the same expression patterns of markers of cell identity. These experiments exploited the observation that lamellipodial activity and migration require the hyaluron synthase Has2 [13]. In embryos in which *has2* expression was