Fucci: Street Lights on the Road to Mitosis

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Tracking cell-cycle progression in live cells within their endogenous environment has been an outstanding challenge. In a recent issue of *Cell*, Sakaue-Sawano et al. describe Fucci, a technique designed to track cell-cycle progression with high spatiotemporal resolution in a multicellular context.

The cell division cycle, which describes the fundamental process by which cells reproduce, directly impacts the operation and development of all living organisms (Nurse, 2000; Nurse et al., 1998). As a consequence, each step in the process of cell division—from replication of chromosomal DNA during S phase to segregation of sister chromatids during mitosis—must be coordinated in a manner that ensures the faithful transmission of hereditary information from one generation of cells to the next. Indeed, the timely execution of each stage of the cell cycle is intimately linked to key developmental processes such as differentiation and organogenesis. On the other hand, failure to precisely regulate cell-cycle progression leads to various diseases such as cancer.

To ensure that events such as S phase and mitosis proceed both in an orderly fashion and with high fidelity, cells have developed a series of checkpoints that act as quality control centers at each stage of the cell cycle. These checkpoints, which govern the transitions between G_1/S and G_2/M, are designed to monitor cellular parameters such as genomic integrity and cell size throughout the division cycle. If a cell fails to meet minimal requirements at any point during the process, regulatory factors prevent the onset of the next phase until the task at hand has been completed.

Until recently, it has been difficult to precisely track cell-cycle progression in a live, multicellular context. This is because most of the techniques currently used to monitor the cell cycle—such as BrdU incorporation or immunostaining of cell-cycle markers—require cell fixation prior to analysis. As a consequence, these methods do not permit the dynamic behaviors of cycling cells to be visualized in real time. Recently, several techniques have been developed to track cell-cycle progression in live cells (Leonhardt et al., 2000; Essers et al., 2005; Easwaran et al., 2005). Unfortunately, each of these methods relies upon relatively small changes in the subcellular distribution and/or patterning of fluorescently-tagged proteins. While these approaches may be useful for monitoring phase transitions in cultured cells, they may not translate well to a multicellular context such as live tissues where a high degree of contrast is required to discriminate between cells at various stages of the cell cycle. Ideally, imaging techniques used to visualize cell-cycle progression would provide a means of easily distinguishing between cells engaged in different stages of the cell cycle with minimal perturbation to the system under study, allowing the dynamic behavior of cycling cells to be monitored in real-time.

In a report published in a recent issue of *Cell*, Sakaue-Sawano and colleagues describe an exciting technique designed to track cell-cycle progression with high spatiotemporal resolution in a multicellular context (Sakaue-Sawano et al., 2008). This method, termed *f*luorescent *u*biquitination-based *c*ell-cycle *i*ndicator (Fucci), exploits cell-cycle-dependent proteolysis of the ubiquitination oscillators, Cdt1 and Geminin, to specifically mark the G_1/S transition in living cells. By fusing the red- and green-emitting fluorescent proteins mKO2 and Azami Green (mAG) to portions of Cdt1 and Geminin, respectively, the authors are able to achieve striking contrast between various stages of the division cycle. Specifically, the nuclei of cells in G_1 phase (and G_0) appear red, while those of cells in S/G_2/M appear green (Figure 1). During the transition from G_1 to S phase, cell nuclei turn yellow, clearly marking cells that have initiated DNA replication.

The dramatic color changes exhibited by Fucci are based upon the reciprocal activities of the ubiquitin E3 ligase complexes APC^{Cdh1} and SCF^{Skp2} (Vodermaier, 2004). For instance, while APC^{Cdh1} functions primarily during G_1 phase, SCF^{Skp2} is most active during S, G_2, and early M phases (Figure 1). Consequently, the APC^{Cdh1} and SCF^{Skp2} substrates Geminin and Cdt1 are specifically degraded during G_1 and S/G_2/M, respectively (Nishitani et al., 2004). After testing several truncated forms of each protein, the authors identified two protein fragments, Cdt1(30/120) and Geminin(1/110), that exhibit the same oscillatory behavior as the wild-type species. Importantly, neither of these constructs perturbed cell-cycle progression. Therefore, the fusion proteins mAG-Geminin(1/110) and mKO2-Cdt1(30/120) employed by Fucci function as benign cell-cycle markers that effectively label the nuclei of cells in S/G_2/M green and those in G_1 red. Since both chimeras are stabilized to varying degrees as the activities of SCF^{Skp2} and APC^{Cdh1} converge during the G_1/S transition, yellow nuclei are observed in these cells. The high contrast afforded by Fucci allows the cell cycle to be correlated with many important physiological processes. For instance, to examine cell-cycle progression of tumor cells during metastasis, the authors injected Fucci-expressing HeLa cells into live animals and tracked their distribution over time. Although most of the cells in the blood vessels existed in G_1, many underwent a G_1/S phase transition as they migrated into neighboring tissues during extravasation.

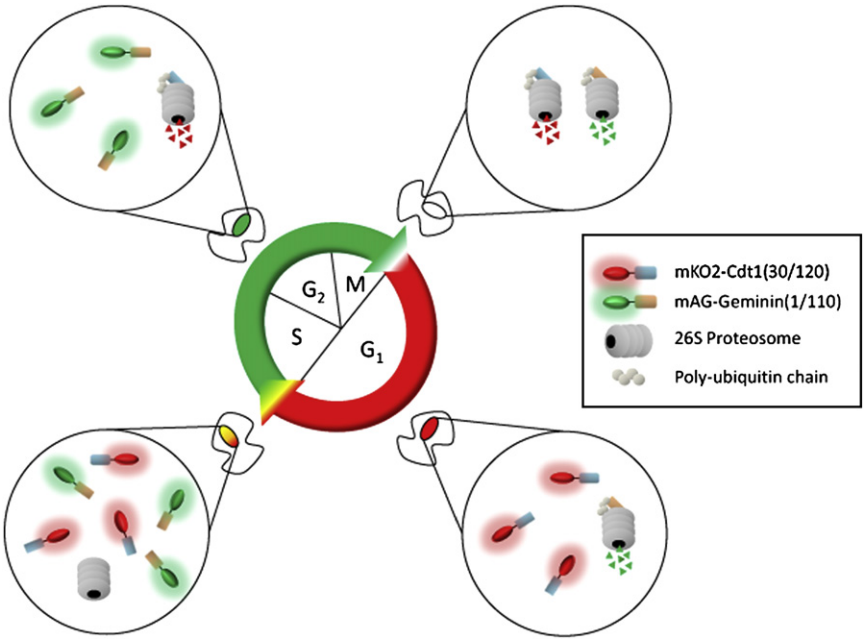

Figure 1. Schematic Representation of the Fucci Technology
Fucci relies upon cell-cycle-dependent proteolysis of the ubiquitination oscillators Cdt1 and Geminin to specifically mark the G_1/S transition in living cells. During G_1 phase, the nuclei of Fucci-expressing cells appear red due to both the stabilization of mKO2-Cdt1(30/120) and ubiquitin-mediated proteolysis of mAG-hGem(1/110). As cells transition from G_1 to S phase, each chimera is stabilized to varying degrees, resulting in nuclei with a yellowish hue. Once the cells have transitioned to S phase, mAG-hGem(1/110) is stabilized and mKO2-Cdt1(30/120) is degraded, causing the nuclei of these cells to appear green. Green fluorescence is maintained throughout S, G_2, and M phases until the fluorescent signal is lost for a brief period between M and G_1 due to the simultaneous destruction of both probes.

Aside from its high contrast, Fucci also exhibits several characteristics that make it particularly well suited for studying cell-cycle progression in a multicellular context. For instance, because it relies upon ubiquitin-mediated proteolysis rather than transcriptional regulation, expression of Fucci can be driven by constitutive promoters. Not only does this property reduce the variability in protein expression levels often observed at different developmental stages, it also facilitates the generation of transgenic animals. Thus, Fucci is a very attractive tool for cell-cycle analysis during development. To this end, the authors used transgenic mice expressing mKO2-Cdt1(30/120) and mAG-hGem(1/110) under the control of the CAG promoter to visualize, in neural tissues, the distribution of proliferating neuronal progenitor cells and postmitotic cells marked with bright red nuclei, a consequence of mKO2-Cdt1(30/120) accumulation after cell-cycle exit. Importantly, because time-lapse imaging of live tissue sections can be achieved over relatively short time intervals using Fucci, the migration patterns of individual neuronal progenitors could be correlated with their cell-cycle progression. Together, these experiments demonstrate Fucci's great promise for studying the coordination of the cell cycle and development.

Fucci also has the potential to significantly enhance our understanding of the molecular mechanisms governing cell-cycle progression. For instance, because the fluorescent chimaeras utilized by Fucci are distributed exclusively in the nucleus, this method is amenable to co-imaging studies using fluorescent biosensors designed to track signaling dynamics in live cells (Zhang et al., 2002). This can be accomplished either by excluding the reporter from the nucleus or by utilizing fluorescent protein variants with emission spectra distinct from those of the Fucci probes. The ability to simultaneously image cell-cycle progression and signal-transduction pathways inside living cells will be vital to unraveling the spatiotemporal dynamics underlying cell-cycle checkpoint control mechanisms (Lukas et al., 2004). Importantly, since Fucci provides a means of easily distinguishing between cells engaged in different stages of the cell cycle, synchronization procedures that may otherwise perturb the cellular system are not necessary using this approach. This property can also facilitate high-throughput analysis of cell-cycle progression in RNAi- or small-molecule-based screens. In this way, Fucci promises to play an integral part in the identification of new cell-cycle regulators as well as chemical biology tools for studying the cell cycle.

Fucci, as a powerful tool to visualize cell-cycle progression in living cells, lays a foundation for studying the cell cycle in a variety of cellular contexts. In particular, its ability to mark the G_1/S transition with high contrast in transgenic animals opens new and exciting avenues of research. Moreover, the anticipated development of complementary cell-cycle probes designed to mark phase transitions other than G_1/S will further expand the applications of this innovative technology. Together, these reporter systems promise to provide unprecedented insights into the regulation and coordination of cell-cycle progression in many physiological processes.

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