intrinsically lead to "replication factories" with DNA moving toward a dual replisome. On the other hand, the twin pump and ploughshare mechanisms could make repairing intrastrand crosslinks difficult given that the helicase complex would presumably arrest with the crosslink in the Mcm2–7 central channel. Only a better understanding of the replication initiation process will distinguish between these models and reveal the mechanism of Mcm2–7 action.

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Transformation Locked in a Loop

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During neoplastic transformation, cells can promote their own growth by activating proto-oncogenes. Reporting in *Cell*, Iliopoulos et al. (2009) now show that in certain cell types, a transient oncogenic signal is sufficient to induce neoplastic transformation and to maintain it through a positive feedback loop driven by the inflammatory cytokine interleukin-6.

To become fully transformed, primary human cells need to bypass several cellular failsafe mechanisms that normally keep cellular growth in check. In addition, transformed cells can become self-sufficient by producing their own growth signals. Activating mutations in proto-oncogenes are a frequent path to cellular neoplastic transformation, and several reports have described the dependence of cancer cells on the oncogene even after transformation (Weinstein and Joe, 2008). However, in this issue of *Cell*, Struhl and colleagues (Iliopoulos et al., 2009) report that transient induction of the Src oncogene in nontransformed human mammary epithelial cells results in production of the cytokine interleukin-6 (IL-6), which drives—and maintains—cells in a transformed state. This is mediated by a positive feedback loop involving IL-6, NF-κB, the let-7 microRNA (miRNA), and its regulator, LIN28B, resulting in a "snowball" effect.

NF-κB is a transcription factor that can induce the expression of IL-6, a cytokine that plays a crucial role in the immune response and inflammation. LIN28B is a stem cell factor and RNA binding protein that inhibits processing of the let-7 miRNA (Viswanathan et al., 2008). Changes in let-7 expression have been associated with tumorigenesis. Although links between inflammation and cancer have been suggested, a link between NF-κB and let-7 has not been reported so far. However, the new work links all of these four players and demonstrates that they cooperate to maintain cellular transformation in response to transient oncogene activation (Iliopoulos et al., 2009).

How transient is the oncogenic signal and how does it work? Iliopoulos et al. expressed a tamoxifen-inducible estrogen receptor-Src (ER-Src) fusion protein in nontransformed human mammary epithelial cells. They show that a 5 min treatment with tamoxifen is sufficient to drive the cells into the transformed state. Next, they provide evidence that this transient treatment induces expression of the inflammatory cytokine IL-6 in an NF-κB-dependent manner. Specifically, they show that Src activates NF-κB, which directly activates the transcription of LIN28B. Indeed, the expression of the mature let-7 miRNA rapidly decreases in response to Src activation, through induction of LIN28B. As a result, IL-6, a direct target of let-7, is derepressed, causing cellular transformation through STAT3, a transcriptional activator that is phosphorylated and activated in response to IL-6 signaling. A positive feedback loop is generated through IL-6, the expression of which is induced by NF-κB, but IL-6 can itself activate NF-κB (Figure 1A). This loop explains why the transformed state is maintained even after Src expression is shut off. Confirming this model, the authors report that

inhibition of any of the components of the feedback loop reverses the transformed phenotype.

The authors provide evidence that their findings may be relevant to some human cancers. In both normal and cancer cells (derived from breast, prostate, and hepatocellular tissues), the authors find a negative correlation between the expression of the let-7 miRNA and that of IL-6. In normal cells, let-7 levels are high and IL-6 levels are low, whereas in cancer cells let-7 levels are low and IL-6 levels are high. These observations suggest that the feedback loop involving IL-6 and let-7 may be active in these cancer cells. Furthermore, interference with any component of the inflammatory feedback loop inhibited the tumorigenicity of several human cancer cell lines. However, despite this striking negative correlation in certain human cancers, in vivo tumor models will be needed to prove a causal role for this regulatory circuit in driving tumorigenesis. Intriguingly, mice lacking IL-6 are more resistant to the formation of tumors driven by the Ras oncogene and to developing tumors of the colon associated with inflammatory colitis than normal mice, suggesting causality (Ancrile et al., 2007; Grivennikov et al., 2009). Future experiments should address whether the IL-6/NF-κB/LIN28B/let-7 loop is involved in the formation of tumors in these animal models.

Many reports have shown that IL-6-mediated inflammation and cancer are linked. However, both tumor-suppressive and tumor-promoting activities have been ascribed to IL-6. In accordance with the observations of Iliopoulos et al. suggesting a tumor-promoting role for IL-6, a recent study showed that IL-6 expression, induced by the Ras^{V12} oncogene, is required for tumor growth of human kidney cells in vivo (Ancrile et al., 2007). On the other hand, oncogene-induced IL-6 upregulation in primary human fibroblasts results in senescence (Kuilman et al., 2008). This antiproliferative response is mediated, at least in part, by the induction of the cyclin-dependent kinase inhibitor $p15^{INK4B}$ (Figure 1B). How can these results be reconciled with the findings of Iliopoulos and colleagues? Remarkably, the spontaneous immortalization of the mammary epithelial cells used by Iliopoulos et al. is associated with a deletion on chromosome 9p21. This deletion includes part of the INK4/ARF locus, such that these cells lack the genes encoding the cyclin-dependent kinase inhibitors $p15^{INK4B}$ and $p16^{INK4A}$ (Cowell et al., 2005). This raises the exciting possibility that the type of IL-6 response that is induced after oncogene activation depends on the status of the INK4/ARF locus. Consistent with this hypothesis, the human kidney cells used in the study of Ancrile et al. in which IL-6 promoted tumor growth, expressed SV40 large T antigen. SV40 large T antigen inactivates the ARF/p53 and INK4/Rb tumor suppressor pathways, thereby neutralizing any IL-6-dependent cell cycle-inhibitory effects via $p15^{INK4B}$ and $p16^{INK4A}$. It would be interesting to determine whether $p15^{INK4B}$ and $p16^{INK4A}$ are still transcriptionally activated in response to oncogene expression. In contrast to mammary epithelial cells, transient activation of the Ras^{V12} oncogene in a primary fibroblast transformation model (Voorhoeve and Agami, 2003) was not sufficient to maintain transformation, perhaps indicating that the IL-6 response might be cell-type dependent.

Iliopoulos et al. made another interesting observation. They report that their positive feedback loop is required for the self-renewing capacity of tumor-initiating cells, so-called cancer stem cells (Figure 1C). A small proportion of the mammary epithelial cells expressing the ER-Src fusion protein that were transiently treated with tamoxifen acquired properties of cancer stem cells, such as self-renewal capacity. The inflammatory loop was more active in this population compared to cells that did not self-renew. When the authors blocked the function of several components in the feedback circuit, they saw a decrease in the self-renewal capacity of the cancer stem cells. This is not the first time a transient signal was found to induce stem cell formation. For example, it is well established that reprogramming of adult fibroblasts back to a stem cell-like state can occur after transient expression of the reprogramming factors OCT4, SOX2, c-MYC, and KLF4 (Soldner et al., 2009), pointing to the involvement of a positive feedback loop. The results of Iliopoulos et al. suggest that the IL-6/NF-κB/LIN28B/let-7 loop could also be at work in the reprogramming of fibroblasts. Given that LIN28B can also be used as a reprogramming factor, it is tempting to speculate about the involvement of the positive feedback loop identified by Iliopoulos et al. in stem cell maintenance. Intriguingly, the INK4/ARF locus serves as a barrier to efficient reprogramming (Li et al., 2009), again implying that this locus plays an important role in the effects observed by Iliopoulos et al.

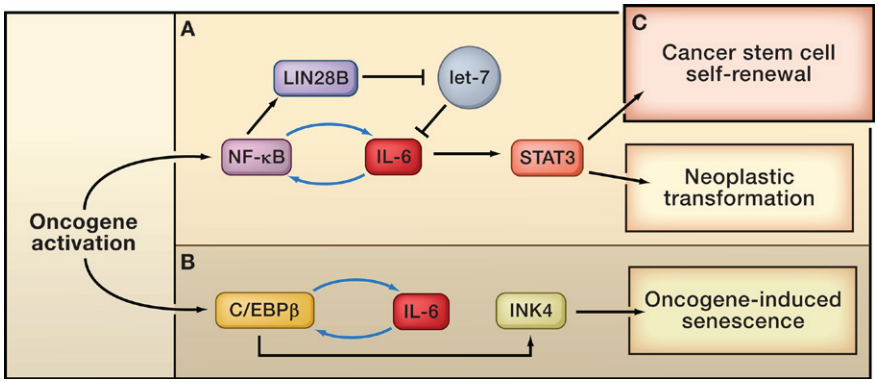

Figure 1. Inflammation and Tumorigenesis

Signaling via oncogenes activates an inflammatory response that drives cells along several different pathways. The response may depend on the cell type or genetic background.

(A) An oncogenic trigger activates a positive feedback loop involving IL-6, NF-κB, let-7 miRNA, and LIN28B. IL-6 signaling activates the transcriptional activator STAT3, which drives cells into the transformed state; IL-6 also activates NF-κB and so completes the loop (Iliopoulos et al., 2009).

(B) Oncogene expression can also activate the transcription factor C/EBPβ. C/EBPβ activates transcription of IL-6 and expression of the INK4 locus, resulting in cellular senescence. IL-6 positively regulates C/EBPβ, thereby generating a positive feedback loop.

(C) A proportion of cells in which the positive feedback loop (IL-6, NF-κB, let-7 miRNA, LIN28B) is activated acquire characteristics of stem cells, such as self renewal, and may act as cancer stem cells in tumors.

The Iliopoulos et al. study shows that a transient oncogenic trigger can lead to cellular transformation. This is mediated through an inflammatory signal and subsequent activation of a positive feedback loop containing IL-6, NF-κB, let-7, and LIN28B. From a therapeutic point of view, this study and others raise the possibility that tumors with this overactive positive feedback loop—LIN28B^{HIGH}/Let-7^{LOW}/IL-6^{HIGH}—may be eradicated efficiently by interference with this loop, which also may inhibit the growth of cancer stem cells.

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Compete Globally, Bud Locally

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How cells generate a single axis of polarity for mating, division, and movement is unknown. In this issue, Howell et al. (2009) use a synthetic biology approach to demonstrate that rapid competition for a soluble signaling component (Bem1) is essential to ensure a unique axis of polarity in budding yeast.

Cell polarity underlies many fundamental cell processes such as directional migration, cell division, and mating. Because of its powerful genetics, the budding yeast *Saccharomyces cerevisiae* is one of the most thoroughly understood examples of cell polarity. Budding yeast undergo isotropic growth (uniform growth in all directions) for most of their lives but polarize their growth under two circumstances: when they divide by forming a bud and when they mate by forming a mating projection called a "shmoo." In both cases, activation and recruitment of the small GTPase Cdc42 in one part of the cell directs trafficking and secretion of cell wall-modifying enzymes to that single location at the cell periphery. By rewiring the yeast signaling cascade that directs Cdc42 polarization, Howell et al. (2009), reporting in this issue, demonstrate that competition for the rapidly diffusing Bem1 protein is essential to ensure that each yeast cell has only a single bud site.

Cdc42 activation in yeast is normally biased by internal landmarks such as the previous bud site in the case of cell division or by external landmarks such as the pheromone gradient in the case of shmoo production. Remarkably, even in the absence of internal and external landmarks, there is spontaneous polarization of Cdc42 activation and recruitment, demonstrating the strongly self-organizing nature of cell polarity in these cells. Genetic and pharmacological analyses of budding yeast have revealed two positive feedback loops that may contribute to polarity (Figure 1A). The first is a signaling-based positive feedback loop in which active Cdc42 locally recruits the scaffolding protein Bem1 from the cytoplasm to the plasma membrane. Bem1 interacts with a guanine nucleotide exchange factor (GEF) for Cdc42, known as Cdc24, which locally activates more Cdc42, thereby leading to autocatalytic activation of Cdc42 (Butty et al., 2002). The second feedback loop is based on the ability of active Cdc42 to generate actin cables, which act as tracks to locally transport vesicular Cdc42 to the plasma membrane, also resulting in autocatalytic recruitment of Cdc42 (Wedlich-Soldner et al., 2003). GTPase-dependent positive feedback loops may also form the core of polarity in other contexts, such as movement and morphogenesis in neutrophils, the slime mold *Dictyostelium*, and neuronal cells (Brandman and Meyer, 2008). Furthermore, evidence in these systems for both soluble signaling and cytoskeletal-based feedback loops suggests a broadly conserved architecture of signaling for directing cell polarization.