sient uncapped state. In mammalian cells, capping is carried out by a protein complex, dubbed "shelterin" (de Lange, 2005), which may act in part by promoting the formation of a protective structure, the T loop, in which the chromosome 3′ single-strand terminus is folded back and buried in a more internal sequence. Thus, it will be interesting to see if and how mammalian KEOPS (whose existence is still hypothetical) impinges upon shelterin or other telomeric factors in mammalian cells.

This elegant study is certain to lead to additional important insights into both the nature of the telomere cap and the mechanism of telomerase regulation and highlights the close relationship between the two that was first revealed by studies of Cdc13 (Nugent et al., 1996). The discovery of KEOPS as a new key player in telomere function is an exciting development that underscores our still incomplete understanding of telomere biology, as well as the utility of the budding yeast *S. cerevisiae*, which continues to provide new genes with which to build models.

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Cellular Conference Call: External Feedback Affects Cell-Fate Decisions

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Cells often need to respond to multiple opposing signals simultaneously. In this issue of *Cell*, Janes et al. (2006) show that challenging cells with multiple extracellular signals induces an external feedback that involves the release of and response to additional secreted factors with antagonistic functions. These results suggest that an individual cell's decision to die or survive is not wholly independent but depends, at least in part, on feedback from its neighbors.

Life requires us to make decisions every day. Usually, our first step is to take the information available and process it ourselves. However, when crucial decisions arise, we often ask advice from friends, family, and colleagues. Many times we get varying, even opposing, advice and we are left with the challenge of weighing these suggestions with our own perspective of the situation (or with our own instincts).

Cells have this problem too. They often need to respond simultaneously to multiple external signals, many of which are contradictory and promote opposite cellular events. For example, different cytokines that activate or inhibit cell survival can compete with each other in the context of tissue homeostasis. The response may also differ from cell to cell, depending on the cell's own internal state. How do cells cope with extracellular signals that antagonize each other, and how does the pre-existing state of the cell affect the final outcome?

In this issue of *Cell*, Peter Sorger and colleagues (Janes et al., 2006) study cellular responses to opposing extracellular signals using high-throughput time-resolved measurements of multiple signaling proteins. The results are surprising: processing an extracellular signal is not only an intracellular event but also involves the release of and response to additional secreted factors with antagonistic functions. This finding brings to the forefront the importance of autocrine signaling in determining

cell fate and suggests that an individual cell's decision to die or survive includes a degree of active consultation with like-minded neighbors.

Janes et al. (2006) studied the response of human epithelial cells to a combination of the pro-death cytokine Tumor Necrosis Factor (TNF) and the pro-survival factors Epidermal Growth Factor (EGF) and insulin. The TNF family of cytokines is produced by the immune system and has cytotoxic effects in many tissues. TNF has multiple effects on cells, one of which is to promote apoptosis via cleavage of caspase 8 and activation of JNK and NF-κB. In contrast, EGF and insulin stimulate proliferation of various cell types and contribute to the survival and growth of many cancers. Each of these cytokines has been well studied, both biochemically and using "omics" approaches. From previous studies, we have a clear idea of the linear pathways of responses triggered in response to an individual cytokine (see, for example, Chen and Goeddel, 2002; Downward, 2001; Yarden and Sliwkowski, 2001), and a number of maps of protein-protein interactions around the pathways of interest (see, for example, Bouwmeester et al., 2004). By studying cellular responses to multiple inputs over time, Janes et al. (2006) have now been able to demonstrate functional interactions between these signaling pathways and have been able to show that autocrine and paracrine signals are an integral component of the overall cellular response.

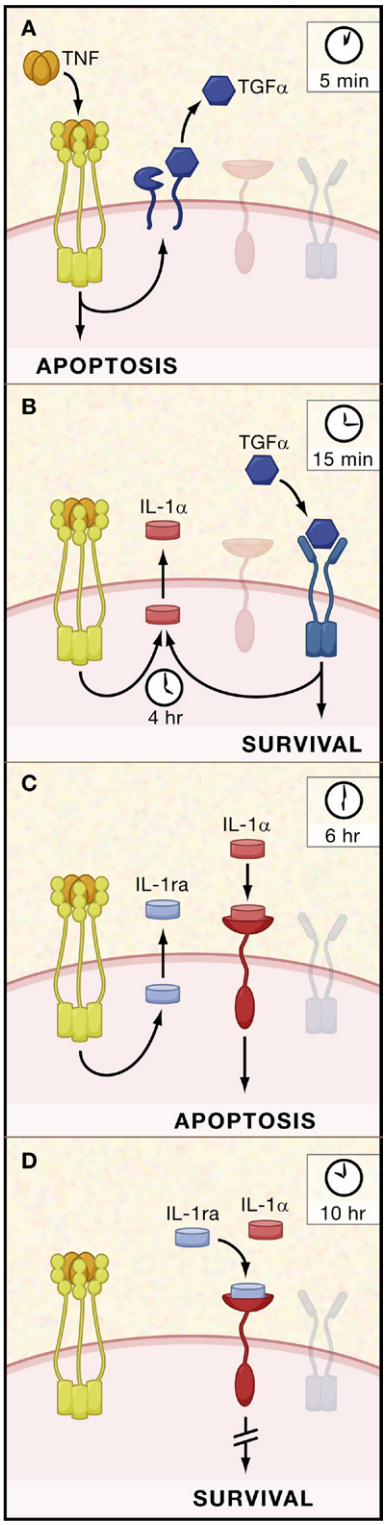

Figure 1. TNF Signaling Induces Contradictory External Feedbacks

(A) After binding to its receptor, TNFα activates a well-known intracellular signaling pathway that induces apoptosis via activated caspase 8. In addition, it promotes the release of the EGF receptor ligand, TGFα, in an immediate response, most likely by activating matrix metalloproteinases.

(B) TGFα binds to its receptor in an autocrine fashion, starting the MAP kinase cascade and thereby providing a pro-survival signal to the cell. Together with the TNF receptor activity, TGFα induces the subsequent secretion of a third cytokine, IL-1α.

(C) IL-1α activates its receptor and sends, via the NF-κB transcription factor, a pro-apoptotic signal to the cell. This signal is balanced, as TNF induces the release of IL-1ra, a receptor antagonist, at later time points.

(D) IL-1ra completes the autocrine cascade induced by TNF signaling by competing with IL-1α for receptor binding and thereby again providing an anti-apoptotic signal. For simplicity the figure shows the secreted cytokines affecting the same cell, but as discussed in the text it is possible that neighboring cells are affected as well or instead.

Janes et al. (2006) selected nineteen signaling species, from kinases to proteases, that represent the information flow generated by exposure of human epithelial cells to one of ten combinations of TNF, EGF, and insulin and then measured the signals at ~20 time points over 24 hr. The analysis is far from comprehensive for technical limitations: ideally, the authors would have been able to measure the formation of key species such as the TNF-dependent DISC complex and the panoply of TNF-mediated transcriptional events. Nonetheless, the almost 8,000 data points collected in this study represent one of the most complete data sets on a dynamic cellular response available to date.

To make sense of this flood of data, Janes et al. (2006) used a variant of principal component analysis (Gaudet et al., 2005; Janes et al., 2005) in which factors responsible for the activation of individual signaling species are ordered with respect to cellular outcome. As applied by Janes et al. (2006), this analysis revealed the extent to which kinases, such as ERK or the EGF receptor, are activated by TNF, by EGF, or by both cytokines. Remarkably, EGF receptor is activated to a similar extent by TNF and by its cognate ligand EGF.

The astonishing upshot from these analyses is that TNF induces a cascade of extracellular signals, alternately promoting death or survival of the cell. In addition to activating well-characterized intracellular pathways, TNF induces the shedding of the EGF receptor ligand TGFα (a pro-survival factor) as an immediate response (see Figure 1). This new combination of the TNF and EGF receptor pathways acts as an AND gate to stimulate the secretion of a third cytokine, the pro-apoptotic IL-1α. And finally an antagonist of the IL-1α receptor, IL-1ra, is secreted, providing a second pro-survival signal. In separate experiments, Janes et al. (2006) were able to confirm the existence of each of these external signaling events

using a variety of antagonists, for example antibodies against the relevant cytokine receptor.

What are we to make of this intricate concert of cytokine signals? Janes et al. (2006) have demonstrated a new mechanism for crosstalk between pathways: signal integration and information processing can occur via cytokine secretion. This opens the possibility that cell-fate determination does not depend only on intracellular events: because some of the positive and negative feedback loops used for information processing are external (not just internal), cell-fate decisions may be the result of a community effort.

It is well known that negative and positive feedback circuits are important for the dynamic behavior of many intracellular signaling pathways (see, for example Ferrell, 2002; Harris and Levine, 2005). It is exciting to discover that external feedback also affects cell survival. It is often assumed that autocrine signaling represents a pathological state found primarily in transformed cells. However, growing evidence points to a key role for EGF autocrine signals in a variety of cell-fate decisions in normal cells. Context-dependent extracellular positive feedback on the MAP kinase pathway has been shown to involve EGFR, Ras-MAPK signaling, and a ligand-releasing protease (Shvartsman et al., 2002). Very recently, VEGF-mediated angiogenesis has been shown to involve EGF, probably acting in an autocrine fashion (Semino et al., 2006). It seems likely that external feedbacks that allow two-way communication between cells and their neighbors will be important in other signaling pathways.

In some ways it might seem that the work of Janes et al. (2006) has only made our lives more complicated. We have exchanged the problem of how signals from two or three cytokines are integrated for even a more complicated problem: How does the cell integrate four sequential opposing signals that arise at different times (Figure 1)? What this work makes clear is that in order to understand the responses of mammalian cells we need to consider the environment in which cellular decisions are made, as well as the evolution of the response over time. The history and state of the cell will presumably also be part of determining the pattern of responses. Even the different epithelial cell lines that Janes et al. (2006) studied had subtly different dynamic responses; if we consider the distinct environments that different cell types will experience in the body, it is clear that these differences could be amplified to generate wildly varying behavior.

Because Janes et al. (2006) used biochemical methods to study populations of cells, they were unable to determine whether the responses are uniform across the entire population or vary at a single-cell level. It seems highly likely that there is some variability in the responses of individual cells; the question is, how much? Are the cells that secrete TGFα the same as the ones that respond to it, or is it only the neighbors of the secreting cell that produce IL-1α? Single-cell studies will be needed to determine how far, if at all, these signals spread, and how exposure to the different signals correlates with cell fate. We also need to find ways to probe how the cell's original internal state (for example, its cell-cycle stage, age, or the integrity of its genome) affects the response and the final outcome: does the cell have the equivalent of an "instinct" about how it should behave in a certain situation, and if so what does it consist of? Increasingly, we have the ability to ask and answer these fascinating questions, and the next few years should see many advances in this area.

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