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Preview

Of Elections and Cell-Death Decisions

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A new study in the journal *Nature* (Spencer et al., 2009) argues that cell-to-cell variation in the decision to undergo apoptosis is not due to genetic, epigenetic, or cell-cycle differences, nor due to random molecular noise, but instead is determined by differences in protein abundances.

Anyone who has followed recent U.S. presidential elections is aware of the important distinction between "swing voters" and the "party base." Swing voters are those who have not decided for whom they will cast their vote and whose decision may yet be swayed by the merits of future arguments; they receive the bulk of each campaign's attention. However, the majority of votes are cast by each campaign's base; these voters appear to be refractory to argument and persuasion, insensitive to political messages, and predetermined in how they will cast their votes.

The cellular decision to undergo apoptosis was generally thought to rely on careful weighing of intracellular biochemical arguments for and against cell suicide. Within a monoclonal population, not all cells reach the same decision, a phenomenon thought to be physiologically important for organ homeostasis but frustrating for cancer therapy. Stated formally, the "fractional cell kill hypothesis" (Skipper, 1978) argues that exposure to a particular dose of drug will kill a constant fraction of tumor cells, irrespective of the total number of cells present. Surviving cells treated a second time will again show the same fraction of apoptosis. The mechanism underlying this phenomenon was thought to be so-called "intrinsic molecular noise," inherent stochasticity in the molecular signaling reactions triggered by the death-inducing stimulus. In a thorough combined experimental and computational analysis, a recent *Nature* paper from the National Institutes of Health (NIH)-funded Cell Decision Process Center turns this assumption on its head (Spencer et al., 2009). Their analysis argues that even cell-death decisions are largely predetermined, more akin to the political base than to swing voters (Figure 1). Variability in this decision is not due to genetic, epigenetic, or cell-cycle differences, nor due to random molecular noise, but instead is determined by differences in protein abundances at the time of stimulation.

In their new study, the authors make use of a biochemically inactive reporter wherein RFP is fused to the Smac N-terminal mitochondrial import sequence (Albeck et al., 2008a), thus facilitating detection of mitochondrial outer membrane permeabilization (MOMP), a switch-like point of no return in the intrinsic cell-death pathway (Albeck et al., 2008b). Using this reporter in HeLa and MCF10A mammary epithelial cells, the authors observe a high degree of variation in the time (T_d) between addition of the TRAIL ligand and MOMP. This variation vanishes when considering only recently divided sister cells, demonstrating that closely related cells tend to die at the same time in response to TRAIL, whereas unrelated cells do not. TRAIL responsiveness, therefore, is a heritable state, but it is only transiently inherited. By 50 hr, the correlation in T_d between sister cells is no greater than that between any two cells selected at random.

This simple but fundamental observation, also made in an independent study published earlier this year (Rehm et al., 2009), posed several new questions. How is TRAIL responsiveness encoded? How is it passed from mother to daughter? Why does it degrade over time? As the authors point out, by virtue of its transience, neither genetic nor epigenetic encoding is likely (Rando and Verstrepen, 2007). By leveraging a previously constructed mathematical model of the biochemical events governing the cellular response to TRAIL, the authors demonstrate that variability in the abundances of key signaling proteins is sufficient to explain the observed variability in T_d. This suggests that TRAIL responsiveness is in fact encoded in the proteome; symmetric partitioning of the proteome between daughter cells ensures that recently divided daughter cells respond similarly. Random fluctuations in protein synthesis and degradation erode that symmetry over time, reducing the correlation in T_d.

The idea that the cellular homeostatic state affects cell signal processing is not new. In 2006, collaborating groups from the same Cell Decision Process Center demonstrated that infection with an E1/E3-deleted adenoviral vector sensitizes human epithelial cells to tumor necrosis factor-induced apoptosis by elevating the basal activity of the prosurvival kinase Akt (Miller-Jensen et al., 2006). Similarly, a combined computational and experimental study demonstrated that the basal activity of the NFκB-activating kinase IKK determines this signaling system's responsiveness to ribotoxic stress (O'Dea et al., 2008). Cells with elevated IKK activity, due to conditioning in an inflammatory environment, show synergistic NFκB activation, whereas cells with no prior inflammatory history do not. This work and others like it have clearly established that stimulus responsiveness is a function of the cellular homeostatic state and that cellular state can be altered by extrinsic factors such as viral infection and inflammation. Now Spencer et al. (2009) provide compelling evidence that variation in the cellular state does not result solely from a definable perturbation but may arise spontaneously in an isogenic cell population. This state is heritable, transient, and encoded by the proteome.

One significant implication of this work pertains to our understanding of the role of noise in the cell-death decision, and

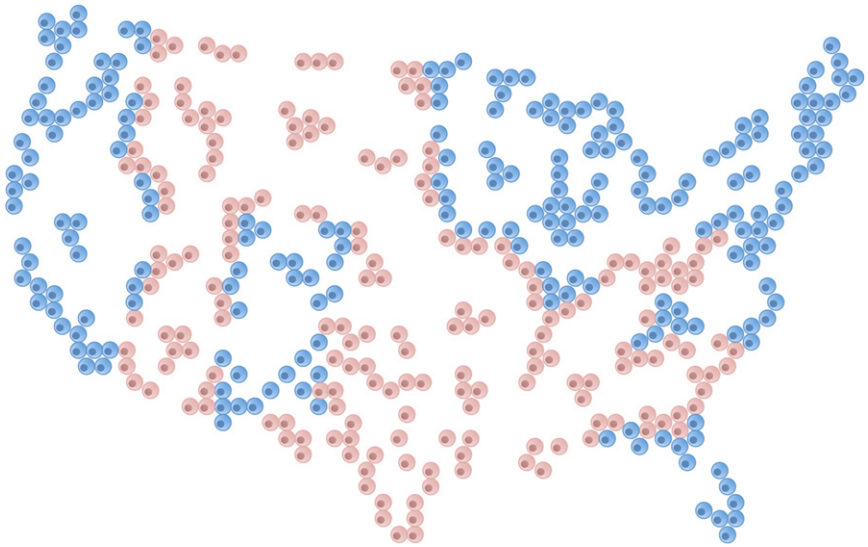

Figure 1. Homeostatic Predeterminism in Presidential Candidates and Cancer Therapies
U.S. presidential election voting patterns are largely predetermined and insensitive to campaigning, often having been established long before election season. New experimental and theoretical work by Sorger and colleagues at the Cell Decision Process Center (Spencer et al., 2009) argues that an isogenic population of cells responds to the external apoptotic stimulus TRAIL in much the same way. Abundances of key proteins determine the sensitivity to the stimulus, as measured by the time elapsed between addition of the stimulus and MOMP. Variability in TRAIL sensitivity is caused by variability in these protein abundances, not by intrinsic stochasticity in the biochemical events mediating the response. This observation has implications for the design of anticancer therapies, as it suggests that drug sensitivity is knowable, deterministic, and protein encoded.

perhaps many other cellular decision processes as well. Prior to this study, variability in the cellular response to stimulus was viewed as a function of random fluctuations in the processing of the stimulus itself. Here the authors argue that TRAIL processing is completely deterministic but dependent on the state of the cell prior to stimulation. This state, encoded by the abundances of key signaling molecules, is itself a function of gene expression. Gene expression is noisy (Raser and O'Shea, 2005), and this noise manifests itself as variability in protein abundance and, over time, proteomic drift between once-similar sister cells (Sigal et al., 2006). One consequence of this hypothesis pertains to attempts to mathematically represent biological processes: though much effort has been invested in developing mathematical modeling approaches that mimic molecular stochasticity, the new work suggests that the cell-death decision may be modeled by using a traditional deterministic framework.

While these considerations may seem academic, the realization that there is little intrinsic noise in the cell-death decision may impact therapeutic design. With regards to TRAIL, for which a number of mimics are in early clinical trials, can we determine a priori whether a target cell is sensitive to the drug? Can we improve sensitivity using a cotreatment? To borrow a page from politics, how can we appeal to the other side's base? If stimulus responsiveness is indeed a cellular state imparted by the abundances of many proteins, this is not a state that can be effectively measured with current technology. As demonstrated by the authors, however, a mathematical model can be used to identify the most predictive features of the cellular state, alleviating the need for complete knowledge of the cellular proteome in order to predict the effectiveness of a particular stimulus.

In addition, the findings of Spencer and colleagues lead to several new questions. First, while the authors have convincingly demonstrated that T_d is a transiently heritable trait encoded in protein abundances, we cannot say for certain that the same is true of fractional killing. Time to MOMP is undoubtedly related, but not equivalent, to the cell-death decision itself. Second, and relatedly, much of their study used conditions of negligible protein turnover. However, the timescale of T_d is very much on the order of inducible gene expression, and the TRAIL ligand is known to activate a number of transcription factors that may well impact the death decision. Therefore it remains to be investigated how protein synthesis may affect MOMP and the ultimate decision of whether or not to apoptose. Progress in answering these questions is likely to involve combined computational and experimental studies of the sort so effectively employed by Spencer and colleagues.

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