between HIF-1α and Notch 1 ICD. To further characterize this, a series of truncated HIF-1α constructs were expressed in P19 cells together with a Notch ICD plasmid, finding that there are two interaction domains in HIF-1α, one located in the N-terminal domain of HIF, spanning residues 1–390, and the second between residues 390 and 531. Interestingly, cotransfection of HIF-1α and Notch 1 ICD resulted in increased expression of the 12XCSL-luc plasmid, both at normoxia and hypoxia, but the truncated forms of HIF-1α failed to do so, demonstrating that only a transcriptionally active form of HIF-1α can increase Notch signaling. Finally, the authors demonstrate with chromatin immunoprecipitation assays that HIF-1α is recruited to the Hes Notch-responsive promoter under activation of both Notch signaling and low oxygen levels; they also show, using HIF-1α mutant cell lines, that Notch transcriptional activation during hypoxia requires HIF-1 function.

These findings suggest a new mode of action of HIF-1α under hypoxia that differs from the canonical response, in which it needs to dimerize with HIF-1β in order to activate the transcription of hypoxia-responsive genes. Based on these data, the authors propose a model in which HIF-1α, once stabilized by hypoxia, interacts with the Notch 1 ICD and is an active part of the Notch 1 ICD/CSL transcriptional complex. There, HIF-1α would contribute to stabilize Notch 1 ICD and would enhance the transcriptional activity of the complex through the recruitment of coactivators such as CBP/p300. This model has strong similarities with the mechanism of interactions between Notch and BMP/TGF-β signaling pathways, in which the intracellular mediators SMAD1 and SMAD3 interact with Notch 1 ICD, and there is no need of SMAD binding to DNA to promote a response (Blokzijl et al., 2003; Dahlqvist et al., 2003). Whether this model of interaction with Notch is followed by other stimuli promoting stem cell dedifferentiation remains to be elucidated.

Despite the evidence suggesting a major role for HIF-1α in the interaction between hypoxia and Notch, the involvement of other hypoxia-related molecules cannot be ruled out. Whereas Notch regulation seems to be independent of pVHL, immunoprecipitation experiments showed FIH-1 physically interacts with Notch 1 ICD. Also, FIH-1 expression in P-19 cells decreased 12xCSL-luc activity when coexpressed with HIF-1α, but also in its absence, suggesting a more direct role of FIH-1 in the regulation of Notch signaling; this will certainly be worthy of study in the future.

It would also be interesting to study the effect of Notch in the hypoxic response. Even though Notch 1 ICD was not recruited to the promoter of the hypoxia responsive gene PGK-1, the absence of the Notch ligand Serrate-1 in the culture caused a decrease in the amount of HIF-1α bound to the promoter, and incubation with L-685,458 decreased the mRNA expression of the HIF-1 target PGK-1 at normoxia and hypoxia. The interaction between these two essential pathways thus appears to be robust, and will likely spawn a great deal of further effort to understand what was first approached in embryo cultures more than 30 years ago: the role played by oxygen in regulating developmental fate.

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Developmental Cell, Vol. 9, November, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.10.004

Tracing the Sources of Cellular Variation

As the adage says, variety is the spice of life, and despite our best attempts, cells, even those with the same genome, never seem to behave the same. By combining mathematical and experimental analyses, Colman-Lerner and colleagues propose, in a recent issue of *Nature*, a method to delicately unravel the sources of this variation (Colman-Lerner et al., 2005). Applying their technique to the pheromone response in budding yeast, they show that much of the observed variation originates from cell cycle effects and is dependent on levels of pathway input.

Ultimately, cell-to-cell differences are caused by the

stochastic nature of biochemical reactions. Reactants come together by diffusion, their motion driven by rapid and frequent collisions with other molecules. Once together, these same collisions alter reactant internal energies, and so their propensity to react. Both effects cause individual reaction events to happen randomly and drive the overall reaction process stochastic.

Is this stochasticity, or "noise," important in vivo? Intuitively, stochasticity is only significant when mean numbers of molecules are low; then, individual reactions, which at most change the numbers of molecules by one or two, matter. Low numbers are not uncommon intracellularly; gene copy number is typically one or two, and transcription factors, at least for bacteria, frequently number in the tens. However, unambiguously measuring stochasticity can be challenging. Naively, one could place green fluorescent protein (GFP) downstream of a promoter that is activated by the system of interest. By measuring the variation in fluorescence across a population of cells, the noise in the system could be estimated. However, every biochemical reaction is potentially noisy. Fluorescence variation could be due to noise in the process under study or could result from the general background "hum" of stochasticity: stochastic effects in ribosome synthesis lead to different numbers of ribosomes and so to differences in gene expression in each cell, stochastic effects in the cell cycle machinery desynchronize the population, stochastic effects in signaling networks make each cell's response its own, and so on.

Variation has, then, two classes: intrinsic stochasticity, which arises from fluctuations in the reactions of the system of interest, and extrinsic stochasticity, which originates from fluctuations in other cellular processes that interact with this system (Swain et al., 2002; Elowitz et al., 2002). To determine whether variation is intrinsic or extrinsic, it helps to visualize an identical second copy of the system, present in the same cell and exposed to the same intracellular environment. For example, take a simple system like constitutive expression. Imagine another copy of the gene, with an identical promoter and ribosome binding site, present in each cell. Variation in the number of free ribosomes will equally affect both system copies; expression from both genes will fall if the number of free ribosomes falls, and will rise if the number of free ribosomes rises—an extrinsic variation. Variation in the number of actively translating ribosomes, however, is intrinsic; it can be varied independently for each gene system. The same technique works experimentally (Elowitz et al., 2002): Two distinguishable alleles of GFP are placed downstream of identical promoters. The intrinsic noise is given by the variation in the difference in concentration of the two alleles, the total noise is determined from the variation in either one of the alleles, and then a simple relationship between these measurements gives extrinsic noise (Swain et al., 2002). Stochasticity in gene expression has thus been quantified in both bacteria (Elowitz et al., 2002) and yeast (Raser and O'Shea, 2004).

The work by Roger Brent's group cleverly extends this technique and applies it to an endogenous cellular network, the yeast pheromone pathway. In budding yeast, pheromone activates a G protein-coupled receptor, the MAP kinase pathway, and ultimately a transcription factor, Ste12. By placing yellow fluorescent protein (YFP) downstream of a Ste12-activated promoter, Colman-Lerner et al. (2005) have a read-out for path-way activity. Pheromone response is funneled through two subsystems: a signaling pathway subsystem and a gene expression subsystem, which synthesizes reporter. Variation in observed fluorescence is therefore set by intrinsic and extrinsic stochasticity in both the pathway and the expression subsystems.

By placing cyan fluorescent protein (CFP) downstream of an identical copy of the Ste12 activated promoter, Colman-Lerner and colleagues used the variation in the difference in the CFP and YFP levels to quantify intrinsic stochasticity in the expression subsystem. To go further than previous work, however, they created a new strain with CFP placed downstream of another promoter entirely unconnected to the pheromone response. They realized that variation in the difference between CFP and YFP levels in this strain is not only determined by the intrinsic stochasticity in the gene expression subsystems, but also by the stochasticity in each pathway subsystem. By creating a collection of strains, each having CFP and YFP downstream of carefully chosen combinations of promoters, they subdivided and quantified total observed variation into three contributions: total stochasticity in the pathway subsystem, and both intrinsic and extrinsic stochasticities in the gene expression subsystem.

What then generates variation? Colman-Lerner et al. (2005) determined that both subsystems contributed significantly. Interestingly, however, they find that their relative importance shifts as the strength of pathway input changes. At lower pheromone concentrations, stochasticity in the pathway subsystem contributes three times as much to total variation as it does at higher pheromone concentrations. In agreement with other studies (Elowitz et al., 2002; Raser and O'Shea, 2004; Rosenfeld et al., 2005; Pedraza and van Oudenaarden, 2005), intrinsic gene expression noise played a minor role.

While quantifying the different contributions to variation is important, a greater challenge is to discover specific sources of stochasticity. The Brent group found that inhibiting cyclin-dependent kinase Cdc28 caused variation to almost halve in cells exposed to high pheromone levels. As cells respond variably to pheromones at different stages of the cell cycle, differences in cell cycle position could increase variation in pheromone response. Similar results also hold for bacteria (Rosenfeld et al., 2005), where correcting for desynchronization of cells significantly reduced noise in the output of a simple gene network.

Their study raises several intriguing questions. Once the role of Cdc28 in setting stochasticity is factored out, where is the rest of the variability being generated? What are both the total and phosphorylated protein concentrations of the mating pathway components at different pheromone concentrations? Does their cellular location influence pathway stochasticity? The principal reporter promoter used, PRM1, normally drives a membrane protein that localizes to the schmoo tip (Heiman and Walter, 2000; White and Rose, 2001). What biological consequence does the variable expression of PRM1 have on the mating efficiency of cells? If cells expressing low levels of PRM1 mate fine, then the variability of pheromone response seen through this reporter could be of little biological significance. Stochasticity is often postulated as both a hindrance to reliable cellular information processing and as a potential resource for cells to exploit. The onus is now not to further demonstrate the existence of stochasticity, but to pin-point its biological relevance.

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