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# Bursting the Notch Bubble: New Insights into *In Vivo* Transcriptional Dynamics

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**In this issue of *Developmental Cell,* Lee et al. (2019) and Falo-Sanjuan et al. (2019) examine the transcriptional dynamics of Notch signaling *in vivo*. Both groups show that Notch signaling produces "bursty" transcriptional responses and that variance in these responses stems from changes in duration, not frequency, of transcriptional bursts.**

Signaling cascades that instruct transcriptional changes are highly dynamic and context specific. In particular, induction of transcription can be sustained, with relatively constant production of mRNA, or "bursty," with sequential, episodic, on and off periods of transcription. The pattern of transcription, and subsequent gene expression, is critical for instructing cellular behavior. For example, oscillatory expression of Notch-dependent genes instructs neural stem cell self-renewal, while sustained expression causes differentiation (Kageyama et al., 2018). Embryonic development is a particularly interesting context to study the mechanisms of signaling cascades, because signaling molecules are present in known gradients that govern cell behavior. However, the molecular mechanisms and dynamics of signal-induced gene transcription require further investigation, especially *in vivo*. The Notch signaling pathway is a major regulatory signaling pathway that is central in developmental processes and mature organisms. Binding of ligands to the Notch receptor on the cell surface of the signal-receiving cell leads to cleavage of the receptor that frees the Notch intracellular domain (NICD), which can enter the nucleus and directly activate transcription of Notch-regulated genes. The molecular mechanisms of how Notch, through NICD, induces transcriptional dynamics such as bursting is largely unknown. A single previous study analyzed the stochastic nature of Notch-activated transcription using smFISH (Lee et al., 2016). This work set the stage for the first live imaging analysis of Notch-dependent transcriptional dynamics *in vivo*. In this issue of *Developmental Cell*, two independent studies by Lee et al. (2019) and Falo-Sanjuan et al. (2019) use *C. elegans* and *Drosophila*, respectively, to show that Notch signaling is bursty *in vivo* and that Notch regulates the burst duration (ON time), rather than pause duration (OFF time), of transcription (Figure 1). Furthermore, Falo-Sanjuan and colleagues explore exciting new insights into the molecular aspects of enhancer regulation that mediate Notch-induced transcriptional bursting dynamics.

Both studies use the MS2/MCP system (Abbaszadeh and Gavis, 2016), which employs MCP-GFP fusion proteins that bind to MS2 stem loops in nascent mRNA to visualize endogenous transcript production in developing embryos. Lee et al. (2019) used this system to study the Notch target *sygl-1* in germline stem cells (GSCs) of live *C. elegans*. They first showed that the graded response to Notch signaling seen in the GSC niche occurs due to the duration of transcriptional bursts being longer where NICD is higher, while burst intensity and transcriptional pause duration do not change. Unlike noisy transcription seen in *in vitro* systems (Corrigan and Chubb, 2014), transcriptional activity outside of the GSC niche was extremely low or undetectable, indicating that *in vivo* co-repressors or other silencing factors help to define boundaries for Notch signaling during development. They further found that transcription at a single locus is stochastic in that the duration of one transcriptional burst does not determine the duration of the subsequent burst, though there is a moderate correlation between consecutive burst intensities at any particular locus. Next, the authors used a mutant GLP-1/Notch receptor with weakened NICD transcriptional activity to confirm that *sygl-1* transcriptional burst duration and intensity, but not pause duration, are directly regulated by Notch signaling through NICD-dependent transcriptional activation. The major takeaway from this study is that native Notch signaling in nematode GSC is in fact bursty, and that despite its dynamic nature, this signaling is still highly regulated—not noisy as previously thought (Desponds et al., 2016; Padovan-Merhar et al., 2015). More specifically, Notch regulates transcriptional dynamics by elongating the transcriptional burst length, but not the pause length, which is in contrast to other transcriptional regulators such as Wnt signaling (Kafri et al., 2016).

In parallel, Falo-Sanjuan et al. (2019) also found that transcriptional dynamics are regulated by NICD-dependent lengthening of the burst length, with no change in pause length, in embryonic *Drosophila* mesectoderm. They used both mathematical modeling and an MS2/MCP system with Notch downstream target enhancers, *singleminded* (*sim*) and *Enhancer of split-Complex*

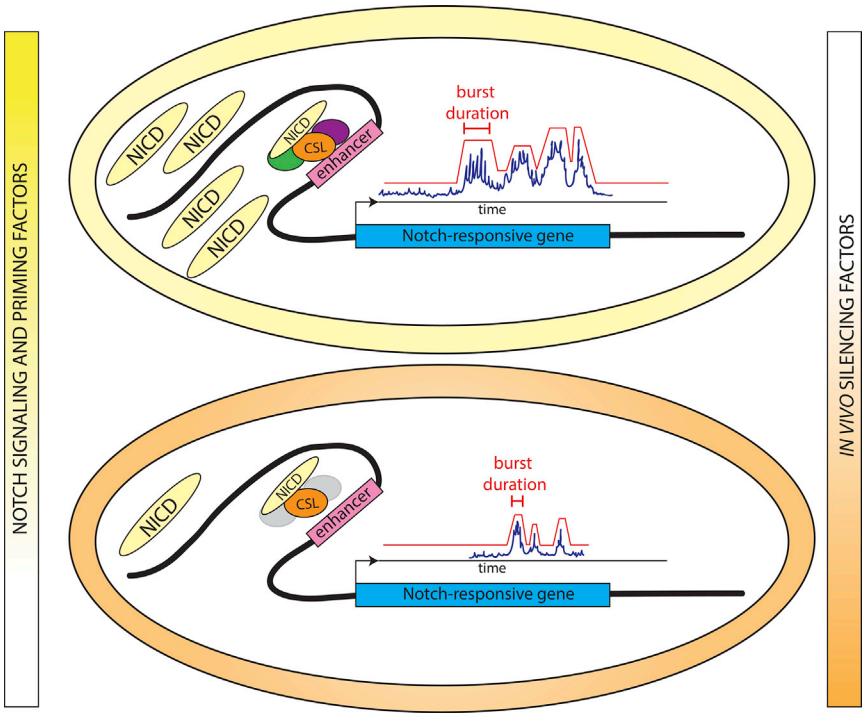


**Figure 1. Cells Respond to Notch Signaling through NICD by Altering the Transcriptional Burst Duration of Target Genes**

Burst duration is increased when high levels of NICD are present (top) and is short or stochastic when low levels of NICD are present (bottom). *In vivo* silencing factors can repress transcriptional responses to Notch signaling, while priming co-factors can bind enhancers to promote sustained responses to Notch signaling.

(*m5*/*m8*). They showed that the temporal dynamics of transcription activation are surprisingly shared between *sim* and *m5*/*m8*, despite these enhancers having different activation thresholds and unique molecular compositions. In particular, the enhancers produce a synchronized onset to transcription that is sustained for long bursts of about 30–50 min. Upon ectopic NICD expression, each enhancer responds if the concentration of ectopic NICD is above the necessary signal threshold to activate the enhancer, but the response is distinct from the response to comparable levels of endogenous NICD. This suggests that other factors likely bind to the enhancers to "prime" them for a particular response to Notch signaling (Figure 1). The authors identified two factors, Twist and Dorsal, which can bind both the *sim* and *m5*/*m8* enhancers and lower the threshold of NICD necessary for enhancer activation. These factors also promote sustained rather than bursty transcription. Finally, the authors found that CSL binding motifs in *sim* and *m5*/*m8* enhancers do not alter threshold detection of NICD but do regulate the transcriptional burst size (amplitude and duration) upon activation. The major findings of this work are that the *sim* and *m5*/*m8* enhancers act as threshold detectors of NICD and modulate transcriptional burst duration, and that the dynamics of sustained versus bursty response to Notch signaling depends on the individual enhancer of the target gene. Enhancer response dynamics vary depending on priming factors like Twist or Dorsal, which can toggle enhancer response between stochastic or sustained transcriptional responses to allow for heterogenous responses to Notch signaling across an embryo.

Both of these papers support the theory that burst duration, but not frequency, is the primary parameter modulated by Notch signaling *in vivo* and that this is conserved across genes and species. This is an important contribution to the field, which to date has posited that burst frequency is what mediates the dynamic nature of transcription. It also highlights the importance of studying signaling in a native context, as both papers show that cells are primed to respond to Notch in a particular pattern and that this contributes to clean boundaries of transcriptional response during embryonic development. It is highly likely that these findings apply to other canonical signaling pathways, which would be worth examining *in vivo* in future studies.

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