**A revised Yan network model**

In light of our novel quantitative observations of dynamic Yan expression in both progenitor and differentiating cells, we have prepared a revised model of the cell autonomous Yan network progression during development. In particular, we chose to simulate the stochastic dynamics within a cell with the widely used Gillespie algorithm [149,150] as it allows us to compare noise features to experiment. Our revised model is focused on providing insight into the counterintuitive effects of the experimental modulation of EGFR-Ras signaling and downstream components. Specifically, we allow low levels of Pnt proteins to activate Yan transcription in addition to their typically accepted role in accelerating Yan decay as the means of producing the suggested dual role for EGFR-Ras signaling in modulating Yan expression. We also note that our chosen network configuration is monostable, and demonstrates that such a model is consistent with experimental observations with appropriate signaling inputs.

Our stochastic model is comprised of a core set of molecular species with known roles in Yan regulation (NETWORK SCHEMATIC FIG). Based on our earlier finding that monomeric Yan is severely impaired in function while dimeric Yan is nearly as functional as wildtype [151], we model Yan dimerization but not higher-order oligomerization. We then use thermodynamic equilibrium binding site models to determine the occupancies of the Mae, miR-7, and Yan promoters, which are subject to competition between repression by Yan dimer and activation by PntP1 and phosphorylated PntP2 (pPntP2). We parametrize these binding site models such that Pnt proteins bind the Yan promoter more strongly than the Mae or miR-7 promoters. This allows low levels of Pnt protein to activate Yan transcription, while higher levels activate Mae and miR-7 transcription. We model the formation of Mae-Yan and Mae-pPntP2 complexes to incorporate inactivation by Mae interaction. Yan transcription and translation are modeled separately, with translation subject to inhibition by miR-7 - Yan mRNA complex formation. The production of Mae and miR-7 as well as the induction of PntP1 by phosphorylated PntP2 are approximated as one-step processes. The influence of external Notch and EGFR inputs are represented by manually changing levels of Su(H) and dpERK in time. In our model, Su(H) activates Yan transcription, while phosphorylation of PntP2 and Yan by dpERK is approximated with Michaelis-Menten enzyme kinetics. In total, our model consists of 33 individual induction, degradation, or complex formation reactions.

Results from our stochastic simulations reproduce experimentally observed Yan dynamics (MODEL FIG). Initially, Yan is produced and maintained at a low baseline level. At 10 hr, low levels of Su(H) and dpERK are introduced to multipotent cells. The dpERK phosphorylates a small fraction of PntP2, and following experimental observations made from EGFR mutants (EGFR FIG), in our model PntP1 activates Yan transcription at low copy number. While some Mae and miR-7 are produced, their level of induction is not high enough to repress initial Yan induction. This low level of dpERK is removed from multipotent cells at hour 25, and Yan levels slowly decrease (MODEL FIG). When we input a strong pulse of dpERK from 20-25 hr, simulating photoreceptor induction, it results in rapid production of PntP1 (MODEL FIG)**.** The resulting high levels of miR-7 and Mae repress Yan mRNA and protein. Low Yan levels are maintained in differentiated cells by persistent low dpERK.

To emulate the *EGFRts* mutant, we scaled dpERK levels to 30%. Simulated multipotent cells with 30% dpERK show Yan levels achieve a lower maximum and are reduced more rapidly (MODEL FIG). Simulated differentiating cells with 30% dpERK degrade Yan more slowly, and Yan ultimately reaches a higher copy number (MODEL FIG). When dpERK levels are scaled by 2.0, emulating the *RasV12* mutant, multipotent cells reach a higher maximum level of Yan and differentiating cells degrade Yan more rapidly (MODEL FIG). In this way, we see how the overall modulation of EGFR signaling can produce seemingly opposite effects on Yan expression in different contexts. With low levels of EGFR pathway activation in progenitor cells, low levels of Pnt proteins are primarily enhancing Yan expression, and so the increase or decrease of EGFR signaling leads to enhanced or diminished Yan expression, respectively. In contrast, with high levels of EGFR pathway activation in differentiating cells, high levels of Pnt proteins are primarily activating Mae and miR-7 which degrade Yan, and so the increase or decrease of EGFR signaling leads to enhanced or diminished Yan degradation, respectively.

The dynamics and magnitude of noise in Yan copy number are quite similar to those measured in experiments (MODEL FIG). Noise tends to increase immediately following peak Yan levels in simulations with deficient dpERK, a trend that appears in experiments with the *EGFRts* mutant(MODEL FIG). Interestingly, in simulated multipotent cells, noise tends to increase in later times, while in experiment, it tends to remain constant. This discrepancy suggests that other factors may be necessary to further suppress Yan noise in progenitors late in development.

**Model Figure Legend**

**Figure XXX.** Stochastic simulation of the Yan network in response to Notch and EGFR input. **Left:** Low level of transient EGFR activation simulates signaling interpreted by multipotent cells. **Right:** Strong pulse of EGFR activation simulates signaling interpreted by differentiating cells. **(A-B)** Copy numbers of select regulatory species in time. Average copy numbers represented by thick lines and an example individual simulation represented by thin lines. **(C-D)** Fluctuations in total Yan in response to varied dpERK levels. Median total Yan shown with overlaid interquartile range. **(E-F)** Noise in total Yan in response to varied dpERK levels.

**Model Supplementary**

We simulate the stochastic dynamics within a cell with the Gillespie algorithm [149, 150]. The inputs to this method are (i) a set of possible molecular species (including complexes) and initial copy numbers and (ii) the allowed reactions with their rate parameters. The Gillespie algorithm is a continuous-time Monte Carlo method, in which each step involves the selection and execution of one event over a variable increment of time. By definition, the “propensity” for a reaction (*ai*) multiplied by an infinitesimal time interval is its probability of happening in that time interval. In practice, at each step, the reactions that can occur are enumerated, their total propensity (*a*tot) is computed, the time increment Δ*t* is drawn from an exponential distribution for fixed *a*tot, and the specific reaction to be executed is selected with probability proportional to its propensity *ai*. The procedure is repeated until the desired total time is reached.

The stochastic model parameters used come either from default parameters for general biological processes or information specific to the Yan network (PARAMETER TABLE). We expect that tuning of default parameters could alter the timescales of copy number fluctuations or the relative timing of induction and downregulation, but would not affect the basic actions of regulatory mechanisms in the model. Measured network parameters can determine other rate parameters in the context of specific biological mechanisms. For example, assuming the estimated promoter region binding energies, ~500 copies of Yan are required for efficient repression by Yan dimer (this number is likely an underestimate because a real cell has many competing binding sites). Given the basic rate for protein degradation, the induction rate for Yan is then determined to achieve the required copy number. Similarly, given the Mae-Yan SAM domain interaction energy and default protein and RNA copy numbers, the Michaelis constant describing Mae-Yan phosphorylation by dpERK and the dissociation constant describing the binding of miR-7 to Yan mRNA are both chosen to ensure effective Yan downregulation. The relative strength of these two reactions can be tuned by adjusting their respective parameters. For rapid degradation of phosphorylated Yan (pYan), we choose the Mae-pYan dissociation time and the pYan lifetime to both be short (~30 sec and ~5 min, respectively), compared with a default protein lifetime of 5 hr.

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