Genetic redundancy in the Wnt pathway promotes robust control of neuronal migration through interlocked feedback loops

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***Short Abstract* — The intriguing observation that functionally redundant genes are highly conserved despite little selective pressure has puzzled biologists for decades [1-4]. To explore emergent functions of genetic redundancy, we examine the migrating Q neuroblasts in *Caenorhabditis elegans* where multiple Wnt receptors function redundantly to induce two patterns of cell migration. Through systematic perturbation of the Wnt pathway and quantitative single-cell transcription profiling, we found redundant Wnt receptors to be differentially regulated through feedback within the Wnt pathway. We employ a mathematical algorithm to quantitatively infer network topology and use computational analysis to identify key regulatory motifs. We show computationally and experimentally that differentially regulated redundant receptors form two distinct signaling modules: a negative feedback loop that filters initial input noise, and a subsequent ultrasensitive positive feedback loop that ensures bistability.**

***Keywords* — robustness in cellular decision, genetic redundancy, feedback regulation in signaling pathways.**

I. Purpose

GENETIC redundancy, where two or more genes share overlapping functions, is highly prevalent across genomes [1-4]. Intuitively, individual genes in a redundant group are non-essential and therefore expected to diverge through evolution. The intriguing observation that redundant genes are highly conserved through evolution has motivated many to believe that genetic redundancy have acquired emergent properties to improve the fitness of the organism [1-3]. As few experimental studies exist, the functional significance of genetic redundancy remains enigmatic.

In this study we use the Wnt signaling-directed migration of the *C.elegans* Q neuroblast as a model to probe the function of redundant genes. Q neuroblasts are born at bilaterally symmetric positions and migrate oppositely in response to Wnt signaling. Multiple Wnt receptors function redundantly to regulate the expression of a Wnt target gene *mab-5/Hox*. Importantly, asymmetric expression of mab-5 in only the left Q cells is critical in establishing the wild-type migratory pattern.

II. RESULTS

A. Redundant Wnt receptors transcriptionally respond to variations in Wnt signaling level

We found three paralogous Wnt receptors to be jointly expressed in both left and right Q cells and function partially redundantly in activating the target gene *mab-5/Hox*. The expression level of each receptor varies dynamically in response to changes in Wnt signaling level, suggesting active feedback regulation within what was thought to be a linear pathway.

B. Differential feedback onto redundant receptors sets up interlinking negative and positive feedback loops

Applying a novel network inference algorithm [6], we obtained a quantitative map of functional interactions among the redundant Wnt receptors and Wnt signaling targets. Surprisingly, we found the redundant receptors to occupy non-similar position in the network. Two receptor genes receive negative feedback while the remaining one is positively regulated by Wnt signaling. Together the three receptors form a series of interlocked negative and positive feedback loops.

C. Ordered activation of feedback loops is required to induce and maintain asymmetric target gene expression

Quantitative expression profiling over the course of migration indicates that the activation of the negative feedback loop precedes that of the positive feedback. Computational modeling predicts that the negative feedback loop functions to integrate signaling input and dampen transient noise, while the ensuing positive feedback initiates signal amplification only upon persistent activation. Receptor mutant phenotypes support the above predictions. Ongoing experiments aim to specifically eliminate feedback regulation.

D. Attenuation of Wnt signaling reveals underlying stochasticity in target gene activation

Finally, we show that attenuating Wnt signaling by reducing functional receptor leads to increased variability in target gene expression and final cell position. We recapitulate this observation in our computational model and highlight the interesting role of redundant genes in forming non-redundant functional modules to promote signaling robustness.

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References

[1] Thomas JH (1993) Thinking about genetic redundancy. Trends Genet **11**, 395-399.

[2] Kafri R (2009) Genetic redundancy: New tricks for old genes. *Cell* **136**, 389-392.

[3] Hittinger CT and Carroll SB (2007) Gene duplication and the adaptive evolution of a classic genetic switch. *Nature* **449**,677-682.

[4] van Wageningen *et al.* (2011) Functional overlap and regulatory links shape genetic interactions between signaling pathways. *Cell* **143**,991-1004.

[5] Maloof JN *et al.* (1999) t migration in *C. elegans*. *Development* **126**, 37-49.

[6] Kholodenko B *et al.* (2002) Untangling the wires: a strategy to trace functional interactions in signaling and gene networks. *PNAS* **99**, 12841-12846.

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