Stochastic gene expression and the partial penetrance of developmental phenotypes

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***Short Abstract* — We investigated the connection between stochastic gene expression and the phenomenon of the partial penetrance of mutant phenotypes, in which genetically identical mutant organisms display varying phenotypes. In particular, we studied a mutation in *C. elegans* that causes gut malformation in some—but not all—early embryos. We traced the cause to the stochastic expression of key genes in the gut developmental pathway by using a new single molecule mRNA detection method. In particular, we found that bimodal expression of regulators of the gene primarily responsible for gut formation helps determine the probability of gut malformation.**

***Keywords* — stochastic gene expression, development, partial penetrance, C. elegans, mRNA, single molecule.**

THE phenomenon of partial penetrance, in which genetically identical mutant organisms show variability in the expression of the mutant phenotype, is commonly encountered when studying the effects of mutations. Researchers have long speculated that partial penetrance could be due to stochastic effects, particularly in gene expression [1], and indeed stochastic gene expression has been found to underlie phenotypic variability in bacteria [2-3]. To determine whether stochastic gene expression could cause such behavior in multicellular organisms, we studied the partial penetrance of a mutation to the gene *skn-1* in *C. elegans* [4]. This mutation results in the phenotype of malformed gut precursor cells during early embryonic development, but only some fraction of these genetically identical mutant embryos actually have malformed guts; i.e., the mutation is partially penetrant.

The formation of gut is controlled by a transcriptional cascade beginning with the maternal gene *skn-1*, which activates (in turn, roughly) *med-1,2*, *end-3*, *end-1*, and finally *elt-2*, the master regulator of intestinal development [5]. To probe the activity of this pathway in both wild-type and mutant organisms, we counted the number of mRNA molecules transcribed from the relevant genes at all developmental stages by using a new fluorescence in situ hybridization (FISH) method that allows one to visualize single mRNA molecules from particular genes in individual embryos via fluorescence microscopy [6]. We found that the wild-type organism is able to buffer fluctuations to produce reliable outcomes whereas the mutant organism displays stochastic, bimodal expression of key genes in the pathway, resulting in the variability in the occurrence of the mutant phenotype.

The particular mutant we examined has a premature stop codon in the gene *skn-1* that results in an attenuation of its function as a transcriptional activator of *med-1,2* as well as *end-1* and *end-3*. Our first goal was to link the previously used phenotypic marker for gut formation (the presence of birefringent gut granules [4]) to the expression of a gene in the developmental pathway. We found that the expression of *elt-2*, the master regulator of gut development, was distinctly bimodal and that the percentages corresponded quite closely to what is observed in the gut granule assay. We then examined the expression of the rest of the genes in the pathway to determine their role in causing this bimodal expression pattern. We found that the expression of *med-1,2* was completely abrogated in the mutant strain as compared to wild-type in all embryos. Surprisingly, the expression of *end-1* and *end-3* was not zero but instead showed bimodality (indicating the presence of another, unknown activating pathway). Interestingly, though, the percentage of embryos displaying *end-1* or *end-3* expression was different than the percentage of embryos displaying *elt-2* expression. Instead, the stochastic expression of these two genes appears to combine to control the ultimate penetrance of the phenotype.

In summary, we have demonstrated that the stochastic expression of certain genes is an important factor leading to the partial penetrance of a mutation involved in gut formation. We expect this fact to hold more broadly and to help explain the partial penetrance of many other mutant phenotypes. Moreover, our results indicate that redundancy is an important means by which the wild-type organism is able to buffer variability in order to produce reliable cell fate determination.

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