**Research Interests**

We study the translational control of gene expression in order to learn how cells change the translation of specific genes and better understand the role of this regulation in the cell. Our research makes extensive use of genome-scale approaches and deep sequencing. We are also interested in the systems-level properties of translational control and its integration into gene expression regulatory systems to provide useful properties.

The translation of mRNA into protein is a central step in gene expression. Recent studies suggest that translation affects protein levels as much as transcription. Translational control acts in diverse processes from cellular stress responses to synaptic plasticity. However, there are many gaps in our knowledge of translational regulation. We cannot predict the expression level of an mRNA from its sequence; we do not have a general framework for understanding how RNA-binding proteins affect translation; and we do not know what advantages the cell achieves by regulating translation as opposed to other steps of gene expression.

**Current Projects**

*Start site selection.* Ribosome profiling revealed an unexpected degree of variability in translation initiation in mammalian cells, including substantial use of non-AUG codons. These diverse start sites led to the translation of alternate protein isoforms as well as short reading frames that likely served principally as decoys repressing translation of a protein-coding gene. Alternate initiation has been recognized as a mechanism for controlling protein expression level and isoform choice for a few specific genes. Our finding that alternate initiation was pervasive posed questions about the ways in which start site selection is controlled and its impact on expression genome-wide. We are using ribosome profiling to study the effects of modulating the factors that control start site usage.

We are also pursuing one specific, interesting mode of alternate protein production: the regulated expression of truncated dominant negative isoforms from the same transcript that encodes a full-length protein. This mode of regulation may provide rapid and precise regulatory responses that are intrinsically buffered against cell-to-cell variation in mRNA abundance (i.e., transcriptional noise. We would like to assess these properties through quantitative, single-cell analyses.

*Internal ribosome entry sites as cis-acting regulatory elements.* Cells respond to diverse stresses by inhibiting most translation while continuing to synthesize specific proteins that enable them to cope with the challenges they face. This rapid translational response often results from inhibition of the canonical pathway of translation initiation accompanied by the translation of specific messages that contain RNA elements capable of recruiting ribosomes and initiation factors directly. These elements can bypass the 5‘ cap dependence of the canonical initiation pathway, thereby promoting translation in the middle of a transcript and serving as internal ribosome entry sites (IRESes). These IRESes serve as cis-acting regulatory elements that specify continued gene expression during cellular stress.

We are taking complementary approaches to understand the scope of non-canonical and IRES-mediated translation initiation. We use ribosome profiling to measure changes in translation during physiologically induced stress responses and following directed molecular disruption of the canonical translation initiation pathway. In parallel, we are developing functional genomic techniques to identify IRESes and related *cis*-regulatory elements in transcripts. The intersection of these data sets will allow us to characterize and explain stress-responsive control of protein synthesis.

*RNA-binding proteins as trans-acting regulators of translation.* Recent studies in yeast and in mammalian cells have emphasized that hundreds of proteins bind to specific subsets of mRNAs. However, we do not know the impact of these proteins on the transcripts they bind. Ribosome profiling and mass spectrometry data both indicate that translation levels vary across messages and, for a single mRNA, change in a regulated manner. It seems likely that many of these RNA-binding proteins act, directly or indirectly, to alter the translation of bound transcripts. The ways in which they may alter translation, however, are not well understood. We are interested in identifying the effector domains of RNA-binding proteins, particularly those that modulate translation, and understanding the co-factors they employ to modulate translation.

Cytoplasmic mRNAs can coalesce into distinctive structures within the cell under many circumstances. This aggregation is a prominent feature of many cellular stress responses and may be driven by RNA-binding proteins. From a molecular perspective, these aggregates may result from the spontaneous association of low complexity sequence domains found within many RNA-binding proteins. Emergent properties of these interactions may explain the apparent phase separation of RNA granules from the surrounding cytoplasm. We wish to understand how these phenomena affect translation of mRNAs in normal regulation and pathological aggregation.

**Selected Publications**

**Ingolia NT†**. Ribosome profiling: new views of translation, from single codons to genome scale. *Nat Rev Genet* advanced online (2014).

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Hsieh AC, Liu Y, Edlind MP, **Ingolia NT**, Janes MR, Sher A, Shi EY, Stumpf CR, Christensen C, Bonham MJ, Wang S, Ren P, Martin M, Jessen K, Feldman ME, Weissman JS, Shokat KM, Rommel C, Ruggero D. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* 485: 55 (2012).

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