Signal transduction: Putting translation before transcription

A recent microarray-based study shows that Ras/Akt signaling rapidly alters the pattern of existing mRNAs that are recruited to polysomes. This response precedes the effects of transcription on total cellular RNA, suggesting that the primary effect of Ras/Akt signaling on gene expression may occur mainly at the level of translation.

Given the enormous literature that encompasses signal transduction research, it is daunting to consider how much still remains to be learned. While some questions have received considerable attention, many others have barely been touched. For example, in cancer, we have gained a broad outline of the major signaling pathways that control cancer pathophysiology (sometimes referred to in cartoon form as roadmaps or subway maps). However, we know little about the signal trafficking pathways that dictate how signal transduction molecules are moved about in cells and how such trafficking pathways may be hijacked in cancer. By controlling the movement of signaling molecules, signal trafficking molecules can control the efficiency of information transfer along a pathway—in digital terms, the information "bandwidth" rather than the information itself. This distinction is important because signal trafficking is an obvious realm for modifier effects: one should expect this realm to become more important to cancer investigations given mounting evidence from mouse genetics and human epidemiological studies that modifier effects can dominate signaling by tumor suppressors and oncogenes (Dove, 2003; Dragani, 2003). Another illustration of the work yet to be done comes from considering how little we know about how the major signaling pathways influence most of the systems that make up cellular physiology. For example, in cancer, a large number of studies have focused on how the major signaling pathways control transcription. In contrast, fewer studies have focused on how these pathways control other fundamental processes, such as translation, membrane dynamics, and energy metabolism (to name only a few). Thus, although it might seem as though our knowledge about signal transduction in cancer is fairly extensive, the existing knowledge probably represents only a small tip of the iceberg of what remains to be learned.

Given this situation, one would expect significant new perspectives on signal transduction in cancer to continue to emerge. One example is offered by a recent report on Ras and Akt signaling published by Eric Holland and colleagues in *Molecular Cell* (Rajasekhar et al., 2003). Reference to signaling roadmaps might lead some investigators to think that the primary effect of Ras/Akt signaling on gene expression is mediated by altering the transcription of downstream target genes. However, the results of Holland and colleagues argue that the primary effect of Ras/Akt on gene expression occurs mainly at the level of translation. Specifically, when Ras/Akt signaling was augmented or disrupted in primary brain cells, there was a rapid change in the pattern of mRNAs loaded onto polysomes, the factories of ribosomes that mediate efficient translation of many cellular messages. This response was documented by gene microarray analysis of polysomal RNAs that were fractionated from total RNA isolated from cells under different conditions. Using a 12,488-gene microarray and a 3-fold cutoff, 705 mRNAs were found to be differentially loaded onto polysomes in cells where signaling was augmented by ectopic expression of activated K-Ras and/or Akt (a myristoylated Akt construct). This set of mRNAs was then compared in two ways to the mRNAs that were loaded onto polysomes when Ras/Akt signaling was disrupted in stably transformed cells by small molecule inhibitors of MEK, PI3K, or mTOR (three downstream effectors of Ras and Akt signaling). By comparing the different sets of mRNAs identified, a "union set" of 426 mRNAs was judged to be regulated by Ras/Akt at the level of polysomal loading. Slightly more than half of the mRNAs identified represented known genes, which encompassed a wide spectrum of functions (Rajasekhar et al., 2003).

Notably, changes in polysomal loading patterns of these mRNAs occurred rapidly upon Ras/Akt blockade, within 2 hr of inhibitor addition. In contrast, during the same period, there was little effect on the complexity of total cellular RNA that would be affected by transcriptional changes (such changes were apparent by 24 hr as would be expected, however). Thus, it appeared that Ras/Akt signaling acted on gene expression and cell transformation primarily at the level of translation by altering the composition of mRNAs loaded onto actively translating polysomes. The likelihood that translational effects preceded transcriptional effects was reinforced by the finding that transcription factors were encoded by ~20% of the mRNAs that were differentially recruited to ribosomes within 2 hr of Ras/Akt blockade (Rajasekhar et al., 2003).

This study extends the evidence that translational modulation by Ras and Akt is necessary and perhaps in some cases even sufficient for cell transformation. Activated Ras stimulates through ERK the activation of

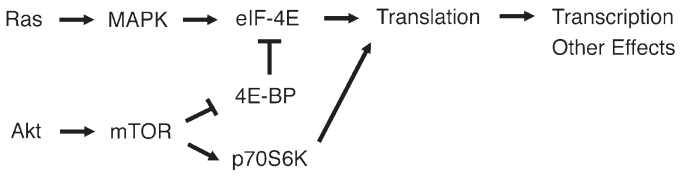

Figure 1. Ras/Akt signaling to translation

Ras signaling through ERK and Akt signaling through mTOR lead to the control of translational effects at the level of cap-dependent recruitment of mRNAs to polysomes and perhaps other cap-independent effects. Patterns of polysomal mRNA recruitment are altered rapidly, before the complexity of total cellular RNA is altered significantly by changes in transcription patterns. Holland and colleagues observed that ~20% of the mRNAs influenced by Ras/Akt at the level of polysomal recruitment encoded transcription factors (Rajasekhar et al., 2003), in support of the idea that translational effects may precede transcriptional effects of Ras/Akt signaling.

the mRNA cap binding translation initiation factor eIF-4E (Gingras et al., 2001). Strikingly, ectopic expression of eIF-4E is sufficient to phenocopy the ability of activated Ras to transform primary rodent cells in cooperation with Myc or E1A (Lazaris-Karatzas and Sonenberg, 1992). eIF-4E is inhibited by binding to 4E-BP and a set of related proteins, and this inhibition is relieved by Akt signaling through mTOR at the level of 4E-BP phosphorylation (Gingras et al., 1999). eIF-4E and 4E-BP have opposing effects on apoptosis, including roles in mediating the antiapoptotic effects of activated Ras (Polunovsky et al., 1996, 2000). Interestingly, recent work suggests that malignant transformation may be associated with a higher requirement for cap-dependent translation to inhibit apoptosis (Li et al., 2002). The Akt/mTOR pathway also activates the key translational regulatory kinase p70S6K (Jefferies et al., 1997), and the ability of Akt to transform cells is tightly linked to upregulation of p70S6K and downregulation of 4E-BP (Aoki et al., 2001). Taken together, these and other studies reveal a causal association between cell transformation and translational control by Ras/Akt signaling.

Holland and colleagues advance the association between Ras/Akt signaling and translation in cancer cells in two ways: first, by showing that Ras/Akt signaling rapidly influences the patterns of mRNA loading on polysomes and second, by defining the identity of a large set of genes that are regulated in this manner. As many receptor signaling pathways impinge on Ras and Akt, and thereby on translation initiation factors, it will be interesting to learn how much overlap there may be in the genes subjected to regulation at the level of polysomal mRNA recruitment. Furthermore, given the rapidity with which cells can respond to signaling through this level of regulation, it will be interesting to learn whether other signal transduction pathways use the same mechanism to drive transcription-independent programs of gene expression.

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Developmental biology informs cancer: The emerging role of the hedgehog signaling pathway in upper gastrointestinal cancers

The hedgehog (Hh) signaling pathway plays many roles in invertebrate and vertebrate development. For example, specific inhibition of sonic Hh expression is critical during early stages of pancreas organogenesis, but an active Hh pathway appears to be required for maintenance of adult endocrine functions. Mutational inactivation of the Hh pathway has been demonstrated in human malignancies of the skin, cerebellum, and skeletal muscle. Now, two papers implicate aberrant Hh signaling in human upper gastrointestinal cancers including those developing from the esophagus, stomach, biliary tract, and pancreas.

Malignancies involving the upper gastrointestinal tract (esophagus, gastric, biliary, liver, and pancreas) represent some of the most biologically aggressive and therapeutically challenging cancers. In the United States, the number of patients diagnosed with these cancers in 2003 has been estimated at 70,000 with approximately 54,000 deaths. Pancreatic cancer alone will account for 30,000 deaths this year and remains one of the most treatment-refractory cancers despite aggressive use of conventional modalities such as surgery, radiation therapy, or chemotherapy.

Originally described in *Drosophila*, the hedgehog (Hh) signaling pathway is one of the most fundamental in embryonic development. Three mammalian Hh genes have been identified (sonic Hh [*SHh*], Indian Hh [*IHh*], and desert Hh [*DHh*]). Generally, this signal transduction pathway is responsible for patterning numerous structures including the axial skeleton, neural tube, limbs, lungs, skin, hair, and teeth. In addition, SHh has been demonstrated to be essential to