Elongation factor Tu as a control gene for mRNA analysis of lung development and other differentiation and growth regulated systems

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The analysis of steady state mRNA levels in cells or tissues that are undergoing growth and developmental changes can be problematic due to the lack of invariant control genes. Direct staining of agarose gels for 18s and 28s ribosomal RNAs is often used to control for loading errors. This approach does not control for transfer and hybridization artifacts. Several genes including actin, GAPDH, and *B2* microglobulin have been used successfully as control genes. However, these genes are not always suitable controls when growth state is changing or limited amounts of mRNA are available.

The analysis of mRNA levels in developing lung is especially difficult. Between 16 and 21 days of gestation the fetal rat lung undergoes rapid growth and differentiation (1). The steady state levels of growth-dependent (e.g. c-myc and actin) and differentiation-specific (e.g. surfactant proteins) mRNAs can modulate dramatically during this period. In the process of identifying and cloning novel differentiation-specific genes we cloned a cDNA whose expression is invariant between 16 and 21 days of gestation. Sequence analysis indicated that the clone is the rat homologue of the protein synthesis elongation factor Tu (EFTu).

To verify that EFTu is constitutively expressed during the time course of lung development, mRNAs from 16, 18, and 21 day fetal rat lung and neonatal and adult rat lung were probed for surfactant protein A (SPA) (2) and EFTu expression. The results displayed in Figure 1A indicate that in contrast to the developmentally regulated SPA gene, EFTu is constitutively expressed at the mRNA level in developing rat lung. EFTu is also constitutively expressed in F9 teratocarcinoma cells before and after retinoic acid-induced differentiation (Figure 1B), in quiescent and serum-stimulated Balb/c 3T3 cells (Figure 1C), and in a rat lung epithelial cell line that growth arrests at 40°C (data not shown). In these systems, differentiation or growth-dependent genes are induced at least 25 fold whereas EFTu mRNA levels remain relatively constant.

EFTu has several properties that makes it an excellent control gene for growth and differentiation studies. EFTu mRNA is especially prevalent allowing easy detection even when RNA samples are limiting or after multiple blot strippings. All cells actively synthesizing protein including quiescent cells should contain EFTu mRNA. Finally, EFTu is highly conserved and should therefore easily crosshybridize among species.

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Figure 1. Northern blot analysis of EFTu mRNAs. Ten micrograms of total RNA were fractionated on a formaldehyde-agarose gel and hybridized to ³²P-EFTu cDNA (in Bluescript, Stratagene) (5×10^5 cpm/ml) and differentiation or growth-dependent probes. (A) Sprague-Dawley rat lungs at 16, 18, and 21 days of gestation, and from neonate and adult lungs probed with SPA (only the 1.6 kb mRNA is shown). (B) F9 teratocarcinoma cells before (Stem) and after (RACT) treatment with retinoic acid (5×10^{-7} M), cAMP (500 μ M) and theophylline (500 μ M) probed with collagen IV (3). (C) Serum starved quiescent and serum stimulated (2 hours) Balb/c 3T3 cells (A31) probed with the serum-dependent gene, JE (4). Specific bands from duplicate experiments were quantitated by densitometry and the mean and standard deviation from each set of experiments are shown.