Quantitative comparison of mRNA levels in mammalian tissues: 28S ribosomal RNA level as an accurate internal control

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Gene expression levels in mammalian tissues can be quantitatively investigated by Northern blot analysis of specific mRNAs. For such an analysis to be truly quantitative, the absolute amount of total RNA in each sample must be accurately determined or reliable internal controls must be included in the study. Due to the often limited amount of RNA isolated from cell cultures or mammalian tissues, spectrophotometric determination (A_{260}) is not always possible. In those cases one relies heavily on internal controls, such as genes that are constitutively expressed in each cell or tissue. However, such housekeeping

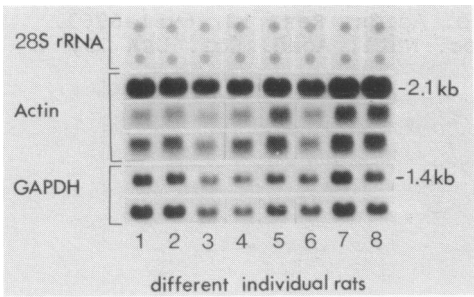

Figure 1. Northern blot analysis of β-actin and GAPDH, and dot blot analysis of 28S rRNA in total liver RNA isolated from 8 individual inbred WAG/Rij rats. mRNA sizes are indicated in kilobases (kb). Blots were rehybridized with different probes.

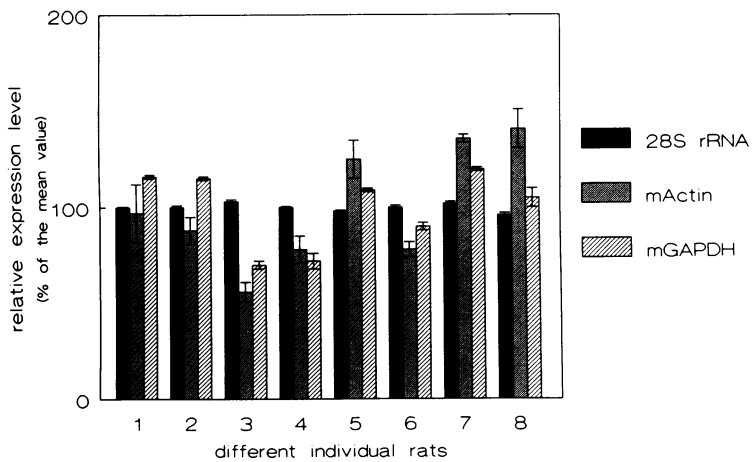

Figure 2. Relative expression levels of β-actin, GAPDH and 28S rDNA obtained by densitometric scanning, as a percentage of the mean level of the hybridization signals (see Fig. 1). Bars indicate the experimental error (SD).

genes are themselves subject to considerable variation and therefore no reliable standards in the liver of different individual rats. This was demonstrated by quantifying mRNA levels of β-actin (1) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (2), relative to the level of 28S rRNA (3), RNA concentrations of the samples were determined spectrophotometrically at 260 nm and compared with dot blot hybridization analysis of equal amounts of total RNA (Fig. 1). The 28S rRNA level appears to be a constant fraction of total RNA, whereas the level of β-actin and GAPDH mRNA in the same samples varied considerably (Fig. 2), indicating interindividual variation in these mRNAs. In comparative analysis of specific mRNAs, the 28S rRNA level is a reliable internal control and a good indicator for the relative amount of total RNA.

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