

S26 ribosomal protein RNA: an invariant control for gene regulation experiments in eucaryotic cells and tissues

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In the course of studies looking for differentially expressed genes, we isolated as a control a cDNA hybridizing to a highly and constantly expressed RNA. Further characterization of the hamster and human full-length cDNAs showed that they correspond to ribosomal protein S26 RNA. Sequence comparison revealed a very high degree of conservation at the proteic level amongst distantly related eukaryotic organisms such as man and *Drosophila*. The S26 gene is expressed as a 600−700 bases long RNA at high and *constant* level in human adult tissues and in mammalian cell lines cultured in different physiological contexts. This mRNA therefore represents a very suitable invariant standard for all experiments involving RNA quantification.

Only a few probes, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin and β2-microglobulin, are available for controlling RNA levels on Northern blots, in RNAse mapping procedures or run-on assays. Several years ago, we isolated and used a set of GAPDH cDNAs as a control to monitor gene regulation experiments, as the level of the corresponding RNA remains unaltered in cells treated by various drugs, such as cycloheximide, or actinomycinD (1−3). Since then, the use of GAPDH probe became so widespread that detection kits have been made available for the monitoring of RT-PCR reactions. Nevertheless, in some instances, the use of this probe suffers several drawbacks: i) GAPDH RNA accumulates in cells exposed for several hours to various hormones or growth factors such as Insulin (4), Epidermal Growth Factor or Fetal Calf Serum (5), ii) there is some degree of tissue specific expression of total and poly(A)$^+$ GAPDH RNA in rat and in man (6), and iii) its size (1,3 kb) is within the average of mRNA size distribution, and might impair a proper detection of low abundance class mRNA during subsequent hybridization of Northern membranes.

We have isolated a cDNA clone whose corresponding RNA level remains strictly invariant in fibroblastic cells treated with growth factors, or with biosynthesis inhibitors (7). This 0.7 kb fully polyadenylated RNA turned out to be the hamster counterpart of the rat RNA encoding ribosomal protein S26 (8). As no human sequence had been yet reported, we also isolated the S26 homologous clone from an HeLa cDNA library. Both cDNAs probably represent full-length copies, as their 5′ non coding regions exhibit the same length than the full-length rat one. Complete nucleotide sequences of the human and hamster cDNAs, respectively stretching over 438 bp and 435 bp (to the exclusion of the poly(A) tail) will appear in the EMBL, GenBank and DDBJ Nucleotide Databases under the accession numbers X69654 (man) and X63389 (hamster). Deduced proteic sequences from human, hamster, rat, *Drosophila* and yeast exhibit a very high degree of identity (78% between man and *Drosophila*).

Assays for S26 RNA revealed identical levels in hamster CCL39 fibroblastic cell line (7), human HeLa, Daudi and Peer cells, or mouse C2 muscle cells (9), OB17 preadipocytes and Ltk$^-$ cells. S26 mRNA half-life is more than 20 hours, as measured by actinomycinD chase experiments (7), and run-on assays revealed a low but constant rate of transcription in hamster CCL39 cells treated with various growth factors (not shown). In addition, S26 mRNA was found to be expressed at high and comparable levels in various adult human tissues, as shown in the figure. This RNA therefore represents a very suitable internal control for gene regulation experiments. Both cDNAs have been cloned in phagemid vectors, and as it was the case for human, rat and hamster GAPDH DNA probes, the S26 probes will be freely available from our laboratory.

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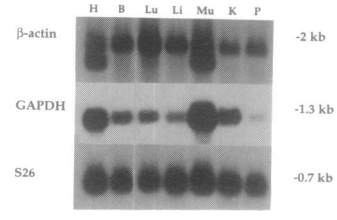

Figure 1. Levels of expression of GAPDH, β-actin and S26 RNAs in various adult human tissues. Multiple Tissues Northern (MTN, Clontech), containing 2 μg poly(A)$^+$ RNA per slot, was sequentially hybridized to S26, GAPDH and β-actin probes. H: heart, B: brain, Lu: lung, Li: liver, Mu: skeletal muscle, K: kidney and P: pancreas.