Use of Metafectene transfection reagent for multiple transfection experiments

Sandy Mosig and Ignacio Rubio
Research Unit “Molecular Cell Biology”, Medical Faculty, Friedrich-Schiller-University, 07747 Jena, Germany

The Transient transfection approach is arguably the most widely used strategy to analyse the function of proteins and mutants thereof in a physiological background. Very often the action of one or more mutated versions of a protein are analysed to dissect the function of individual domains that harbour independent enzymatic activities or interaction motifs. Furthermore, in most cases additional plasmids are expressed, e.g. in order to provide a biochemical readout to the assay. Such an experimental setting poses a number of requisites on the transfection technique of choice as there are, among others a stable transfection potency which is ideally independent of the plasmid sequence in order to ensure equal levels of expression. This prerequisite gains importance in the case of multiple plasmid transfections in which two or more plasmids are delivered to the cells.

We have performed multiple transfections of COS-7 monkey fibroblast-like cells using the metafectene transfection reagent. We have analysed the levels of expression of various point mutants of the Ras GTPase to assess the suitability of the metafectene reagent for comparative studies of Ras/Ras-mutant function. Using 5 ?g each of Ras/Ras-mutant cDNA we obtained almost equal levels of expression for all Ras versions (Fig. 1). Transcription of all Ras versions is driven by the CMV promoter albeit in the background of different plasmid types (pexv3, pcDNA3 and pNRTIS21). The mutant Ras-versions in lanes 2 and 3 exhibit a reduced electrophoretic mobility due to the presence of an N-terminal tag sequence. Also note that overexpression of all Ras proteins was too high for efficient and quantitative post-translational processing in COS-7 cells giving rise to a second low-mobility band which represents non-farnesylated Ras protein.

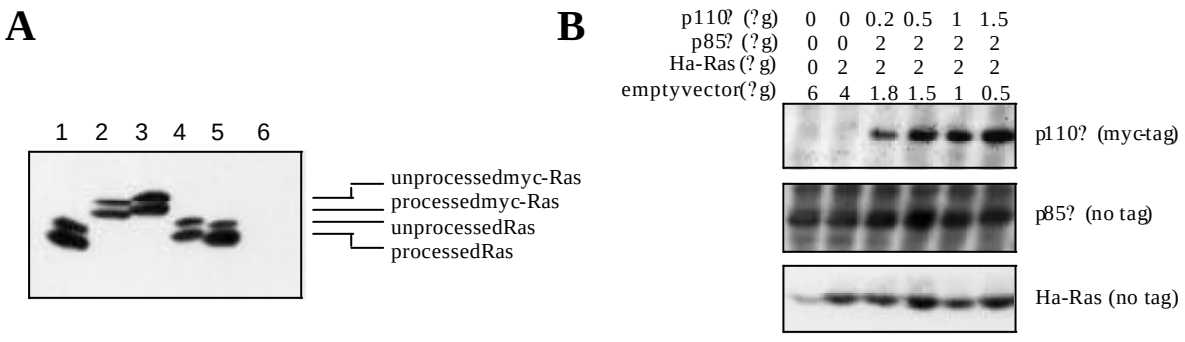

Figure 1. Transient transfection of COS-7 cells using the metafectene transfection reagent. Sub-confluent Cos-7 cells seeded in 6-well plates were transfected employing 6 ?l metafectene reagent per well according to the manufacturer's protocol. (A) 6 ?g each of individual Ha-Ras constructs were transfected into COS-7 cells. Protein expression was assessed by immunoblotting out of cell extracts 48 h after transfection. *Lane1*: wt-Ras, *lane 2*: myc-tagged N119-Ras, *lane 3*: myc-tagged N17-Ras, *lane 4*: V12-Ras, *lane 5*: V12/A38-Ras, *lane6*: empty vector. Note that endogenous Ras is not detectable in lane 6. (B) The indicated amounts of plasmids coding for Ha-Ras, p85? and p110? were co-transfected as indicated and processed as described for (A) using specific antibodies.

We have also conducted experiments to evaluate the suitability of this approach for multiple transfections. We transfected Ras in conjunction with either empty vector or two additional proteins and analysed the levels of expression (Figure 2). Ras overexpression remained stable in all points. Note that all heterologous Ras protein was post-translationally processed in this case due to the lower levels of expression as compared to Fig. 1 (owing to the use of less Ras plasmid DNA/point). Importantly, Ras expression levels were not affected by the coexpression of additional plasmids coding for the p85 adapter subunit of phosphoinositide 3-kinase (PI3K) and increasing amounts of the PI3K catalytic subunit p110, two proteins that do functionally interact with Ras in vivo.

We have since conducted numerous co-transfection experiments of this kind and have always obtained more than satisfactory reproducible and stable expression patterns for various plasmid combinations. Based on this experience we recommend metafectene reagent for multiple transfection experiments.