

## ASSESSMENT OF A NUCLEAR TARGETING PEPTIDE SCAFFOLD (M9) TO IMPROVE PLASMID DNA TRANSFECTION EFFICIENCY

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REPEATED APPLICATION of certain growth factors has been shown to improve wound healing, but inefficient delivery methods and the short half-lives of agents limit this approach. Gene therapy has the potential to allow the continuous production of adequate amounts of these growth factors encoded by DNA. Viral means of introducing the DNA genes are efficient, but there are serious concerns about their safety and immunogenicity. Currently, the major impediment to nonviral transfection is the inefficiency of gene delivery. Liposomes can mediate the transport of DNA plasmid into the cytoplasm, but the nuclear membrane remains a barrier to macromolecules and efficient plasmid DNA transfection.<sup>1</sup> Cells routinely use specific transport mechanisms to translocate macromolecules in and out of the nucleus. A 38 amino acid sequence (termed M9) confers the nuclear import and export function of the mRNA carrier, heterogeneous nuclear ribonucleoprotein-A1. When the M9 component is complexed to a DNA plasmid, increased transfection has been demonstrated in endothelial cells.<sup>2</sup> We assessed this construct in 3T3 fibroblasts and evaluated its mechanism of action.

### METHODS

3T3 fibroblasts were grown to confluence in DMEM + 5% FBS. Prior to transfection, a  $\beta$ -galactosidase ( $\beta$ -gal) plasmid or a rhodamine-labeled plasmid were complexed to varying amounts of the M9 peptide with or without Lipofectamine. Cells were transfected for 2 hours before the growth medium was replaced. Quantitative  $\beta$ -gal activity was determined using a commercially available  $\beta$ -gal assay kit at 24 hours posttransfection. Also at 24 hours, cell nuclei were stained in situ, with the fluorescent DNA stain 4', 6-diamidino-2-phenylindole dihydrochloride, and the intracellular location of the rhodamine-labeled plasmid was determined by fluorescent microscopy.

### RESULTS

Individually, neither M9 nor Lipofectamine at low doses increased plasmid-mediated transfection as evidenced by  $\beta$ -gal activity. However their combination increased transfection dramatically by approximately 26-fold, from  $0.07 \pm 0.01$  to  $1.85 \pm 0.61$  ( $P < .001$ ; ANOVA) at  $2 \mu\text{L}/\text{mL}$  of Lipofectamine (Fig 1). This effect

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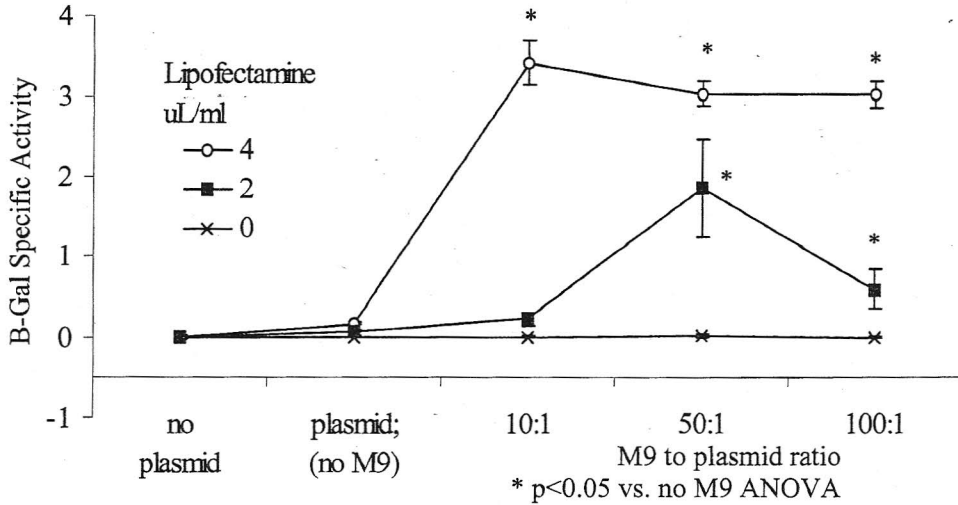


Fig 1— $\beta$ -Galactosidase ( $\beta$ -Gal) activity following transfection with plasmid with varying concentrations of Lipofectamine and M9.

was most dramatic at the lower doses of Lipofectamine. The higher doses of Lipofectamine and M9 seemed to be toxic to cells.

In the fluorescent microscopy experiments, rhodamine-labeled plasmid alone did not enter the cells. In the Lipofectamine-treated cells, rhodamine-labeled DNA plasmid was sequestered in the cytoplasm, but gained access to the nucleus with the M9 (Fig 2).

CONCLUSION

These experiments indicate that the M9 peptide improves the efficiency of gene expression in 3T3 fibroblasts when used in conjunction with Lipofectamine. The Lipofectamine seems to deliver plasmid to the cytoplasm, whereas the M9 shuttle increases transfection efficiency by delivering plasmid to the nucleus. As expected, based on their actions, the combination of the 2 agents increases transfection efficiency synergistically. Fibroblast transfection, either ex vivo or in vivo may have important applications in wound healing.

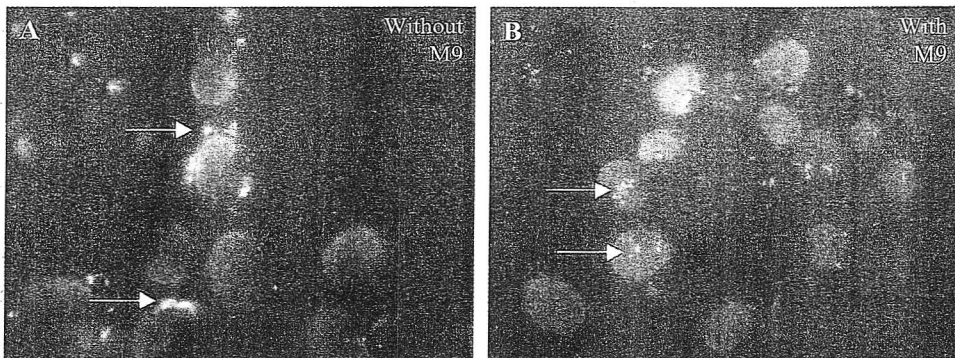


Fig 2—Fluorescent micrographs after transfection with rhodamine-labeled plasmid. Without M9 (A), the plasmid remained in the cytoplasm (arrows), whereas in the presence of M9 (B) some plasmid was seen in the nucleus (arrows).

## REFERENCES

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  2. Subramanian A, Ranganathan P, Diamond SL: Nuclear targeting peptide scaffolds for lipofection of non-dividing mammalian cells. *Nat Biotechnol* 17:873-877, 1999
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