

Smooth Muscle-Specific Gene Delivery in the Vasculature Based on Restriction of DNA Nuclear Import

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The two currently employed approaches restricting gene delivery and/or expression to desired cell types *in vivo* rely on cell surface targeting or cell-specific promoters. We have developed a third approach based on cell-specific nuclear transport of the delivered plasmid DNA. We have previously shown that plasmid nuclear import in non-dividing cells is sequence-specific and have identified a set of cell-specific DNA nuclear targeting sequences that can be used to limit DNA nuclear import to desired cell types. Specifically we have identified elements of the smooth muscle gamma actin (SMGA) promoter that direct plasmid nuclear import selectively in smooth muscle cells (SMCs) *in vitro* (Vacik et al, 1999, *Gene Therapy* 6:1006–1014). In the present study, we demonstrate that the SMC-specific DNA nuclear targeting sequence from the SMGA promoter drives nuclear accumulation of plasmids and subsequent gene expression exclusively in the smooth muscle cell layer of the vessel wall in the intact vasculature of rats using electroporation mediated delivery. These results demonstrate that certain DNA nuclear targeting sequences can be used to restrict DNA nuclear import to specific cell types providing a new, novel means of cell targeting for gene therapy. *Exp Biol Med* 233:840–848, 2008

Key words: electroporation; nuclear import; cell-specific; plasmid; vasculature

Introduction

Currently, a number of gene delivery systems for use *in vivo* are being studied, but as yet their low efficiency of gene transfer and lack of cell-specific targeting and expression are major limitations. The two major approaches that have been used to target gene transfer and/or expression to desired cell types have used either cell surface receptor-ligand interactions to promote cell-specific internalization of the DNA into the cytoplasm or cell-specific promoters to restrict transcription to desired cell types (1, 2). A third, but as yet unvalidated, approach is based on the fact that the nuclear import of plasmids can be regulated in a cell specific manner. We have shown that in the absence of mitosis, plasmids are imported into the nucleus in a sequence-specific manner, and we and others have identified several DNA sequences that mediate this nuclear import (3–7). The 72 bp SV40 enhancer supports nuclear import of plasmid DNA in a variety of non-dividing cells in culture and in numerous cell types in animal models (3, 5, 8, 9). Since this DNA sequence binds to a number of general transcription factors that are expressed in most cell types, we proposed a model in which the SV40 enhancer (termed a “DNA nuclear targeting sequence” or “DTS”) is coated with newly synthesized nuclear localization signal-containing transcription factors in the cytoplasm, and that this DNA-protein complex is then imported into the nucleus using the nuclear protein machinery (5, 6, 10).

Based on this model, we reasoned that if transcription factor binding sites contained within a sequence were specific for factors expressed exclusively in a certain cell type rather than ubiquitously, nuclear import of that sequence would be limited to cells expressing those cell-specific transcription factors. Indeed, we have identified several DNA sequences from cell-specific promoters that mediate nuclear import with cell specificity, including the smooth muscle gamma actin (SMGA) promoter that supports plasmid nuclear import specifically in cultured smooth muscle cells (SMCs) (6). This promoter is regulated transcriptionally by the complement of positive and negative

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transcriptional regulators present within smooth muscle cells including SRF and Nkx factors (11, 12). We have demonstrated that binding of these factors to the DNA is needed for DNA nuclear import activity in smooth muscle cells. We have shown that the majority of the nuclear import activity resides in the first 176 bp of the promoter, proximal to the start site, since plasmids containing either the full length 2294 bp, or two truncated promoters consisting of the first 404 bp or 176 bp are all capable of causing plasmid nuclear import in smooth muscle cells following cytoplasmic microinjection (6). Moreover, we have used this SMGA nuclear targeting sequence to restrict gene transfer and expression to cultured smooth muscle cells following transfection (6).

In the present study, we have tested the effects of the SMGA, SMC-specific DNA nuclear targeting sequence in the intact vasculature of rats using electroporation mediated delivery. Using this approach, we have previously demonstrated that a DNA nuclear targeting sequence is required for efficient *in vivo* gene delivery and further that the SV40 DNA nuclear targeting sequence mediates nuclear import and increased gene expression in all cell types of the neurovascular bundle (9). Here we demonstrate that the SMC-specific DNA nuclear targeting sequence from the SMGA promoter increases gene expression exclusively in the smooth muscle cell layer of the vessel wall, confirming the function of DNA nuclear targeting sequences *in vivo*. These results demonstrate that certain DNA nuclear targeting sequences can be used to restrict DNA nuclear import to specific cell types to provide a novel means of cell targeting for gene therapy.

Materials and Methods

Plasmids. The nomenclature of all plasmids is promoter-gene product-DNA Nuclear Targeting Sequence (DTS). pCMV-Lux, pCMV-Lux-DTS, pCMV-Lux-SMGA₂₂₉₄, and pCMV-Lux-SMGA₁₇₆ express firefly luciferase from the CMV immediate early promoter (CMV_{iep}) and contain no DTS, the SV40 DTS, the 2294 bp SMGA DTS, or the 176 bp SMGA DTS downstream of the luciferase gene, respectively (6). pSMGA₂₂₉₄-GFP was created by subcloning the 2319 bp SMGA promoter fragment (−2294 to +25) from pGL3-SMGA₂₂₉₄ into the SmaI site of pEGFP-1 (Clontech, Palo Alto, CA). pCMV-GFP-DTS and pCMV-GFP express green fluorescent protein (GFP) from the CMV_{iep} with or without the SV40 DTS downstream of the GFP gene, respectively (5, 9). Plasmids pCMV-GFP-SMGA₂₂₉₄ and pCMV-GFP-SMGA₁₇₆ were constructed by subcloning the blunt-ended 2319 bp or 201 bp SMGA promoter fragments (−2294 to +25 and −176 to +25) from pGL3-SMGA₂₂₉₄ and pGL3-SMGA₁₇₆, respectively (12), into the SmaI site of a modified pCMV-GFP plasmid containing a polylinker inserted into the NotI site, downstream of the GFP gene.

In Vivo Gene Transfer. Rat mesenteric vessels were electroporated as previously described (9, 13). Briefly, male

Sprague-Dawley rats (200–400 gm) were anesthetized with isoflurane, a midline incision was made, and the small intestine was exteriorized. Generally, 8–10 mesenteric vessels per animal were electroporated and each animal received only 1 of the DNA constructs ($n = 4$ animals per DNA construct). DNA was suspended in 10 mM Tris, pH 8.0 containing 1 mM EDTA and 140 mM NaCl at a concentration of 2 mg/ml and placed into a 55 μ l spoon-like electrode into which vessels were draped. Vessels were electroporated with 8 square wave pulses lasting 10 milliseconds each at the optimum field strength of 200 V/cm using a BTX830 electroporator (Genetronics, San Diego, CA). After all vessels were electroporated within a given animal, the incision was closed, the animal was given buprenex as analgesia, allowed to recover, and returned to the vivarium. At 2 days post-transfer, vessels were harvested for analysis of gene expression and the animals were euthanized. All animal experiments were performed with the approval of the Northwestern University Animal Care and Use Committee and according to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals.

Visualization of Reporter Gene Expression. GFP expression was detected directly in vessels that were rinsed extensively with cold PBS, dissected away from the surrounding adipose tissue, and viewed using a low power objective on an upright Leica DMR fluorescence microscope. Alternatively, vessels within the mesenteric neurovascular bundle were embedded in OCT and frozen. Thin sections were prepared for direct GFP detection or immunofluorescence using antibodies against GFP or smooth muscle alpha actin (SM α A) as a smooth muscle marker. Electroporated vessels were also fixed in formalin and embedded in paraffin for sectioning and immunohistochemistry. Deparaffinized sections were blocked with normal serum and reacted with antibodies against GFP, luciferase, or SM α A, and visualized using Vector Laboratory's ABC system and either Vector Red followed by hematoxylin counterstaining or Vector Blue and eosin counterstaining. Fluorescent images of different vessels were captured, all with the same exposure time and gain settings, using a Hamamatsu ORCA cooled CCD camera and OpenLab software (Improvision, Lexington, MA). For immunohistochemistry, images were captured with an INSIGHT color camera using SPOT software (Diagnostic Instruments, Sterling Heights, MI). All images were compiled using Adobe Photoshop.

Measurement of Luciferase Expression. Luciferase expression was measured in lysates from excised vessels and quantified using a purified luciferase protein standard as previously described (9, 13).

In Situ Hybridization. *In situ* hybridizations were performed on 10 μ m sections, using biotin-labeled, nick translated luciferase or GFP gene probes as described (9). Following hybridization and washes, sections were treated with RNase H to eliminate detection of mRNA. Electroporated plasmids were detected using the TSA Biotin

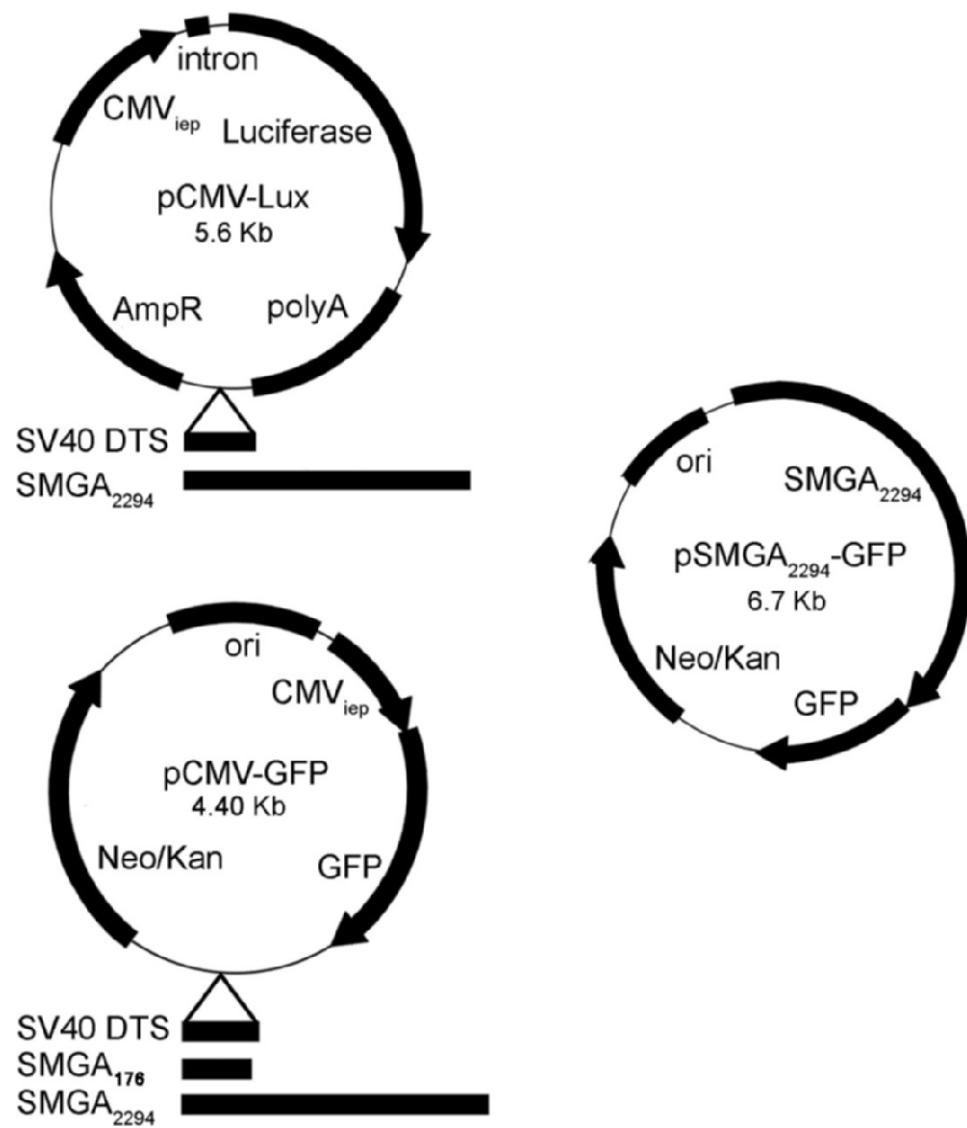


Figure 1. Plasmids. Cartoons of the plasmids used in this study are shown. Abbreviated elements are as follows: CMV_{iep}, CMV immediate early promoter/enhancer; SMGA₂₂₉₄, SMGA₄₀₄, 2294 bp, or 176 bp SMGA promoter fragments, respectively; SV40 DTS, SV40 DNA nuclear targeting sequence.

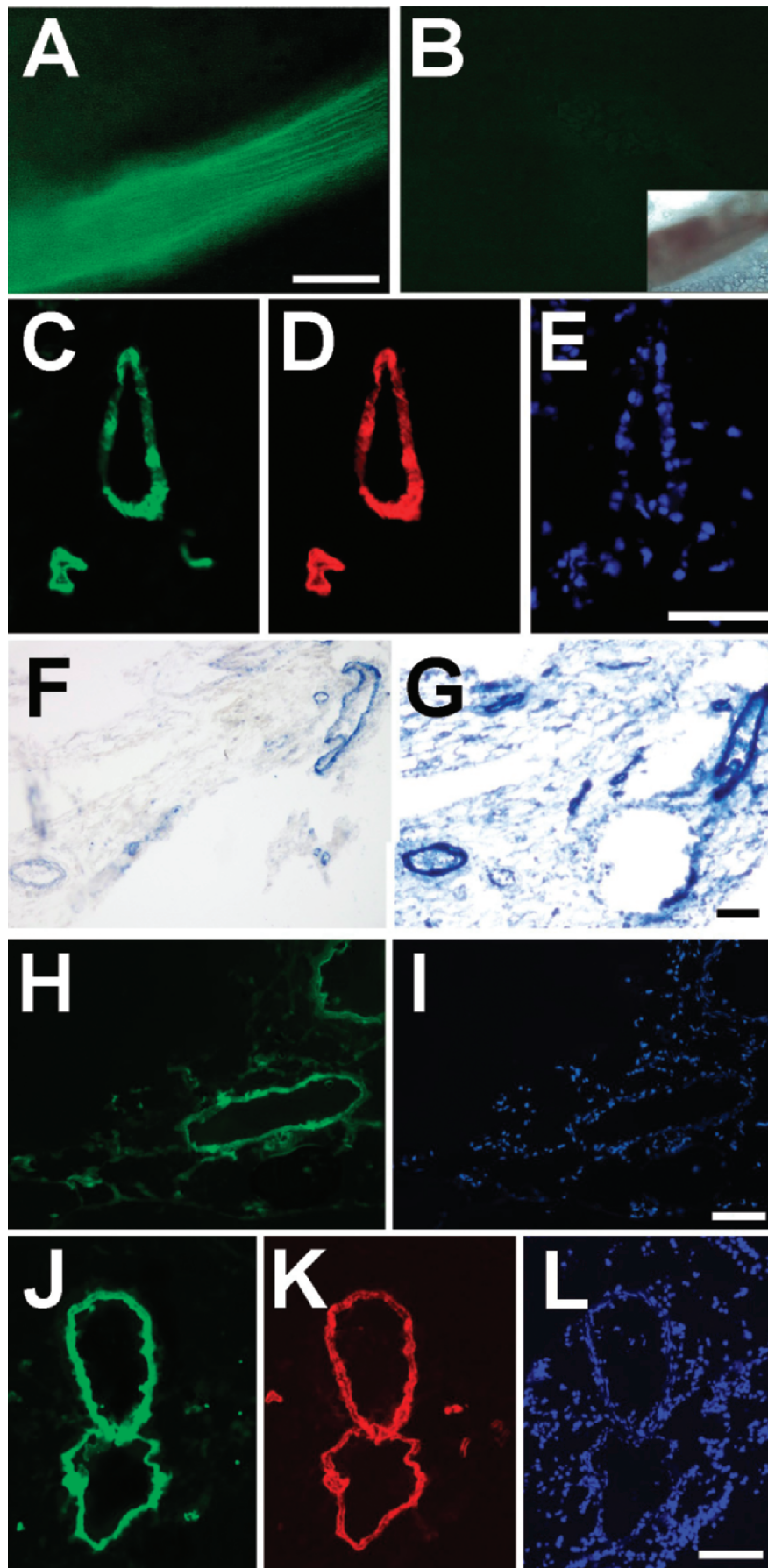
System (Perkin-Elmer, Boston, MA) and Alkaline Phosphatase-Vector Blue ABC system. All tissue sections were counter-stained with DAPI/DABCO.

Results

Effect of the SMGA SMC-Specific Nuclear Import Sequence on Gene Transfer *In Vivo*. To validate

the function of the SMC-specific, SMGA nuclear targeting sequence *in vivo*, we constructed a set of plasmids, each containing various lengths of the SMGA promoter downstream of a reporter gene (Fig. 1). Since these various lengths of the SMGA promoter appear to be equivalent in *in vitro* nuclear import assays, both full length and the shorter versions were used where indicated (6). In most cases, the

Figure 2. The SMGA DNA targeting sequence functions *in vivo* to increase gene expression specifically in smooth muscle cells. Plasmids containing the 2294 bp SMGA DNA nuclear import sequence (pCMV-GFP-SMGA₂₂₉₄, A, C–G, or pSMGA₂₂₉₄-GFP, J–L), the SV40 DTS (pCMV-GFP-DTS, H and I), or lacking any import sequence (pCMV-GFP, B) were delivered to rat mesenteric arteries by electroporation and harvested 2 days later for visualization of GFP expression. GFP fluorescence from representative whole mounts from 8–14 vessels per condition are shown (A and B). The inset in panel B shows the vessel imaged for GFP expression. Frozen thin sections of vessels electroporated with pCMV-GFP-SMGA₂₂₉₄ (C–E), pCMV-GFP-DTS (H and I), or pSMGA₂₂₉₄-GFP (J–L; *n* = 4 vessels per plasmid) were visualized for GFP fluorescence (C, H, and J, green) and/or stained with antibodies against smooth muscle alpha actin (D and K, red). Nuclei were counterstained with DAPI (E, I, and L, blue). Immunohistochemistry for GFP (F, blue) or the smooth muscle marker, smooth muscle alpha actin (G, blue), was performed in adjacent paraffin-embedded sections of vessels electroporated with pCMV-GFP-SMGA₂₂₉₄. Bars = 100 μm.



CMV immediate early promoter was used to drive gene expression since *in vitro* and *in vivo* data have demonstrated that this promoter does not mediate nuclear import in non-dividing cells but gives high levels of gene expression (5, 6, 9). As a consequence, since the SMGA sequence is downstream of the reporter gene and thus cannot drive transcription, it acts only as a nuclear targeting sequence in these plasmids. To test the cell-specificity of these sequences, we used the mesenteric vasculature as our model tissue, since multiple cell types are present within the neurovascular bundle, including endothelial cells, fibroblasts, neurons, adipocytes, as well as smooth muscle cells, all of which have been shown to uptake and express plasmids following electroporation (9, 13). When a GFP-expressing plasmid containing the 2294 bp SMGA promoter (pCMV-GFP-SMGA₂₂₉₄) was delivered to the intact vasculature of rats using electroporation, GFP expression was robust at 2 days post-transfer (Fig. 2A). By contrast, vessels receiving pCMV-GFP which lacks a DNA nuclear targeting sequence, had nearly undetectable levels of GFP expression (Fig. 2B) (5, 6, 9).

In order to quantify the effects of the different DTSs on gene transfer and expression, we generated a set of CMV promoter-driven luciferase-expressing plasmids that carried the individual DTSs downstream of the reporter gene. When the level of expression in electroporated vessels was quantified using luciferase-expressing plasmids that contained either the SV40 DTS or the SMGA₂₂₉₄ sequence, we found that pCMV-Lux-SMGA₂₂₉₄ expressed 1.19 ± 0.45 pg (mean $\pm$ SEM) of luciferase per vessel segment which was approximately 8% of the levels achieved using a similar plasmid carrying the SV40 DTS (pCMV-Lux-DTS; 15.4 ± 6.76 pg/vessel; $P < 0.05$). Although the absolute levels of luciferase produced per vessel with pCMV-Lux-DTS are lower than those previously reported (9, 13), almost 70% of these electroporated vessels showed expression that was greater than 1700-fold over background ($P < 0.001$ for pCMV-Lux-DTS, as well as all other plasmids, compared to background). The overall percentage of vessels expressing luciferase was similar between the two groups: 69% of pCMV-Lux-DTS electroporated vessels and 84% of pCMV-Lux-SMGA₂₂₉₄ electroporated vessels showed luciferase activity. Therefore, the decreased level of gene expression observed in vessels receiving the SMGA-containing plasmid is likely due to the fact that only a small subset of cells within the vessel wall and surrounding tissue are targeted for gene expression (i.e., SMCs only).

Effects of the SMGA Targeting Sequence on Localization of Gene Expression *In Vivo*. To determine which cell types in the neurovascular bundle were expressing the transgene, vessels were electroporated with the nuclear import reporter plasmid pCMV-GFP-SMGA₂₂₉₄ and GFP expression was detected directly in sectioned vessels 2 days later (Fig. 2C). GFP expression was limited to SM α A-expressing smooth muscle cells in the vessel wall (Fig. 2D). Immunohistochemical analysis using an antibody

directed against GFP confirmed that the GFP expression coincides with the SMC layer of the vessel wall (Fig. 2F), as detected by SM α A staining (Fig. 2G). Almost none of the cells of the adventitia expressed GFP. In contrast, vessels receiving the SV40 containing plasmid pCMV-GFP-DTS expressed GFP from the CMV promoter at high levels in multiple cell types throughout the neurovascular bundle, including the adipocytes, fibroblasts, smooth muscle cells and endothelial cells (Fig. 2H) (9, 13). We also tested whether the SMGA promoter could simultaneously act as an SMC-specific nuclear import sequence and an SMC-specific promoter by placing it upstream of the GFP gene. This approach would restrict gene expression to desired cells on two levels, nuclear import and transcription. Vessels were electroporated with pSMGA₂₂₉₄-GFP and harvested 2 days later to visualize GFP expression in tissue sections by fluorescence microscopy. As predicted, GFP expression was limited to the smooth muscle cell layer in cross sections of treated vessels (Fig. 2J), as visualized by staining for the smooth muscle marker SM α A (Fig. 2K).

To further demonstrate the difference in localization of gene delivery and expression between an SV40 DTS-containing plasmid and an SMGA-containing plasmid, a mixture of plasmids pCMV-Lux-DTS and pCMV-GFP-SMGA₁₇₆ was delivered to the same vessels by electroporation. Since the 176 bp SMGA proximal promoter shows similar nuclear import activity to the full length 2264 bp promoter in cultured SMCs (6), we used the smaller fragment so that both the SV40 and SMGA plasmids were of equivalent length to minimize any effects of plasmid size on nuclear import activity. At 2 days post delivery, the vessels were harvested and the localization of gene expression was determined by immunohistochemical detection of GFP and luciferase. Tissue sections stained with an antibody directed against GFP demonstrated that GFP expression (from pCMV-GFP-SMGA₁₇₆) was limited almost entirely to the SMC layer of the vessel wall (Fig. 3A). This result confirms that the shorter 176 bp promoter also mediates SMC-specific plasmid nuclear import *in vivo* as it does in microinjected cells. By contrast, adjacent thin sections from the same vessels stained with an antibody directed against luciferase revealed that luciferase (from pCMV-Lux-DTS) was expressed in multiple cell types throughout the vessel wall including endothelial cells, smooth muscle cells, cells of the adventitia and adipocytes in the surrounding adipose tissue (Fig. 3B). Control arteries receiving no DNA were negative for both luciferase and GFP staining (not shown). Taken together, these data demonstrate that the SMGA DNA nuclear targeting sequence functions *in vivo* to increase gene expression and that incorporation of this DNA sequence can target high level gene expression to the smooth muscle cells of the vasculature.

Effect of SMGA DTS on Plasmid Nuclear Import *In Vivo*. The above results suggest that the SMGA nuclear import sequence functions *in vivo* to increase nuclear

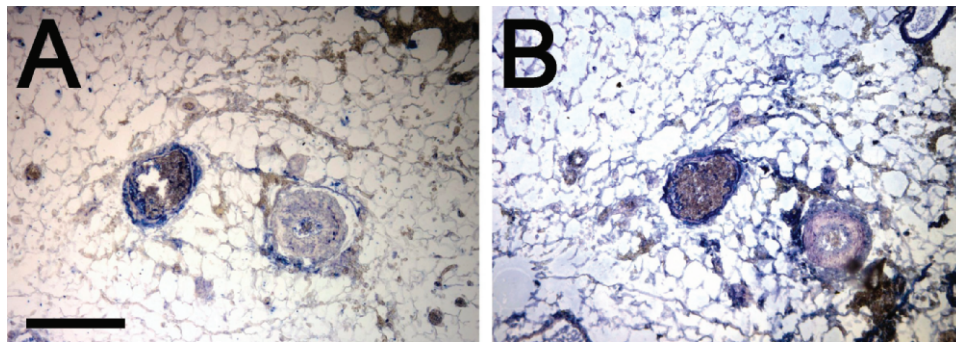


Figure 3. Effects of the SMGA DNA nuclear targeting sequence on localization of reporter gene expression. Mesenteric vessels were electroporated with pCMV-GFP-SMGA₁₇₆ and pCMV-Lux-DTS and harvested 2 days later ($n = 4$ vessels per plasmid). GFP and luciferase expression was detected in adjacent thin sections using antibodies directed against GFP (A) or luciferase (B) followed by Vector Laboratory's ABC Alkaline Phosphatase system and Vector Blue substrate. Bar = 200 μ m.

accumulation of plasmid in smooth muscle cells exclusively. To test this, *in situ* hybridization was carried out on tissue sections of vessels treated with the proximal (176 bp) SMGA-containing plasmid, pCMV-Lux-DTS, or pCMV-Lux 2 days after gene transfer. As previously reported, although plasmids lacking a DTS (pCMV-Lux) were detectable immediately following electroporation (9), no plasmids were detected by 2 days post-transfer (Fig. 4A), presumably due to degradation and clearance. Plasmids containing the SV40 DTS, however, were detected throughout the entire bundle including the SMCs of the vessel wall as well as cells of the adventitia and surrounding tissue (Fig. 4C). By contrast, pCMV-GFP-SMGA₁₇₆ DNA was limited to the SMC layer of the vessel wall within the neurovascular bundle (Fig. 4E and G). These results demonstrate that the SMGA DNA nuclear targeting sequence acts *in vivo* as well as *in vitro* to limit gene transfer to smooth muscle cells.

Discussion

To date there have been numerous attempts to target specific cell types for transgene expression within a given tissue. Targeting cell surface receptors and using cell-specific promoters to drive gene expression are among the most common (1, 2, 14). In the case of cell surface targeting using receptor-ligand interactions, the major drawback is that suitable receptors are often only enriched on, and not unique to, desired cell types, making delivery less than specific. Alternatively, cell-specific targeting may be achieved using cell-specific promoters to drive gene expression exclusively in one cell type. This approach may be more practical assuming that DNA can be delivered to all cells within a given tissue. In this study, we describe a novel third method to achieve cell-specific gene therapy by limiting nuclear import of DNA to a specific subset of cells within a particular tissue. The data presented demonstrate that the SMGA DNA nuclear targeting sequence functions *in vivo* to target gene expression to the smooth muscle cells of the vasculature exclusively. Moreover, this gene expression correlates with specific nuclear accumulation of

the SMGA-containing plasmids in the SMC layer of the vessel wall.

Until now, increasing or limiting the nuclear accumulation of exogenous DNA has been overlooked as a means of targeting specific cell types *in vivo*. It has been shown however, that the nuclear envelope provides a significant barrier to macromolecules in non-dividing cells, which makes restricting gene expression in this way a reasonable possibility (3, 15, 16). We have previously shown that a DNA nuclear targeting sequence is required to achieve high-level gene expression in the vasculature (9). Without incorporation of a nuclear import sequence, plasmids remain cytoplasmic in the non-dividing cells of the vasculature and are eventually degraded. By incorporating the SV40 universal DTS we can direct high-level gene expression by increasing nuclear uptake in all cell types of the vessel wall and surrounding tissue. Incorporation of the SMGA SMC-specific nuclear import sequence functions similarly to increase nuclear import and expression in the vasculature but has the added advantage of selectively targeting the SMCs. While the absolute levels detected are lower than those seen with the SV40 DTS, this is most likely due to the significant contribution towards gene expression of adventitial and endothelial cells in vessels treated with the SV40-containing plasmids.

Although a number of DNA sequences have been identified and shown to increase DNA nuclear import both *in vitro* and *in vivo* by multiple laboratories (4, 6, 7, 17–20), other experiments suggest that these sequences may not be “required” for DNA nuclear import in the absence of cell division, but rather may enhance transport. Studies in mouse muscle using direct injection or electroporation have shown that plasmids lacking any SV40 sequence give robust gene expression from either the CMV promoter or RSV LTR, among other promoters (21–26). This implies that in certain tissues, especially skeletal muscle, plasmids lacking the SV40 enhancer can enter the nucleus, although other studies have reported that the SV40 enhancer can increase gene expression in mouse muscle (8, 27). Another recent study used a series of plasmids containing or lacking various

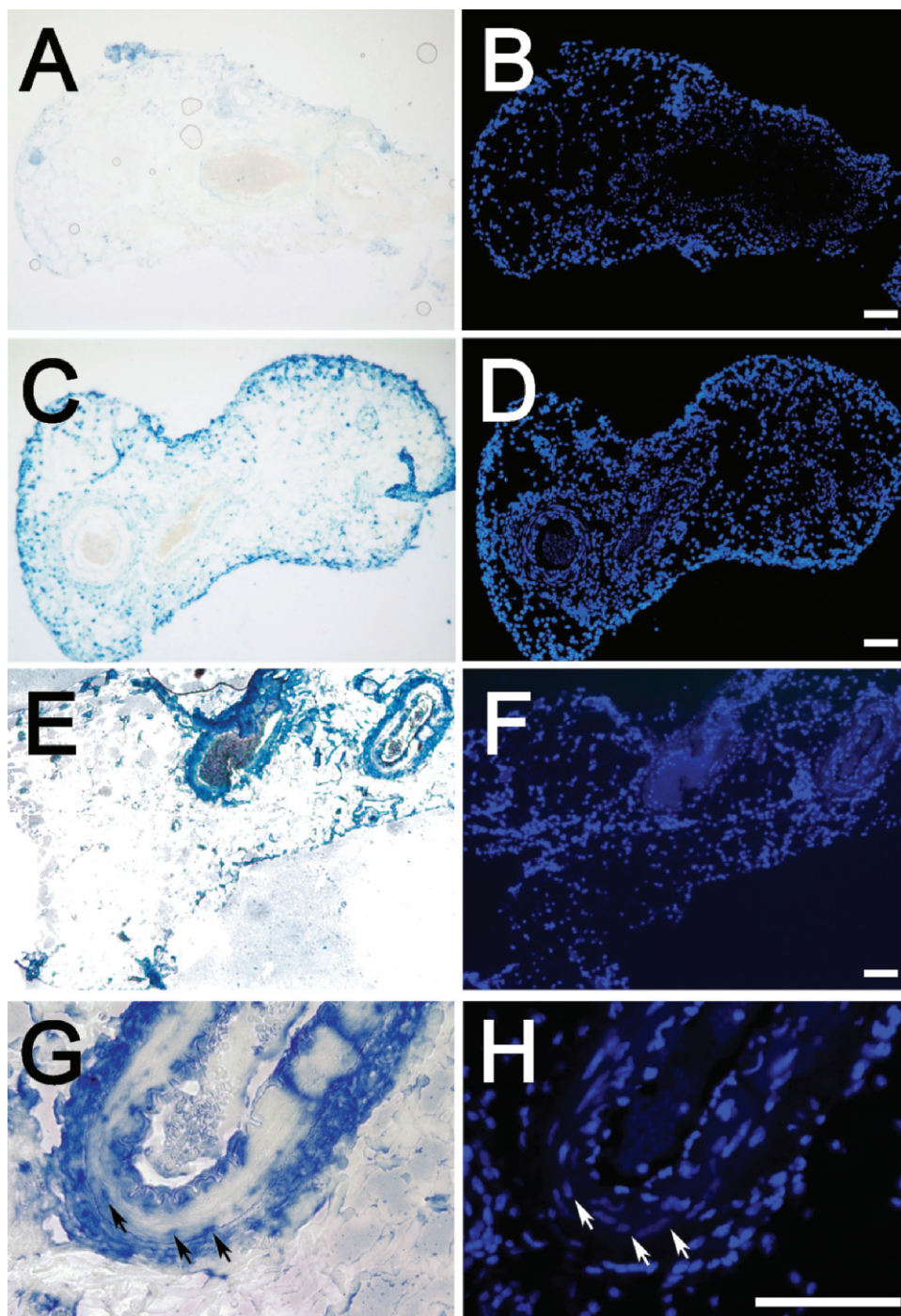


Figure 4. The SMGA DNA nuclear targeting sequence promotes smooth muscle-specific plasmid nuclear import *in vivo*. pCMV-Lux (A and B), pCMV-Lux-DTS (C and D), or pCMV-GFP-SMGA₁₇₆ (E–H) was delivered to mesenteric vessels by electroporation and vessels were harvested 2 days later ($n = 4$ vessels per plasmid). *In situ* hybridizations were carried out on tissue sections using either a probe specific for pCMV-Lux/pCMV-Lux-DTS (A–D) or a GFP-specific DNA probe to detect SMGA₁₇₆-containing plasmid (E–H). Tyramide amplification and Vector Laboratory's ABC, alkaline phosphatase-Vector Blue detection systems were used to visualize plasmid in sections from treated vessels. Sections were counterstained with DAPI (Panels B, D, F, and H). Arrows in panels G and H identify matching SMC nuclei at higher power magnification. Bars = 100 μ m.

segments of the SV40 DTS and found that upon liposome-mediated transfection of several cultured cell types, there was no effect by the import sequence on gene expression (28). However, in this study, effects on nuclear import of the DNA were not evaluated. One possibility is that these DNA

nuclear import sequences increase the rates of nuclear import so as to prevent substantial DNA degradation in the cytoplasm. If this is the case, it could be possible to load the cytoplasm with large amounts of DNA and drive the nuclear import reaction independent of specific DNA sequences. In

support of this, it has been shown that when 10^5 plasmids (lacking SV40 sequences) are injected into the cytoplasm of a single mouse myotube *in vivo*, no gene expression is seen, but when 10^6 plasmids are injected, gene expression is detected (29). Another possible reason for these differing results is that many studies on intracellular trafficking of DNA have used a labeling technique that has been shown to alter DNA trafficking properties, perhaps leading to misinterpretations (30). Finally, it should be stressed that the central caveat to these experiments is that these DNA nuclear import sequences are of consequence only in largely non-dividing tissues and cells. Indeed, we recently demonstrated that while the presence of the SV40 DTS was required for DNA nuclear import in cultured corneal epithelial cells and fibroblasts (31), it had little impact on gene delivery to the cornea following injection and electroporation, due to the fact that the cells in the cornea were actively dividing in a wound repair process induced by the gene transfer process (32).

Experiments using microinjected and transfected cells suggest that smaller fragments of the SMGA promoter (e.g., 404 bp and 176 bp fragments) should act similarly to the 2294 bp full-length promoter to direct nuclear import in the intact vasculature (6). Indeed, all three supported nuclear import and subsequent gene expression. This is in contrast to the transcriptional activity of this and many other cell-specific promoters, where results from cultured cells and animals do not necessarily correspond (33). For example, when the expression and smooth muscle specificity of various lengths of the SMGA promoter were studied in transgenic mice and cultured smooth muscle cells, the promoter fragments behaved differently in the two systems, with 4.9 and 0.6 kb SMGA promoter fragments giving high gene expression in cells but only the larger promoter functioning for SMC-specific transcription in transgenic mice (11, 12, 34, 35). Taken together with studies that have shown that mutations in transcription factor binding sites in the SMGA promoter that do not greatly alter the transcription activity of the promoter in transient transfections but destroy nuclear import activity of the sequence (11), these results suggest that transcriptional and nuclear import activities of the SMGA promoter are separable.

In summary, these results show that cell selectivity of gene expression can be achieved in a non-dividing tissue by limiting nuclear import of DNA to specific subpopulations of cells within a tissue. By combining this approach with the use of cell-specific or regulated promoters driving gene expression, a second level of protection can be achieved so as to further limit gene expression to one cell type within a tissue. The ability of various promoters to express for short (e.g., the CMV promoter) or prolonged (e.g., the UbC promoter) durations can further increase the utility of vectors containing these cell-specific DNA nuclear targeting sequences to allow for smooth muscle targeted expression that lasts days (CMV) or months (UbC), depending on the disease target to be addressed (32, 36). These approaches

will expand our repertoire of gene therapy vectors. Moreover, this finding of selective DNA nuclear uptake will enhance the safety of gene transfer, allowing rational gene therapies to be developed.

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